STANDARD OPERATING PROCEDURES

Complete Collection

Environmental Analysis Program
Teaching and Research Laboratories, Seeley G Mudd
Pomona College

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Pomona College
Claremont, California

STANDARD OPERATING PROCEDURES BINDER

EA Program – Standard Operating Procedures

Biogeochemistry Lab, Pomona College

June 26, 2026

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SOP-L805	Drone Image Processing (Photogrammetry)
SOP-L806	Statistical Analysis Templates (R)
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SOP-XXX	Title of Procedure

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-XXX

Biogeochemistry Lab Resource Page

Standard Operating Procedure

SOP Number	SOP-XXX
Revision	–
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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How to Use This Site

- **Find an SOP:** Use the sidebar navigation or the search bar (top right).
- **Download a PDF:** Each SOP page has a "View as PDF" link at the top.
- **Suggest a change:** Click the "Edit this page" link on any SOP, or open an issue.
- **Create a new SOP:** Copy `lab_sops/SOP-TEMPLATE.qmd`, fill it in, and submit a Pull Request.

SOP Numbering Scheme

SOPs are numbered by topic. Field SOPs use an **F** prefix and Lab SOPs use an **L** prefix, but both share the same numeric ranges:

Category	Field SOPs	Lab SOPs
Safety and administration	F001–F099	L001–L099
Equipment and instrumentation	F100–F299	L100–L299
Water and aquatic methods	F300–F399	L300–L399
Soil methods	F400–F499	L400–L499
Air and atmospheric methods	F500–F599	L500–L599
Biology, ecology, and molecular biology	F600–F799	L600–L799
Data management and analysis	F800–F899	L800–L899

SOPs (pdf)

- Complete Set of SOPs (pdf)

SOP Subsets by Topic

- Safety and Administration (pdf)
- Equipment and Instrumentation (pdf)
- Water and Aquatic Methods (pdf)
- Soil Methods (pdf)
- Air and Atmospheric Methods (pdf)
- Biology and Molecular Biology (pdf)
- Data Management and Analysis (pdf)

Safety and Administration (001–099)

SOP	Title	Current Rev.	Notes
F001	Field Safety	1.1	
F002	Field Equipment Checklist	0.1	
F003	Field Notebook Protocols	0.1	
F004	Site Access and Permits	0.1	
F005	Random Sampling Design	1.0	FW
F006	GPS and Site Navigation	0.1	
F007	Field Decontamination Protocols	0.1	
F008	Sample Labeling, Chain of Custody, and Transport	0.1	
L001	*Lab Orientation and Training Checklist*	0.1	TBD
L002	Laboratory Safety	1.04	
L003	Hazardous Materials Handling	1.04	
L004	Chemical Spill Response	0.1	

SOP	Title	Current Rev.	Notes
L005	Chemical Inventory Management	0.1	
L006	Waste Disposal and Hazardous Waste Pickup	0.1	
L007	Equipment and Instrument Inventory	1.0	
L010	Lab Notebook Recording	0.1	
L015	Foreign Soil Handling (Import Permits)	0.3	
L020	Reagent and Standard Preparation	0.1	

Equipment and Instrumentation (100–299)

Field Equipment (F100–F199)

SOP	Title	Current Rev.	Notes
F100	EA Program Vehicle Use	0.1	
F101	Electrical Power in the Field	0.2	
F102	Waders/Wetsuit	–	TBD
F103	*Canoe / Kayak / Small Watercraft*	–	TBD
F104	*Field Laptop and Tablet Use*	–	TBD

Field Instrumentation (F200–F299)

SOP	Title	Current Rev.	Notes
F200	Eureka Manta2 Multiprobe	0.7	
F201	Pygmy and Type A Velocity Meters	0.1	
F202	SonTek Acoustic Velocity Meters	0.1	
F203	HOBO Water Level Pressure Transducers	0.1	
F204	HOBO Weather Station Deployment	0.1	
F205	Handheld Turbidity Meter	0.1	
F207	Secchi Disk and Secchi Disk Tube	0.1	
F208	Portable Spectrophotometer / Colorimeter (Field)	0.1	
F209	Dissolved Oxygen Meter (Field)	0.1	

Lab Equipment (L100–L199)

SOP	Title	Current Rev.	Notes
L100	*Milli-Q Water System*	0.2	Broken
L101	Glassware Cleaning and Preparation	0.1	
L102	Using Balances	0.2	

SOP	Title	Current Rev.	Notes
L103	Micropipettes	0.3	
L104	Using a Vortex Mixer	0.1	
L105	Drying Ovens	0.1	
L106	Refrigerators and Freezers	0.1	
L107	Hot Plates and Dry Bath	0.12	
L108	*Clinical Centrifuge*	0.1	
L109	Sorvall ST8R Centrifuge	0.1	
L110	*Ozone Generator Operation*	0.1	
L111	Muffle Furnace	0.1	
L112	Autoclave / Sterilization	0.1	
L113	Laminar Flow Cabinet	0.1	
L114	Vacuum Filtration Apparatus	0.1	
L115	Sonicator	0.1	
L116	Sample Grinding and Homogenization (Ball Mill, Mortar and Pestle)	0.1	

Lab Instrumentation (L200–L299)

SOP	Title	Current Rev.	Notes
L200	Analytical Balance Calibration	1.0	
L201	*Spectrophotometer Operation*	–	
L202	Accumet pH Meter	0.4	
L203	Trilogy Laboratory Fluorometer	0.4	
L204	Shimadzu TOC/TON Analyzer	1.0	
L205	XRF (X-Ray Fluorescence)	0.1	
L206	*ICP-MS*	0.1	
L207	*LA-ICP-MS (Laser Ablation)*	0.1	
L208	ThermoFisher Delta V IRMS	0.2	
L209	*Radiometric Dating*	NA	TBD
L210	*Ion Chromatograph Operation*	NA	TBD
L211	Echo Labs Revolve Microscope	0.1	
L212	NanoDrop Spectrophotometer	0.1	
L213	Qubit Fluorometer	0.1	
L214	QX200 Droplet Digital PCR System – Instrument Familiarization	0.1	
L215	Hach DR3900 Benchtop Spectrophotometer	–	TBD
L216	Dissecting and Compound Microscope	0.1	

Water and Aquatic Methods (300–399)

Field – Water / Aquatic Systems (F300–F399)

SOP	Title	Current Rev.	Notes
F300	Surface Water Sampling	0.9	
F301	Stream Discharge Measurement	0.1	
F302	Stormwater Sampling	0.1	
F303	Groundwater Sampling	0.1	
F304	Stream Physical Habitat Analysis	0.1	
F305	Sediment Sampling (Streams and Lakes)	0.1	
F306	Wetland Hydrology Monitoring	0.1	
F306	Wetland Delineation	0.1	
F307	Lake and Reservoir Profiling*	0.1	
F308	eDNA Water Sample Collection	0.1	
F309	*Periphyton / Algae Sampling*	–	TBD

Lab – Water Analysis (L300–L399)

SOP	Title	Current Rev.	Notes
L300	pH and Conductivity (Bench)	1.0	
L301	Alkalinity Titration	0.1	
L302	*Turbidity Measurement*	–	TBD
L303	Hach Colorimetric Analysis	1.0	
L304	*Nutrient Analysis (Water Samples)*	–	TBD
L305	Bacteria Screening (Colilert Quanti-Tray)	4.3	
L306	BOD5 (Biochemical Oxygen Demand)	0.3	
L307	ELISA: BPA, BPB, Pesticides	0.3	
L308	Microplastics in Water	0.2	
L309	Pesticide Analysis – Gas Chromatography	–	
L310	Chlorophyll Extraction and Analysis	0.1	
L311	Dissolved (NPOC) Organic Carbon (Water)	0.1	
L312	Total Suspended Solids / Total Dissolved Solids	0.1	
L313	Metals in Water (ICP-MS Prep and Acidification)	0.1	
L314	*Anion Analysis (Ion Chromatography)*	–	NA
L315	eDNA Lab Processing (Filtration, Extraction, Inhibition Testing)	0.1	

Soil Methods (400–499)

Field – Soil (F400–F499)

SOP	Title	Current Rev.	Notes
F400	Soil Sampling and Processing	1.0	
F401	Texture by Feel	0.1	DLF
F402	Soil Bulk Density (Core Method)	0.1	AC
F403	Soil Infiltration Rate	0.1	
F404	Soil Temperature Profiles	0.1	
F405	Soil Color (Munsell)	0.1	
F406	Soil Respiration Chambers (Field)	0.1	
F407	Soil Profile Description	0.1	

Lab – Soil and Carbon Analysis (L400–L499)

SOP	Title	Current Rev.	Notes
L400	Soil Moisture – Gravimetric Method	0.2	
L401	Soil Texture (Hydrometer Method)	0.1	DLF
L402	Soil pH and Salinity	0.1	
L403	Nitrate and Ammonium – KCl Extraction	0.4	MLH
L404	Nitrate Quick Test	0.1	FW
L405	Extractable Phosphorus	0.1	
L406	Loss on Ignition (SOM)	1.0	
L407	Total Carbon – Soil	1.0	DLF
L408	Solvita CO_2 Burst	1.1	
L409	Microbial Biomass Carbon (CFE)	1.0	AC
L410	Hot-Plate Acid Digestion of Soils for Trace Metal Analysis	0.2	
L411	Soil Lead Analysis (MARS Microwave)	0.2	
L412	Nitrification Potential	0.1	
L413	Solvita Compost Maturity	0.1	
L414	Flash EA Soil dN15 and dC13 Combustion	0.1	
L415	Carbonate Isotope Analysis by GasBench II-IRMS	0.1	
L416	Cation Exchange Capacity (CEC)	0.1	
L417	Soil Aggregate Stability (Wet Sieving)	0.1	
L418	Water-Extractable Dissolved Organic Carbon (Soil)	0.1	
L419	Soil Color (Munsell – Lab Confirmation)	0.1	
L420	Soil Labile Amino-Nitrogen – Solvita SLAN Test	0.1	

Air and Atmospheric Methods (500–599)

Field – Air / Atmospheric (F500–F599)

SOP	Title	Current Rev.	Notes
F501	Air Temperature Monitoring (Sensors)	0.1	
F502	Air Quality Monitoring (Portable Sensors)	0.1	
F503	Particulate Matter Sampling*	0.1	
F504	Picarro GHG Analyzer (Field Deployment)	1.0	AC
F505	Passive Air Samplers (Diffusion Tubes)	0.1	
F506	Noise Level Monitoring	0.1	
F510	Weather Monitoring and Field Meteorology	0.1	

Lab – Air / Atmospheric (L500–L599)

No lab air SOPs exist yet. Range is reserved.

SOP	Title	Current Rev.	Notes
L500	*Gravimetric PM Filter Analysis*	–	TBD
L501	*Passive Sampler Extraction and Analysis*	–	TBD

Biology, Ecology, and Molecular Biology (600–799)

Field – Microbes, Plants, and Animals (F600–F699)

SOP	Title	Current Rev.	Notes
F602	iNaturalist for Biodiversity Documentation	0.3	
F603	Vegetation Transect Surveys	0.1	
F604	Tree DBH and Forest Inventory	0.1	
F605	Leaf Area Index Measurement	0.1	
F606	Canopy Cover (Densiometer & Hemispherical Photography)	0.1	
F607	Tree Tagging for Restoration Monitoring	0.1	
F608	Plant Tissue Sampling	0.1	
F610	Macroinvertebrate Sampling	0.1	
F611	Pollinator Observation Surveys	0.1	
F615	Bird Point Count Surveys	0.1	
F620	Camera Trap (CamPark T20)	0.1	
F621	*Acoustic Monitoring (Bioacoustics)*	–	TBD
F622	*Small Mammal Trapping (Sherman Traps)*	–	TBD
F630	Drone Operations (DJI Matrice/Mavic)	0.1	
F631	*360 Camera*	–	

Field – Molecular Biology and DNA (F700–F799)

SOP	Title	Current Rev.	Notes
F700	eDNA Field Sampling and Preservation	0.1	
F701	Soil Microbial Field Sampling for Molecular Analysis	0.1	

Lab – Microbes, Plants, and Animals (L600–L699)

SOP	Title	Current Rev.	Notes
L600	*TBD*	–	
L601	Wet Mount for Microscopy	0.1	
L602	Macroinvertebrate Sorting, Subsampling, and Identification	0.1	
L603	Plant Tissue Drying, Grinding, and Preparation for Analysis	0.1	
L604	Algae / Periphyton Identification and Enumeration	0.1	
L605	Soil Enzyme Assays (Fluorometric)	0.1	

Lab – Molecular Biology and DNA (L700–L799)

SOP	Title	Current Rev.	Notes
L700	DNA Extraction	0.1	AC
L701	Primer Selection and Design for Nitrogen Cycle Genes	0.1	
L702	ddPCR Applications Guide – Absolute Quantification of Soil Nitrogen-Cycle Functional Genes	0.1	
L703	PCR Amplification and Gel Electrophoresis	0.1	
L704	Amplicon Library Preparation for Sequencing	0.1	
L705	Bioinformatics Pipeline (QIIME2 / DADA2)	0.1	
L706	eDNA Metabarcoding Lab Workflow	0.1	

Data Management and Analysis (800–899)

SOP	Title	Current Rev.	Notes
L801	RStudio Server and GitHub	1.5	
L802	Quality Control and Quality Assurance	1.0	
L803	Data Entry and Database Management	0.1	
L804	GIS	0.1	
L805	Drone Image Processing (Photogrammetry)	0.1	
L806	Statistical Analysis Templates (R)	0.1	
L807	Instrument Data Export and File Management	0.1	

Note

This table is maintained manually. Italicized entries marked *TBD* are planned SOPs that have not yet been written. Entries marked TBD are suggested additions identified during the 2026 renumbering review. The sidebar navigation reflects every `.qmd` file in the repository automatically.



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-XXX

New to the Lab? Start Here

Standard Operating Procedure

SOP Number	SOP-XXX
Revision	–
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Welcome

Welcome to the EA Program lab and field research team at Pomona College. Before you begin any lab or field work, you need to complete the training steps below. This page walks you through everything in order.

2. Step 1: Required Safety Training

Complete these SOPs first. You will not be cleared for independent work until you have read and been signed off on each one.

Order	SOP	What You'll Learn
1	L002: General Laboratory Safety	Lab rules, PPE, emergency procedures, training sign-off form
2	L003: Hazardous Materials Handling	Chemical safety, spill response, waste disposal, storage
3	F001: Field Safety	Transportation, weather hazards, biotic hazards, emergency response

! Before You Touch Anything

You must complete both safety SOPs and be signed off by the PI or Chemical Safety Officer before working independently in the lab or field.

3. Step 2: Lab Equipment Basics

Once safety training is complete, familiarize yourself with the equipment you will use most often.

SOP	What You'll Learn
L400: Soil Moisture	Drying oven, analytical balance, desiccator use
L102: Using Balances	Proper weighing technique
L11: Micropipettes	Accurate liquid dispensing (coming soon)
L14: Milli-Q Water System	Producing ultrapure water (coming soon)

4. Step 3: Software and Data Management

We use R, RStudio, and GitHub for data analysis and collaboration. You will need accounts and basic proficiency before starting a research project.

- **RStudio Server:** Access at your institution's RStudio Server URL (ask the PI)
- **GitHub:** Create an account at github.com and request access to the marclos/SOPs repository
- SOP L801: RStudio Server and GitHub

5. Step 4: Project-Specific Training

Depending on your research project, you will need additional SOPs. Your PI or project lead will tell you which ones apply. Common starting points:

If you're doing soil work:

- F400: Soil Sampling and Processing
- L400: Soil Moisture
- L403: Nitrate and Ammonium (KCl Extraction)
- L408: Solvita CO_2 Burst
- L406: Loss on Ignition
- L407: Shimadzu TOC-L

If you're doing water quality work:

- F300: Surface Water Sampling
- F200: Eureka Manta2 Multiprobe
- LXXX: Accumet pH Meter (coming soon)
- L305: Colilert Quanti-Tray

6. Step 5: Get Signed Off

After completing the required reading and supervised practice for each SOP, get your training record signed by the PI or designated trainer. Training records are maintained in the EA Program files.

7. Questions?

- **SOP questions:** Open an issue on GitHub
- **Lab access:** Contact the PI or lab manager
- **Safety concerns:** Contact the Chemical Safety Officer or Campus Safety

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F001

Field Safety

Standard Operating Procedure

SOP Number	SOP-F001
Revision	1.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP covers field-based research activities and class field trips, addressing transportation, weather-based, and biotic hazards. Although we cannot predict all risks and hazards, it is everyone's responsibility to identify and mitigate potential risks associated with field work (Occupational Safety and Health Administration, 2012).

2. Scope

- **Applies to:** All field-based research activities, class field trips, and off-campus data collection
- **Does not apply to:** Laboratory-only procedures (see relevant Lab SOPs)
- **Location:** All field sites used by the EA Program

3. Responsibilities

Role	Responsibility
Instructor / PI	Approve field plans, ensure training compliance
Trained Personnel	Follow this SOP, report deviations and hazards
New Students	Complete training and document review before field work

Anyone conducting research in the field is required to have documented training before commencing field work. Learning about risks and taking the precautions described here is the first step to reducing the risks associated with field work. Anyone working in the field should identify potential risks and attempt to mitigate them.

4. Definitions

- **Field lead:** The trained, designated person responsible for safety decisions during a field outing; authorizes resumption of work after a stop-work condition, maintains communication with the PI, and is the first point of contact in an emergency.

- **Heat illness:** A spectrum of conditions caused by overheating, ranging from heat cramps and heat exhaustion to heat stroke; heat stroke (core temperature above 40 °C, altered mental status) is a life-threatening emergency requiring immediate 911 activation (Occupational Safety and Health Administration, 2012).
- **Incident report:** A written record of any injury, illness, near-miss, or property damage occurring during field work; submitted to the PI and the college risk management office within 24 hours of the event (Pomona College, 2024).
- **Minimum crew size:** Two persons at all times during field work; one person must be capable of summoning help if the other is incapacitated. Solo field work is prohibited unless explicitly waived in writing by the PI for low-hazard, high-visibility situations.
- **PPE (personal protective equipment):** Equipment worn to minimize exposure to field hazards, including closed-toed shoes, long pants, safety glasses, sun protection, and task-specific items such as hard hats and cut-resistant gloves (National Research Council, 2011).
- **Risk assessment:** A structured evaluation of the hazards at a field site and the probability and severity of resulting harm; used to select appropriate control measures and documented on the Field Work Planning Form (Appendix A).
- **Stop-work authority:** The right – and duty – of any crew member to stop field operations without prior approval when they observe an imminent safety hazard. No crew member may be penalized for exercising stop-work authority in good faith (Occupational Safety and Health Administration, 2012).
- **Two-person rule:** See "Minimum crew size" above.
- **Widow-maker:** A dead branch, dead tree, or tree with structural defects that may fall without warning; a leading cause of fatalities in forest work; avoid working beneath or near hazard trees, especially in high wind.

5. Safety and Hazards

General Field Hazards

Reducing risks is one of our main priorities. Like many endeavors in life, field work has certain risks. We can reduce these risks dramatically by understanding our physical limits and how these can be tested by uncontrolled environmental factors.
We rely on each individual to be mindful of their own limitations and to advocate for themselves and others to reduce risk exposure.

5.1. Required Materials

- First Aid Kit (kits are located in EA vehicles; see EA vehicle checklist to document their presence)
- Cell phone(s) with emergency contacts programmed
- Walkie-talkies (optional but recommended for sites with poor cell service)
- Personal Protective Equipment (PPE) appropriate for the site and task

6. Estimated Time

Preparing to go in the field can take a few minutes to several hours of planning and gathering resources. A good rule of thumb is to double the time you think it will take to get ready.

7. Field Preparation Checklist

7.1. Before Departure

- ☐ Check weather report and air quality index
- ☐ Read and understand emergency procedures for the specific site
- ☐ Know locations of emergency equipment (first aid kit, fire extinguisher if applicable)
- ☐ Be aware of any special medical conditions of team members
- ☐ Report departure time and expected return to supervisor
- ☐ Identify the hospital or urgent care facility closest to the field site
- ☐ Develop a transportation plan with a designated driver
- ☐ Complete a risk assessment and safety plan (Appendix A)

7.2. Supply Checklist

- ☐ PPE appropriate for the task (see §8 for task-specific items)
- ☐ Rain gear (if precipitation is forecast)
- ☐ Closed-toed shoes or field boots (no sandals or open-toed shoes)
- ☐ Additional clothing/gear for site conditions (e.g., long sleeves and gaiters for poison oak areas)
- ☐ Sunscreen and sun hat
- ☐ Adequate water (minimum 1 L per person per 2 hours in warm conditions)
- ☐ Rite in the Rain field notebook and waterproof pen
- ☐ Watch or phone for time-stamping field records
- ☐ First aid kit
- ☐ Cell phone with the following numbers programmed:
  - 911 (emergency)
  - Campus safety: (909) 621-8170 (Pomona College)
  - Lab supervisor and/or instructor
  - Field team members

7.3. On Site

- Identify a meeting time and departure plan if the team splits up
- Leave no trace: ensure the environment remains undisturbed
- Field lead ensures that safety procedures are followed
- Report any potential field hazards to the field lead immediately

8. Hazard-Specific Procedures

8.1. Transportation

- All passengers must wear seatbelts at all times
- Designated driver is identified before departure; no impaired driving
- Allow extra time on unpaved or unfamiliar access roads
- Do not drive into flooded washes or roads

8.2. Weather Hazards

8.2.1. Lightning

Follow the 30-30 rule: if the time between a lightning flash and thunder is 30 seconds or less, the storm is within 10 miles – seek shelter immediately. Remain sheltered for 30 minutes after the last flash or thunder (Occupational Safety and Health Administration, 2012).

When lightning threatens:

1. Move away from tall isolated objects (trees, towers, open hilltops)
2. Avoid open fields and water bodies
3. Seek a low-lying area away from individual trees
4. Do not hold metal equipment
5. If no shelter is available, spread the team out (at least 30 m apart) to reduce the risk of multiple casualties

8.2.2. Heat

Southern California field sites can exceed 40 °C. Carry adequate water, take shade breaks, and monitor for heat-related illness. Symptoms of heat exhaustion include heavy sweating, weakness, cold or clammy skin, weak pulse, nausea, and fainting. Heat stroke (hot/dry/red skin, rapid strong pulse, unconsciousness) is a medical emergency: call 911 immediately and cool the person rapidly (Occupational Safety and Health Administration, 2012).

8.2.3. High Wind

Sustained winds above 25 mph (above 20 mph when working near trees or overhead hazards) require stopping work. High winds increase the risk of widow-makers and can topple equipment.

8.3. Overhead Hazards (Widow-Makers)

Before working under or near any tree:

1. Look up and assess the crown for dead branches, leaning limbs, or structural failure
2. Do not work beneath or near hazard trees in high wind

3. Wear a hard hat when working in forested areas with overhead hazard potential
4. If a hazard tree is identified, flag it and report it to the field lead; do not attempt to remove it

8.4. Sharp Tools

When using hand saws, loppers, pruners, or other cutting tools:

- Keep blades sheathed while walking
- Pass tools handle-first; never toss tools
- Never carry open blades through brush
- Wear cut-resistant gloves when operating cutting tools

8.5. Biotic Hazards

8.5.1. Poison Oak

Wear long sleeves, long pants, and gloves when working in potential poison oak habitat. Learn to identify the plant. Wash exposed skin with soap and water as soon as possible after potential contact. Do not burn plant material that may contain poison oak.

8.5.2. Ticks

Wear long pants tucked into socks and long sleeves. Use permethrin- treated clothing or DEET-based repellent in tick habitat. Perform a full-body tick check at the end of every field day. Remove attached ticks promptly with fine-tipped tweezers.

8.5.3. Stinging Insects

Know whether any team member has a history of anaphylaxis. Ensure that at least one team member knows the location of any prescribed epinephrine auto-injectors (EpiPens) in the group. Call 911 immediately if anaphylaxis is suspected.

8.5.4. Wildlife

Do not approach, feed, or attempt to handle wildlife. Report unusual wildlife behavior (particularly in mammals that could indicate rabies) to the field lead.

9. Emergency Response Procedure

9.1. Responding to a Medical Emergency

1. Survey the scene; identify any risks to other team members
2. Do not move the injured person unless they are in immediate danger

3. Call 911; provide the following information:
    - Suspected type of injury or illness
    - Location (GPS coordinates if known; nearest landmark otherwise)
    - Type of assistance required
    - Number of persons injured
4. Identify a location for emergency vehicle access and send someone to meet the vehicle
5. Notify the PI or instructor as soon as possible (Pomona College, 2024)

9.2. Chronic Conditions

Personnel with chronic conditions (epilepsy, diabetes, heart conditions, severe allergies) must inform the field lead before each field outing. The field lead will ensure the team knows relevant first aid steps and the location of any required medications.

9.3. Injury from Equipment

Handle all equipment with caution. Read operating procedures before use. Do not use any equipment you are unsure of how to handle – ask a trained team member first.

9.4. Documenting the Incident

- Record what happened and how the injury or illness occurred in the field notebook
- Complete a formal incident report and submit it to the PI and the college risk management office within 24 hours
- This information is used to eliminate hazards and prevent future incidents (Pomona College, 2024)

10. Stop-Work Conditions

Stop all field operations immediately if any of the following occur:

- Lightning is detected within about 10 miles, or thunder is audible
- Wind is sustained above 25 mph (above 20 mph when working near trees or other overhead hazards)
- A crew member shows signs of heat illness, injury, or allergic reaction
- An unexpected utility or buried line is found within the work zone
- Crew size drops below the two-person minimum

Resume only when the field lead confirms conditions are safe (Occupational Safety and Health Administration, 2012).

11. References

- Occupational Safety and Health Administration (2012)
- National Research Council (2011)
- Pomona College (2024)

National Research Council. (2011). *Prudent practices in the laboratory: Handling and management of chemical hazards* (Updated). National Academies Press. https://doi.org/10.17226/12654

Occupational Safety and Health Administration. (2012). *Laboratory safety guidance* (OSHA 3404-11R). US Department of Labor.

Pomona College. (2024). *Emergency instructions*. http://www.pomona.edu/sites/default/files/emergency-instructions.pdf

12. Related SOPs

SOP	Title	Relationship
F002	Field Equipment Checklist	Supplies required for safe field work
F003	Field Notebook Protocols	Incident documentation
F004	Site Access and Permits	Site-specific hazard requirements
F100	EA Program Vehicle Use	Transportation safety

13. Training Record

Training is documented in EA Program files. Before conducting independent field work, personnel must:

1. Read this SOP in full
2. Attend a field safety orientation with the PI or field lead
3. Demonstrate understanding of the stop-work conditions and emergency response procedure
4. Be signed off by the instructor or designated trainer

14. Appendix A: Field Work Planning Form

Complete this form before each field outing and retain it in the project file.

Project / Site Name: _____

Date: _____ **Departure time:** _____ **Expected return:** _____

Field Lead: _____

Team Members: _____

Emergency Contacts:

Name	Role	Phone
911	Emergency	911
Campus Safety	Pomona College	(909) 621-8170
PI / Instructor		

Nearest Hospital / Urgent Care:

Name: _____ Address: _____ Phone: _____

Location / GPS of field site: _____

Hazards identified at this site:

- ☐ Lightning / thunderstorm risk
- ☐ Extreme heat
- ☐ High wind / overhead hazard trees
- ☐ Poison oak
- ☐ Ticks
- ☐ Stinging insects / known anaphylaxis in team
- ☐ Swift water / drowning risk
- ☐ Wildlife
- ☐ Other: _____

Risk Mitigation Plan: _____

PI / Supervisor Approval: _____ **Date:** _____

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F002

Field Equipment Checklist

Standard Operating Procedure

SOP Number	SOP-F002
Revision	1.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP provides standardized checklists for packing, verifying, and returning field equipment. Using a checklist before every field outing prevents forgotten items, reduces time lost in the field, and ensures that equipment is returned clean, functional, and ready for the next user. This SOP complements the safety and planning procedures in SOP-F001 Field Safety and the equipment inventory in SOP-L007 Equipment Inventory.

2. Scope

- **Applies to:** All EA Program field outings, including research sampling events, class field trips, and equipment loans

- **Does not apply to:** Laboratory-only procedures; drone operations (see dedicated SOP when available)
- **Location:** Equipment stored in SGM 131, 132, 133, and 134; deployed to all EA field sites

3. Responsibilities

Role	Responsibility
Field Lead	Verify checklist completion before departure; confirm equipment return
Trained Personnel	Pack and check equipment; clean and return after use
New Students	Assist with packing under supervision; learn storage locations

4. Definitions

Not applicable. This SOP covers logistical checklists using standard English equipment names that require no technical definition. For definitions of field measurement terms, consult the method-specific SOPs listed in Section 5 (Equipment Modules).

5. References

6. Procedure

6.1. Before Departure

1. Identify which **activity modules** (Section 5) apply to the planned field work. Most outings require the Core Module plus one or more activity-specific modules.
2. Print or copy the relevant checklists. Check off each item as it is packed.
3. For instruments requiring calibration (Eureka Manta2, pH meter), calibrate in the lab before packing. Record calibration results in the field notebook.
4. Verify that all batteries are charged (Crime-Lite, GPS, walkie-talkies) and that backup batteries are included.
5. Confirm cooler ice or ice packs are frozen and ready.
6. Complete the Field Work Planning Form in SOP-F001, Section 13.
7. The field lead initials the checklist and confirms vehicle reservation per SOP-F100 if applicable.

6.2. On Site

1. Unpack systematically; keep items organized to avoid loss.
2. At the end of the sampling event, re-check the checklist to confirm all items are accounted for before leaving the site.
3. Note any damaged or malfunctioning equipment on the checklist.

6.3. After Return

1. Clean all equipment per the relevant method SOP (rinse sampling equipment with DI water, wipe down instruments, dry sieves).
2. Return every item to its designated storage location (see room assignments below and SOP-L007).
3. Recharge batteries and replace consumables (sample bottles, filters, preservatives) for the next user.
4. Report any damaged, lost, or malfunctioning items to the field lead or lab manager immediately.
5. File the completed checklist with the field data sheets.

6.4. Storage Locations

Storage Room	Equipment Category
SGM 131	Tapes, sieves, survey scopes, infiltrometers, walkie-talkies
SGM 132	Vehicles, coolers, generators
SGM 133	Sampling bottles, sondes, VanDorn bottles, LiCor sensor, microscope, analytical instruments
SGM 134	DI water system, glassware, vacuum filtration supplies

7. Equipment Modules

7.1. Core Module (Every Field Outing)

This checklist applies to every field trip regardless of activity type.

Item	Storage	Check
Completed Field Work Planning Form (SOP-F001)	–	[ ]
Field notebook (Rite in the Rain) and pens	SGM 133	[ ]
Cell phone, charged, with emergency contacts	Personal	[ ]
First aid kit	In vehicle	[ ]
Sunscreen and sun hat	Personal	[ ]
Full water bottles (one per person)	Personal	[ ]
Nitrile gloves (box)	SGM 133	[ ]
GPS unit or smartphone with GPS app	SGM 133	[ ]
Camera (or phone camera)	Personal	[ ]
Vehicle keys and fuel card	In vehicle	[ ]
Walkie-talkies (if splitting into groups)	SGM 131	[ ]
Trash bag (leave no trace)	–	[ ]
Clipboard	SGM 133	[ ]

7.2. Water Sampling Module

Use for surface water grab sampling, stormwater sampling, and bacteria sampling. See SOP-F300 and SOP-F302.

Item	Storage	Check
Pre-labeled sample bottles (poly and/or glass, with preservatives)	SGM 133	[ ]
Cooler with ice packs or ice	SGM 132	[ ]
Chain of custody (COC) forms	SGM 133	[ ]
Sample tags	SGM 133	[ ]
Extension pole with bottle attachment	SGM 133	[ ]
Sterile 100 mL Colilert bottles	SGM 133	[ ]
0.45 µm syringe filters and syringes	SGM 134	[ ]
Deionized water (squeeze bottle)	SGM 134	[ ]
Paper towels	SGM 134	[ ]
Field data sheets (water sampling)	SGM 133	[ ]
VanDorn bottle (if needed)	SGM 133	[ ]
Wading boots (if entering water)	Personal	[ ]

7.3. Soil Sampling Module

Use for soil profile description, bulk density, and grab samples. See SOP-F400 and SOP-F407.

Item	Storage	Check
Soil auger or hand trowel	SGM 131	[ ]
Pre-labeled Ziploc bags or Whirl-Pak bags	SGM 133	[ ]
Munsell color chart	SGM 131	[ ]
Soil core rings (bulk density)	SGM 131	[ ]
Rubber mallet	SGM 131	[ ]
Field data sheets (soil)	SGM 133	[ ]
Cooler with ice (perishable samples)	SGM 132	[ ]

8. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F003	Field Notebook Protocols	Notebook is on checklist
L007	Equipment Inventory	Storage locations
F100	EA Program Vehicle Use	Vehicle reservation

9. Training Record

Before leading a field outing independently, personnel must:

1. Read this SOP in full
2. Identify the correct storage room for each major equipment category
3. Complete a supervised pre-departure checklist for one field outing
4. Be signed off by the field lead or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F003

Field Notebook Protocols

Standard Operating Procedure

SOP Number	SOP-F003
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP establishes the minimum standards for field documentation in the EA Program. Complete, legible, and contemporaneous field notes are the foundation of defensible environmental data (Kanare, 1985). Notes recorded in the field cannot be reconstructed later from memory; what is not written down at the time is effectively lost. This SOP ensures that every field visit produces a record sufficient for another trained researcher to understand what was done, where, when, by whom, and under what conditions.

For laboratory notebook requirements, see SOP-L010 Laboratory Notebook Recording.

2. Scope

- **Applies to:** All EA Program field activities, including sampling, sensor deployment and retrieval, site reconnaissance, equipment installation, and monitoring visits.
- **Does not apply to:** Laboratory work or purely digital data collection on tablets and phones (though digital entries should follow the same content standards described here).
- **Location:** All EA Program field sites.

3. Responsibilities

Role	Responsibility
Lab PI	Set documentation standards; review notebooks periodically
Field Lead	Ensure all team members record required entries
All Field Personnel	Maintain a field notebook per this SOP
New Students	Read this SOP before their first field outing

4. Definitions

- **Chain of custody (COC):** A documented record of sample possession at every transfer point from collection to laboratory analysis; the COC form number must be cross-referenced in the field notebook entry for the corresponding samples.
- **Contemporaneous record:** An entry made at the time the observation or action occurs, not reconstructed from memory afterward; the defining characteristic of a legally defensible field or laboratory record (Kanare, 1985).
- **Deviation:** Any departure from a written SOP during field activities; must be recorded in the notebook with the reason for the deviation and an assessment of its potential effect on data quality.

- **Field notebook:** A bound, weather-resistant notebook with numbered pages used to record all field observations, measurements, and activities; the primary raw-data record for a field project (Kanare, 1985).
- **Good Documentation Practice (GDP):** A set of principles for creating accurate, legible, contemporaneous, original, and attributable records; the foundation of data integrity in regulated and research laboratories (Broman & Woo, 2018).
- **Header block:** A standardized set of fields (date, time, site, personnel, weather, purpose, SOPs in use) recorded at the top of each field visit entry before any observations or data are logged.
- **Rite in the Rain:** A brand of field notebook and paper products printed on water-resistant stock that can be written on with ballpoint or mechanical pencil even in rain; the preferred notebook format for EA Program field work.
- **Sample ID:** A unique alphanumeric code assigned to each sample at the moment of collection, cross-referencing the field notebook, sample label, COC form, and laboratory data file.
- **Tare mass:** Not applicable in field notebooks – see SOP-L400 Soil Moisture.
- **Time stamp:** The date and time of a discrete field action, recorded in the notebook at the moment of the action; use ISO 8601 format (YYYY-MM-DD HH:MM) or a consistent AM/PM scheme throughout the notebook.

5. Notebook Selection and Preparation

5.1. Acceptable Notebooks

Use a bound, weather-resistant field notebook with numbered pages. Acceptable options include:

- Rite in the Rain (top-spiral or side-spiral, any size)
- Any hardbound notebook with numbered pages (if weather protection is not required for the site)

Do **not** use loose-leaf paper, spiral notebooks with perforated pages, or sticky notes for primary field records (Kanare, 1985).

5.2. Front Matter

On the inside front cover or first page of a new notebook, record:

- Your name, phone number, and email
- "If found, please return to: EA Program, Pomona College, SGM 133"
- Project name(s)
- Date the notebook was put into service

5.3. Page Numbering

If pages are not pre-numbered, number them in ink before going to the field. Never remove pages from a field notebook.

6. Required Entries for Every Field Visit

Every field visit entry must begin with the following header information. Write it before you do anything else on site.

6.1. Header Block

Record at the top of a new page or clearly separated section:

Field	Example
Date	2026-05-18 (ISO format: YYYY-MM-DD)
Time of arrival	08:15 PST
Site name / ID	Bernard Field Station, Plot BFS-03
GPS coordinates	34.0975 N, 117.7125 W (WGS84)
Personnel present	M. Los Huertos, J. Smith
Weather conditions	Clear, ~25 °C, light wind from W, no recent rain
Purpose of visit	Soil sampling for CN analysis; sensor download
SOPs in use	F400, F401, F510

6.2. Time Stamps

Record the time of every discrete action: sample collection, instrument reading, equipment start/stop, calibration check, photo taken. Use 24-hour format or AM/PM consistently throughout the notebook (Broman & Woo, 2018).

6.3. Site Observations

Before beginning work, note anything relevant:

- Visible changes since the last visit (new construction, vegetation removal, standing water, animal activity, vandalism, equipment condition)
- Odors or visual indicators of contamination
- Land use activities in progress nearby (mowing, irrigation, construction)

6.4. Sample and Measurement Records

For each sample collected or measurement taken, record at minimum:

- Sample ID (use the project's established naming convention)
- Time of collection or measurement
- Location (plot, transect position, depth, or other descriptor)
- Value or description (e.g., "pH = 7.2," "soil moist, dark brown, loamy texture")
- Preservation method applied (e.g., "filtered, acidified with HNO_3 to pH < 2")
- Container type and label verification

> **Write More Than You Think You Need**
>
> Include observations that seem obvious at the time. "Soil was saturated and smelled of sulfide" or "sensor housing had a spider web across the inlet" may be critical for interpreting data months later (Kanare, 1985).

6.5. Equipment and Instrument Notes

Record for every instrument used:

- Instrument ID (serial number or lab asset tag)
- Calibration status (date of last calibration; field check performed?)
- Settings (logging interval, flow rate, method name)
- Any malfunctions, error messages, or anomalies

6.6. Deviations from SOPs

If you deviate from the standard procedure for any reason, record:

- What you did differently and why
- Whether the deviation may affect data quality
- Who authorized the deviation (if applicable)

6.7. Departure

Before leaving the site, record:

- Time of departure
- Summary of work completed and any work left undone
- Disposition of samples (e.g., "cooler with ice, to lab by 12:00")
- Any follow-up actions needed

7. Sketches and Diagrams

A simple sketch is often more informative than a paragraph of text (Kanare, 1985). Include sketches of:

- Site layout showing sampling locations, landmarks, and compass orientation
- Equipment placement (sensor positions, transect layout, plot boundaries)
- Anything unusual (erosion features, spill boundaries, wildlife sightings relevant to the study)

Label all sketches with a north arrow, approximate scale, date, and your initials.

8. Photographs

8.1. When to Photograph

- At the start of every visit: a general site photo showing conditions
- Each sampling location before and after sampling
- Equipment installations and sensor positions
- Anything unusual or noteworthy (damage, contamination indicators, unexpected conditions)
- Calibration setups

8.2. Photo Naming and Logging

Record in the notebook the photo number or filename and a brief description for every photo taken:

> "Photo IMG_4231: Plot BFS-03 looking NE, pre-sampling, 08:22"

Transfer photos to the project folder within 48 hours and rename using the convention:

`YYYY-MM-DD_SiteID_Description_PhotoNumber.jpg`

Back up photos to the project GitHub repository or shared drive (Broman & Woo, 2018).

9. Corrections and Errors

- Never erase, white-out, or obliterate an entry.
- To correct an error, draw a single line through the incorrect text so it remains legible. Write the correction nearby and initial and date the change.
- If you skip a page or leave blank space, draw a diagonal line through the unused area and initial it to prevent later additions (Kanare, 1985).

10. Chain of Custody

When samples will be submitted to an analytical laboratory (internal or external), complete a Chain of Custody (COC) form. The COC must accompany the samples and include:

- Project name and PI
- Sample IDs matching the field notebook entries
- Date and time of collection
- Number and type of containers
- Analyses requested
- Preservatives applied
- Signatures at each transfer (field to transport, transport to lab)

Record the COC form number in the field notebook entry for cross-reference. See SOP-F008 Sample Labeling, COC, and Transport for the full chain-of-custody procedure.

11. Notebook Archiving

- At the end of a field season or project, submit completed notebooks to the PI for archiving.
- Notebooks are stored in labeled binders in SGM 133 and must be retained for a minimum of 5 years after project completion.
- Transcribe key data from notebooks to digital spreadsheets within 48 hours of each field visit, following the tidy data principles in

    1. Flag any discrepancies between the notebook and the digital record and resolve them with the field lead.

12. References

- Kanare (1985)
- 1

Broman, K. W., & Woo, K. H. (2018). Data organization in spreadsheets. *The American Statistician*, *72*(1), 2–10. https://doi.org/10.1080/00031305.2017.1375989

Kanare, H. M. (1985). *Writing the laboratory notebook*. American Chemical Society.

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Recording GPS coordinates
F008	Sample Labeling and COC	COC cross-referenced in notebook
L010	Lab Notebook Recording	Lab counterpart to this SOP
L801	RStudio and GitHub	Digital data file version control

14. Training Record

Before conducting any field work independently, personnel must:

1. Read this SOP in full
2. Set up a field notebook with correct front matter
3. Complete a practice header block entry under supervision
4. Demonstrate correct error-correction technique (single-line strikethrough, initials, date)
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F004

Site Access and Permits

Standard Operating Procedure

SOP Number	SOP-F004
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the procedures for obtaining site access and research permits for field work conducted by the EA Program. Field research on public or institutional lands almost always requires advance authorization (US Fish and Wildlife Service, 2019). Working without proper permits can

result in fines, confiscation of samples, invalidation of data, and jeopardized relationships with land managers. This document provides an agency-by-agency guide covering the landowners and managers most commonly encountered by EA Program researchers.

2. Scope

- **Applies to:** All EA Program field activities that take place on lands managed by the agencies and institutions listed in this SOP, including class field trips, independent student research, and faculty-led projects
- **Does not apply to:** Laboratory procedures; field work on private land (which requires landowner permission negotiated on a case-by-case basis); marine or offshore research (which may require additional permits from NOAA or the California Coastal Commission)
- **Key principle:** It is the responsibility of the PI (or the supervising faculty member) to determine what permits are required and to obtain all necessary approvals before any field work begins. Students should never assume that their faculty advisor has obtained permits – always confirm.

3. Responsibilities

Role	Responsibility
PI / Faculty	Identify required permits, submit applications, maintain permit files, ensure compliance with all permit conditions
Field Lead	Carry copies of active permits in the field; confirm permit status before each outing
Students	Verify that permits are in place before field work; follow all permit conditions; report any compliance issues to the PI

4. Definitions

- **BFS (Bernard Field Station):** An 86-acre biological reserve of The Claremont Colleges located at 1400 N. Amherst Avenue, Claremont, CA; administered by the BFS Director; all organized access requires an approved Use Request (Robert J. Bernard Biological Field Station, 2024).
- **CESA (California Endangered Species Act):** California state law (Fish and Game Code §2050 et seq.) that protects state-listed threatened and endangered species; take of CESA-listed species requires a separate Memorandum of Understanding from CDFW in addition to a Scientific Collecting Permit (California Department of Fish and Wildlife, 2024).
- **CDFW Scientific Collecting Permit (SCP):** A permit issued by the California Department of Fish and Wildlife authorizing the take or possession of wildlife for scientific, educational, or propagation purposes; required whenever research involves capturing, handling, marking, or collecting wildlife, or when take of sensitive species may occur incidentally (California Department of Fish and Wildlife, 2024).

- **ESA Section 10 permit:** A federal incidental take permit issued by the U.S. Fish and Wildlife Service under Section 10(a)(1)(A) of the Endangered Species Act; required for non-federal activities that involve take of federally listed threatened or endangered species (U.S. Fish and Wildlife Service, 2024).
- **ESA Section 7 consultation:** The formal interagency process under Section 7 of the Endangered Species Act through which a federal agency (e.g., USFS, BLM) consults with USFWS or NMFS to ensure that its authorized activities do not jeopardize listed species or destroy critical habitat.
- **Incidental take:** The unintentional, but not unexpected, harm to a listed species that results from an otherwise lawful activity; must be authorized through a Section 10 permit (private activities) or Section 7 consultation (federal activities).
- **NEPA (National Environmental Policy Act):** Federal law requiring federal agencies to assess the environmental impacts of their proposed actions; complex research proposals on federal lands may trigger NEPA review, substantially extending the permit processing timeline.
- **RAMS (Reserve Application Management System):** The online portal through which researchers and instructors apply for access to University of California Natural Reserve System reserves (University of California Natural Reserve System, 2024).
- **RPRS (Research Permit and Reporting System):** The National Park Service's online system for submitting research permit applications, reporting annual activities, and communicating with park research coordinators (National Park Service, 2024).
- **Special Use Permit (SUP):** A general-purpose permit issued by federal land management agencies (BLM, USFS) authorizing a specific research project or activity on public lands; may take 60–120 days or more to process (Bureau of Land Management, 2024; USDA Forest Service, San Bernardino National Forest, 2024).
- **Take:** Under California Fish and Game Code §86, to hunt, pursue, catch, capture, or kill, or attempt to do so; under the federal ESA, to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to do so.
- **UC Natural Reserve System (UCNRS):** A network of 41+ reserves managed by the University of California for research, teaching, and public service; access requires an approved RAMS application (University of California Natural Reserve System, 2024).

5. General Principles

5.1. Planning Timeline

Permit processing times vary widely. Submit applications at least 60–90 days before the planned start of field work. Some agencies (particularly federal ones involving NEPA review or listed species) may require 6 months or more. Always confirm the current timeline with the specific office.

Agency / Institution	Typical Lead Time
Bernard Field Station	1–2 weeks
California State Parks	60–90 days
UC Natural Reserve System	2–4 weeks
CDFW (General Use SCP)	60–90 days
CDFW (Specific Use SCP)	3–6 months

Agency / Institution	Typical Lead Time
USFWS (Section 10)	3–12 months
BLM	60–120 days
National Park Service	60–120 days
USDA Forest Service	60–120 days

5.2. Permits You May Need Simultaneously

A single field project can require permits from multiple agencies. For example, sampling macroinvertebrates in a stream within a National Forest may require a USFS Special Use Permit, a CDFW Scientific Collecting Permit, and (if listed species are present) a USFWS Section 10 permit. The land management agency will typically not finalize its own permit until copies of all other required permits are submitted.

5.3. Permit Files

The PI must maintain a digital folder (in the EA Program Google Drive or GitHub repository) containing:

- Copies of all active permits (signed, final versions)
- Copies of all applications submitted
- Copies of annual reports and compliance documents submitted to agencies
- Expiration dates and renewal deadlines

Field leads must carry printed or digital copies of relevant permits during every field outing.

5.4. Insurance and Liability

Several agencies require proof of liability insurance as a condition of permit issuance. Pomona College maintains institutional liability coverage; contact the Office of Risk Management to obtain a Certificate of Insurance naming the specific agency as an additional insured party.

6. Agency-Specific Procedures

6.1. Robert J. Bernard Biological Field Station (BFS)

The BFS is an 86-acre facility of The Claremont Colleges located at 1400 N. Amherst Avenue, Claremont, CA. Access requires advance approval from the BFS Director (Robert J. Bernard Biological Field Station, 2024).

When a permit is needed: All research projects, class field trips, and organized activities on the BFS require a Use Request, even for Claremont Colleges faculty and students. Casual, unaccompanied pedestrian access also requires prior authorization.

How to apply:

1. Contact the BFS Director (Dr. Wallace M. Meyer III, wallace.meyer@pomona.edu, 909-621-8577) to discuss the proposed project
2. Complete the online "Apply to Use" form at https://bfs.sites.pomona.edu/using-the-bfs/
3. Allow 1–2 weeks for approval; more complex projects may require additional review

Website: https://bfs.sites.pomona.edu/using-the-bfs/

6.2. California Department of Fish and Wildlife (CDFW)

CDFW issues Scientific Collecting Permits (SCPs) for the take or possession of wildlife (mammals, birds, reptiles, amphibians, fish, certain plants, and invertebrates) for scientific, educational, and propagation purposes (California Department of Fish and Wildlife, 2024).

When a permit is needed: Any time your research involves capturing, handling, marking, or collecting wildlife – or when there is potential for incidental take of sensitive species.

Permit types:

- **General Use SCP** – for more common species, non-invasive methods, and areas outside Marine Protected Areas
- **Specific Use SCP** – for focused studies involving prohibited wildlife, more invasive methods, or sensitive locations including MPAs

How to apply:

1. Review the SCP regulations (Title 14, Section 650, CCR) and Standard Conditions at https://wildlife.ca.gov/Licensing/Scientific-Collecting
2. Apply online through the Scientific Collecting Permit Portal (SCPP)
3. Pay the required permit fee before issuance

CESA-listed species: Requires a separate CESA Memorandum of Understanding from CDFW in addition to the SCP.

Reporting: Each permitholder must submit a Mandatory Wildlife Report (form DFW 1379a) annually.

Website: https://wildlife.ca.gov/Licensing/Scientific-Collecting

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6.3. California Department of Parks and Recreation (State Parks)

When a permit is needed: Any scientific research, data collection, specimen collection, or installation of monitoring equipment in California State Park System lands requires written authorization (California Department of Parks and Recreation, 2024).

How to apply:

1. Download and complete form DPR 65 from https://www.parks.ca.gov/?page_id=21557
2. Prepare a full study proposal following the Study Proposal Guidelines for Research in California State Parks
3. Submit the application, study proposal, proof of insurance, and copies of any other required permits (CDFW, USFWS, etc.) to the appropriate State Parks district office; for multi-district projects, submit to the Natural Resources Division in Sacramento at nrd.research@parks.ca.gov
4. Allow 60–90 days for processing

Reporting: A summary report of activities and findings must be submitted to State Parks; renewal is contingent on receipt of this report.

Website: https://www.parks.ca.gov/?page_id=21557

6.4. University of California Natural Reserve System (UCNRS)

The UCNRS manages 41+ reserves across California for research, teaching, and public service (University of California Natural Reserve System, 2024).

When a permit is needed: All research projects and class visits require an approved application through the Reserve Application Management System (RAMS).

How to apply:

1. Contact the reserve director(s) to discuss project requirements
2. Review the NRS Use Guidelines at https://ucnrs.org/reserve-research-guidelines/
3. Submit a research application through RAMS at https://rams.ucnrs.org
4. Once approved, submit a Reservation for each planned visit through RAMS

Special conditions:

- IACUC approval required if the project involves vertebrate animals
- Additional agency permits (CDFW, USFWS) may be required
- All equipment must be removed and sites restored at project conclusion

Website: https://ucnrs.org/

6.5. U.S. Fish and Wildlife Service (USFWS)

USFWS administers permits for activities involving species listed under the federal Endangered Species Act (ESA), migratory birds (Migratory Bird Treaty Act), and wildlife on National Wildlife Refuges (U.S. Fish and Wildlife Service, 2024).

When a permit is needed:

- Take of federally listed threatened or endangered species requires a Section 10(a)(1)(A) recovery permit
- Research involving migratory birds requires a federal Bird Banding Permit and/or Scientific Collecting Permit
- Research on National Wildlife Refuge lands requires a Special Use Permit from the refuge manager

How to apply:

1. For ESA-listed species: submit form 3-200-55 through the USFWS Endangered Species permits portal
2. For migratory birds: apply through the USFWS Bird Banding Laboratory or the federal permits portal
3. For refuge access: contact the specific refuge manager

Key considerations: Section 10 applications are subject to a 30-day Federal Register public comment period; processing times are 3–12 months. Annual reports are required for all active permits.

Website: https://www.fws.gov/service/apply-permit

6.6. Bureau of Land Management (BLM)

BLM manages extensive public lands in California, including desert, grassland, and mountain areas where EA Program research may occur (Bureau of Land Management, 2024).

When a permit is needed: Any organized research activity on BLM-managed lands, including specimen collection, installation of monitoring equipment, and systematic surveys.

How to apply:

1. Contact the nearest BLM Field Office at https://www.blm.gov/office/directory
2. BLM staff will determine whether a Special Recreation Permit, research authorization, or another approval type is needed
3. Submit required application materials (maps, project description, copies of other agency permits)
4. Allow 60–120 days; complex NEPA projects may take longer

Website: https://www.blm.gov/learn/science-in-the-blm/conducting-science

---

6.7. National Park Service (NPS)

NPS requires a Scientific Research and Collecting Permit for most scientific activities in any unit of the National Park System (National Park Service, 2024).

When a permit is needed: Any study in the natural or social sciences within a national park, monument, seashore, or other NPS unit.

How to apply:

1. Create an investigator account in the Research Permit and Reporting System (RPRS) at https://irma.nps.gov/rprs/
2. Locate the park profile in RPRS and contact the research coordinator for park-specific conditions
3. Submit the application through RPRS; allow 60–120 days
4. Annual reports are submitted through RPRS

Website: https://www.nps.gov/subjects/science/research-and-collecting-permit-overview.htm

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6.8. USDA Forest Service (USFS)

USFS manages National Forest System (NFS) lands through local Ranger District offices (USDA Forest Service, San Bernardino National Forest, 2024).

When a permit is needed: Any organized research activity on NFS lands, including specimen collection, installation of monitoring equipment, and systematic surveys.

How to apply:

1. Contact the Ranger District office for the relevant National Forest (for Southern California: San Bernardino, Angeles, Cleveland, or Los Padres)
2. Download and complete a Research Special-Use Operating Plan/Itinerary from the forest's website and submit it to the district permit coordinator
3. Forest staff will determine whether a full Special Use Permit or a Research Permit Waiver is appropriate
4. Allow 60–120 days; projects requiring NEPA analysis may take longer

Special conditions:

- Projects involving wildlife or threatened/endangered plants may require additional CDFW and/or USFWS authorization
- A summary of findings must be provided to the Forest Service upon completion

Website: https://www.fs.usda.gov/

7. Permit Decision Flowchart

Use the following steps to determine what permits you need:

1. **Identify the land manager.** Who owns or manages the land where you will work?
2. **Obtain the land access permit** from the land manager (Sections 5.1–5.8 above).
3. **Will you handle, capture, collect, or disturb wildlife?** If yes, you likely need a CDFW Scientific Collecting Permit (§5.2).
4. **Are any federally listed species present, or could they be incidentally encountered?** If yes, you likely need a USFWS Section 10 permit or ESA Section 7 consultation (§5.5).
5. **Are any state-listed species (CESA) present?** If yes, you need a CESA MOU from CDFW in addition to the SCP (§5.2).
6. **Does the land manager require other agency permits before issuing its own?** If yes, obtain those permits first and submit copies with your land access application.

> When in doubt, ask
>
> If you are unsure whether a permit is required, contact the agency before beginning field work. It is always better to ask in advance than to be stopped in the field or have your data and specimens invalidated.

8. References

- California Department of Fish and Wildlife (2024)
- California Department of Parks and Recreation (2024)
- Robert J. Bernard Biological Field Station (2024)
- University of California Natural Reserve System (2024)
- U.S. Fish and Wildlife Service (2024)
- Bureau of Land Management (2024)
- National Park Service (2024)
- USDA Forest Service, San Bernardino National Forest (2024)
- US Fish and Wildlife Service (2019)

Bureau of Land Management. (2024). *Conducting science on BLM lands*. https://www.blm.gov/learn/science-in-the-blm/conducting-science

California Department of Fish and Wildlife. (2024). *Scientific collecting permits*. https://wildlife.ca.gov/Licensing/Scientific-Collecting

California Department of Parks and Recreation. (2024). *Scientific research and collecting on state park system lands*. https://www.parks.ca.gov/?page_id=21557

National Park Service. (2024). *Research and collecting permit overview*. https://www.nps.gov/subjects/science/research-and-collecting-permit-overview.htm

Robert J. Bernard Biological Field Station. (2024). *Rules for use and apply to use*. https://bfs.sites.pomona.edu/using-the-bfs/

University of California Natural Reserve System. (2024). *Reserve research guidelines*. https://ucnrs.org/reserve-research-guidelines/

U.S. Fish and Wildlife Service. (2024). *Endangered species act section 10: Incidental take permits*. https://www.fws.gov/service/apply-permit

US Fish and Wildlife Service. (2019). *Quality assurance project plan for eDNA monitoring of bighead and silver carps*. USFWS, Midwest Fisheries Center. https://www.fws.gov/project/environmental-dna-edna

USDA Forest Service, San Bernardino National Forest. (2024). *Research and collection permits*. https://www.fs.usda.gov/r05/sanbernardino/permits/research-and-collection-permits

9. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required reading before any field work
F002	Field Equipment Checklist	Carry permit copies in field kit
F007	Field Decontamination	Required by many agency permits
F610	Macroinvertebrate Sampling	Typically requires CDFW SCP
F700	eDNA Field Sampling	May require CDFW SCP for target taxa

10. Training Record

Before leading field work on any permitted site, personnel must:

1. Read this SOP in full
2. Identify all permits required for their specific project and site
3. Confirm with the PI that all permits are active and in hand
4. Know where to find copies of active permits (digital and/or printed)
5. Understand the reporting requirements for each active permit
6. Be signed off by the PI or designated field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F005

Random Sampling Design

Standard Operating Procedure

SOP Number	SOP-F005
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP provides standardized procedures for designing and executing random sampling in field and laboratory settings. Random sampling ensures that every unit in the target population has an equal and independent probability of selection, eliminating researcher bias and supporting valid statistical inference (Elzinga et al., 1998; Krebs, 1999).

2. Scope

- **Applies to:** Any EA Program study where population estimates are the goal, including vegetation surveys, soil sampling, water quality monitoring, and laboratory subsampling
- **Does not apply to:** Targeted or judgmental sampling designs (e.g., hot-spot investigations); mark-release-recapture studies for mobile organisms (see separate SOP)
- **Sampling types covered:** Simple random sampling, grid-based random sampling, and stratified random sampling

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design, review sampling plan
Trained Personnel	Execute random selection, collect and record data
New Students	Complete training before independent fieldwork

4. Safety and Hazards

> ⚠ Hazards
>
> Field sampling involves site-specific hazards including uneven terrain, heat exposure, wildlife encounters, and sharp equipment (stakes, pins). Review site conditions before each sampling event.

4.1. Required PPE

- ☐ Closed-toe shoes or boots
- ☐ Sun protection (hat, sunscreen)
- ☐ Gloves (when handling equipment or samples)

4.2. Emergency Procedures

Refer to SOP-F001 Field Safety. Carry a first aid kit and charged cell phone to all field sites.

5. Definitions

- **Border rule:** A convention for counting organisms that overlap a quadrat edge; the standard rule is to count organisms touching the top and right edges and exclude those touching the bottom and left edges; must be applied consistently within a study to avoid double-counting (Elzinga et al., 1998).
- **Grid-based random sampling:** A method in which a coordinate grid is laid over the study area and quadrat locations are selected using randomly generated X and Y coordinates; the standard approach for quadrat-based ecological surveys (Elzinga et al., 1998).
- **Power analysis:** A calculation to determine the minimum sample size (n) needed to detect a biologically meaningful effect with acceptable Type I error (α) and Type II error (β) probability; should be completed before fieldwork begins (Krebs, 1999).
- **Quadrat:** A square frame of known area (typically 0.25, 0.5, or 1.0 m^2) used to delineate sampling units in the field (Elzinga et al., 1998).
- **Sampling frame:** A numbered list or spatial grid that enumerates every unit in the population; units missing from the frame have zero probability of selection and introduce bias (Krebs, 1999).
- **Sampling universe (population):** The complete set of units from which a sample will be drawn; must be explicitly defined before generating a sampling frame (Krebs, 1999).
- **Simple random sampling:** A design in which n units are selected at random from a numbered list (sampling frame) such that every possible combination of n units has an equal probability of selection; appropriate when the population is homogeneous (Krebs, 1999).
- **Stratified random sampling:** A design in which the population is divided into internally homogeneous subgroups (strata) and random samples are drawn independently from each stratum; reduces variance compared to simple random sampling when strata differ from each other (Elzinga et al., 1998; Krebs, 1999).
- **Stratum:** A subdivision of the sampling universe into zones that are internally homogeneous (e.g., by elevation band, vegetation type, or soil moisture class); the basis for stratified random sampling.

6. Required Materials

Item	Notes
Two survey tape measures (20–50 m)	For establishing coordinate grids
Ground pins or stakes	To anchor tape measures
Quadrat frame(s)	Size appropriate to target organisms
Random number generator app or R	See §8.1
GPS unit	To record sampling locations
Field data sheets and waterproof markers	Pre-printed with site and date fields
Compass	For establishing grid orientation
Field identification guides	If sampling vegetation or organisms

7. Biases and Interferences

Representative sampling requires strict adherence to randomized procedures. Common sources of bias include:

- **Incomplete sampling frames.** Any unit missing from the frame has zero probability of selection (Krebs, 1999).
- **Haphazard placement.** Never "throw" a quadrat to choose a location; human throws cluster toward the center of the area, violating equal probability assumptions (Elzinga et al., 1998).
- **Environmental heterogeneity.** If the study area contains distinct gradients, simple random sampling may under-represent one end. Use stratified random sampling instead (§8.4).
- **Edge effects.** Establish and apply a consistent border rule when counting organisms in quadrats (§8.3).

8. Procedure

8.1. Generating Random Numbers

Use a software-based random number generator to produce unbiased selections. Never rely on manual or mental number selection (Krebs, 1999).

In R, generate a set of random integers within a defined range:

```
# Generate 15 random integers between 1 and 100
set.seed(NULL)  # ensure a fresh seed each session
random_vals <- sample(1:100, size = 15, replace = FALSE)
sort(random_vals)
```

```
 [1]   3   9  12  15  20  35  44  51  59  63  66  75  78  84 100
```

For field use without a computer, a printed random number table is acceptable. Begin at a pre-selected (arbitrary) starting position and read values sequentially. Table 1 provides a reference table of 200 random numbers between 0 and 1000.

Table 1: Reference table of 200 random numbers (0–1000)

1	2	3	4	5	6	7	8	9	10
924.2	634.1	556.0	247.2	144.7	912.8	778.9	886.8	4.8	373.9
537.2	379.9	597.8	374.3	856.6	972.9	414.4	848.9	367.1	208.8
469.2	942.1	197.0	595.3	509.7	56.6	574.1	244.4	943.6	610.2
288.6	755.0	79.2	751.2	920.4	192.6	399.1	194.8	98.3	188.4
770.1	227.6	971.5	722.2	232.8	578.3	91.7	278.5	457.4	21.5
772.8	914.7	509.7	470.0	195.0	28.6	174.7	224.9	523.8	710.5
39.3	620.4	49.7	176.0	152.5	491.3	525.4	49.3	529.7	164.2

Table 1: Reference table of 200 random numbers (0–1000)

1	2	3	4	5	6	7	8	9	10
434.9	319.1	675.4	111.4	165.3	589.5	345.6	973.2	740.5	826.2
472.2	76.3	776.3	616.9	367.0	55.5	827.1	965.7	508.6	166.7
273.8	260.8	753.4	667.2	85.9	0.5	638.3	696.6	490.0	417.1
674.3	883.8	524.8	999.1	866.6	152.6	594.7	998.7	515.1	667.4
2.0	17.5	883.5	690.5	647.1	48.2	198.4	445.5	535.9	512.7
25.1	314.9	348.6	152.1	519.7	942.0	314.5	224.7	833.1	369.5
432.1	211.8	638.2	878.3	543.3	179.5	228.5	574.9	528.1	357.5
499.4	906.2	737.8	973.9	837.4	931.0	700.3	439.8	755.1	90.1
388.7	650.0	287.8	373.8	126.8	600.6	194.7	755.6	212.7	156.0
395.4	129.3	192.8	390.5	820.9	411.9	767.0	731.3	367.1	195.0
715.7	110.4	978.7	523.3	213.6	109.2	442.0	294.0	190.1	783.1
941.0	177.1	539.3	412.3	886.2	366.6	206.3	524.7	367.8	187.4
827.2	533.1	923.0	932.7	174.4	314.6	426.2	43.7	861.6	117.3

8.2. Simple Random Sampling

Use this approach when the population can be enumerated as a list (e.g., lab samples, batch numbers, individual organisms in a database) (Krebs, 1999).

1. Define the target population and compile a complete, numbered sampling frame.
2. Determine the required sample size (n) using a power analysis.
3. Use `sample()` in R to draw n unit numbers without replacement.
4. Document the random seed (if reproducibility is desired), the selected unit IDs, and the date of selection.
5. Collect the data or specimens from the selected units.

8.3. Grid-Based Random Sampling (Field)

Use this approach when the sampling universe is a spatially defined area (Elzinga et al., 1998).

8.3.1. Establishing the Baseline Grid

1. Locate the boundaries of the study site.
2. Drive a stake at the origin point and lay two tape measures at 90 degrees to create X and Y axes.
3. Secure the tapes with ground pins to define a coordinate grid (e.g., 20 m × 20 m).

8.3.2. Generating Coordinates and Placing Quadrats

4. Generate pairs of random integers within the range of each axis:

```r
x_max <- 20; y_max <- 20; n_quadrats <- 15
coords <- data.frame(
  X = sample(0:x_max, size = n_quadrats, replace = TRUE),
  Y = sample(0:y_max, size = n_quadrats, replace = TRUE)
)
coords <- coords[order(coords$X), ]
coords$QuadratID <- seq_len(nrow(coords))
knitr::kable(coords, row.names = FALSE)
```

X	Y	QuadratID
0	2	1
1	7	2
2	4	3
4	17	4
7	4	5
7	11	6
7	3	7
8	8	8
8	10	9
9	18	10
13	0	11
17	18	12
17	1	13
18	0	14
18	16	15

5. Walk along the axes to each coordinate intersection and place the bottom-left corner of the quadrat at that point.
6. Never reposition a quadrat after placement; if a coordinate falls on an obstruction, record the obstruction and sample as-is (Elzinga et al., 1998).

8.3.3. Recording Data Within Quadrats

7. Quantify the target variable using one of three standardized metrics:
   - **Density:** Count every individual within the frame
   - **Percent cover:** Estimate the fraction of ground area covered
   - **Frequency:** Score presence (1) or absence (0)
8. Apply the border rule consistently (§4).
9. Aim to sample at least 5–10% of total grid area, or a minimum of 30 quadrat placements, for adequate statistical power (Krebs, 1999).
10. Calculate the mean count (or cover) per quadrat and extrapolate:

$$\text{Estimated population} = \bar{x}_{\text{quadrat}} \times \frac{\text{Total site area}}{\text{Area of one quadrat}}$$

8.4. Stratified Random Sampling

Use this approach when the study area contains distinct environmental gradients that would increase variance under simple random sampling (Elzinga et al., 1998; Krebs, 1999).

1. Divide the study area into homogeneous strata based on a relevant environmental variable.
2. Within each stratum, establish an independent coordinate grid following §8.3.
3. Generate random coordinates separately for each stratum.
4. Sample each stratum independently; do not pool random selections across strata.
5. Weight stratum means by the proportion of total area each stratum represents when calculating site-level estimates.

8.5. Map-Based Sampling with Exclusion Zones

1. Obtain or create a georeferenced map of the study area.
2. Delineate exclusion zones and calculate the remaining accessible area.
3. Generate random coordinates across the full extent, then discard any that fall within exclusion zones.
4. Continue generating coordinates until the target sample size is met within the accessible area.
5. Record discarded coordinates in the sampling log.

> R Implementation
>
> An R script for map-based random sampling with exclusion polygons can be built using the `sf` package. Generate random points with `sf::st_sample()`, then filter against exclusion polygons with `sf::st_difference()`.

9. Data Management

1. Record all sampling metadata (date, site, grid dimensions, random seed or table starting position, personnel) on the field data sheet.
2. Enter field data into the project database within 48 hours.
3. Archive original data sheets (scan if paper).
4. Version-control all analysis scripts per SOP-L801 RStudio and GitHub.

10. References

- Krebs (1999)
- Elzinga et al. (1998)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Elzinga, C. L., Salzer, D. W., & Willoughby, J. W. (1998). *Measuring and monitoring plant populations* (BLM Technical Reference 1730-1). Bureau of Land Management.
Krebs, C. J. (1999). *Ecological methodology* (2nd ed.). Benjamin Cummings.

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Navigate to random points
F603	Vegetation Transect Surveys	Applies this sampling design

12. Training Record

Before designing or executing random sampling independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Demonstrate proficiency with random number generation in R
4. Participate in a supervised field sampling session using the grid-based procedure
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F006

GPS and Site Navigation

Standard Operating Procedure

SOP Number	SOP-F006
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standardized procedures for recording geographic coordinates, navigating to field sites, and managing waypoint data. Accurate and consistent location data are essential for linking field observations to spatial analyses, returning to the same sampling points across seasons, and sharing site information among researchers. This SOP covers handheld GPS receivers, smartphone GPS applications, and post-field data management (U.S. Forest Service, 2012).

2. Scope

- **Applies to:** All EA Program field activities that require geographic coordinates, including sample collection, transect establishment, monitoring well location, equipment installation, and site reconnaissance
- **Does not apply to:** High-precision survey-grade GNSS work (sub-centimeter accuracy); drone-based georeferencing (see SOP-F630); GIS post-processing and spatial analysis (see future SOP-L804)
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve coordinate system and datum for each project
Trained Personnel	Record waypoints per this SOP, upload data after fieldwork
New Students	Complete training before independent use

4. Definitions

- **Datum:** A mathematical model of the Earth's surface used as the reference for geographic coordinates; the EA Program default is **WGS 84** (World Geodetic System 1984), the datum used by consumer GPS and Google Maps; NAD 83 is nearly identical for most field purposes but must be specified explicitly if used (U.S. Forest Service, 2012).
- **Decimal degrees (DD):** A coordinate format expressing latitude and longitude as a single decimal number (e.g., 34.0975 N, 117.7125 W); the EA Program default format; preferred for data entry in spreadsheets and GIS.
- **GNSS (Global Navigation Satellite System):** The general term for satellite-based positioning systems; includes the US GPS, Russian GLONASS, European Galileo, and Chinese BeiDou constellations; modern receivers use multiple constellations simultaneously to improve accuracy.
- **GPS (Global Positioning System):** The US-operated GNSS constellation of 31+ satellites; provides horizontal accuracy of 3–5 m under open sky with a modern consumer receiver (U.S. Forest Service, 2012).
- **GPX file:** A standard XML-based file format for exchanging GPS waypoints, tracks, and routes between devices and software; the preferred export format for EA Program waypoints.
- **HDOP (Horizontal Dilution of Precision):** A dimensionless number indicating GPS positional uncertainty due to satellite geometry; values below 2 indicate excellent geometry; values above 5 indicate poor geometry and reduced accuracy (U.S. Forest Service, 2012).
- **Offline map:** A map tile set downloaded to a smartphone or device before the field visit, enabling navigation without cellular data; required for EA Program field sites with unreliable coverage.
- **UTM (Universal Transverse Mercator):** A projected coordinate system that divides the world into 60 zones and expresses positions in meters east (easting) and north (northing) of a zone origin; useful for measuring distances directly but requires specifying the UTM zone.

- **Waypoint:** A named, saved geographic location stored in a GPS receiver or smartphone app; defined by coordinates (latitude, longitude, and optionally elevation), a name, and often a description or icon.
- **WGS 84 (World Geodetic System 1984):** The global coordinate datum used by GPS satellites, Google Maps, and most modern consumer GPS devices; the EA Program default (U.S. Forest Service, 2012).

5. Safety and Hazards

⚠ Hazards

- **Physical:** Navigating to remote waypoints may involve uneven terrain, dense vegetation, or stream crossings. Do not fixate on the GPS screen while walking.
- **Environmental:** Reliance on GPS in areas with poor satellite coverage (dense canopy, steep canyons) can lead to navigation errors. Always carry a paper map or site sketch as a backup.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. If lost or disoriented, stop moving, note your last known coordinates, and contact the field lead by phone or walkie-talkie.

6. Equipment and Materials

Item	Notes
Handheld GPS receiver (Garmin or equivalent)	Check battery level; carry spare batteries (Garmin International, 2019)
Smartphone with GPS app	Avenza Maps, Gaia GPS, or similar app with offline map capability (Avenza Systems, 2024)
Backup paper map or site sketch	Annotated with known landmarks and access roads
Field notebook	For recording waypoint IDs and descriptions
USB cable or Bluetooth adapter	For downloading waypoints to a computer
Spare batteries (AA or rechargeable)	Matched to the GPS unit model

7. Procedure

7.1. Pre-Field Setup

1. Confirm the coordinate system and datum for the project. The EA Program default is **WGS 84, latitude/longitude in decimal degrees** (U.S. Forest Service, 2012). If a project requires UTM or NAD 83, configure the device accordingly and note the setting in the field notebook.
2. On a handheld GPS, verify the datum setting: navigate to Setup > Position Format and confirm WGS 84 (Garmin International, 2019).
3. On a smartphone app, enable offline maps for the study area. Do not rely on cellular data for map tiles in the field (Avenza Systems, 2024).
4. Clear or archive old waypoints from the device so the day's data are easy to identify.
5. Pre-load target waypoints if revisiting known sites. Use the naming convention described in §6.3.

7.2. Recording Waypoints in the Field

1. At the sampling location, hold the GPS receiver at chest height in an open area away from dense canopy, cliff faces, and vehicles.
2. Wait until the GPS reports an accuracy of 5 m or better (HDOP < 2 preferred). If accuracy remains above 5 m after two minutes, record the waypoint and note the reported accuracy in the field notebook (U.S. Forest Service, 2012).
3. Save the waypoint using the naming convention in §6.3.
4. In the field notebook, record:
   - Waypoint name
   - Date and time
   - Coordinates (as displayed on the device)
   - Reported accuracy (HDOP or meters, as available)
   - A brief description (e.g., "upstream riffle, transect 3 start")
5. If using a smartphone, take a screenshot of the GPS display as a backup record.

7.3. Waypoint Naming Convention

Use the following format: `SITE_TYPE_NN`

- `SITE` = three- or four-letter site abbreviation (e.g., BFS, THW, MRW)
- `TYPE` = two-letter code for the point type (see table below)
- `NN` = sequential two-digit number

Code	Point Type	Example
WQ	Water quality sampling	BFS_WQ_01
SO	Soil sampling	THW_SO_03

Code	Point Type	Example
TR	Transect endpoint	MRW_TR_01
WL	Monitoring well	BFS_WL_02
RF	Reference / benchmark	THW_RF_01
GN	General / other	MRW_GN_05

If a project requires a different convention, document it in the project README and field notebook.

7.4. Navigating to Existing Waypoints

1. Load the target waypoint on the GPS receiver or smartphone app.
2. Use the "Navigate" or "Go To" function. Follow the bearing indicator, checking your surroundings periodically.
3. When the GPS indicates you are within the reported accuracy of the waypoint, look for physical markers (flagging, rebar, painted rocks) to confirm the exact location.
4. If the physical marker cannot be found, record a new waypoint at the location you judge to be correct and note the discrepancy in the field notebook.

7.5. Post-Field Data Management

1. Download waypoints from the GPS receiver to the project computer within 48 hours of returning from the field.
2. Export waypoints as a `.gpx` or `.csv` file and save to the project GitHub repository under `data/gps/`.
3. Use the file naming convention: `YYYY-MM-DD_SiteID_waypoints.gpx`
4. Verify that waypoint names match the field notebook entries. Correct any discrepancies before committing the file.
5. Back up the raw GPS file to the lab shared drive.

8. Quality Control

- Compare GPS coordinates against known benchmarks or previously recorded waypoints when revisiting sites. Discrepancies greater than 10 m should be investigated and noted (U.S. Forest Service, 2012).
- At least once per semester, verify the GPS receiver against a known benchmark (e.g., a USGS survey marker or campus reference point).
- Record the GPS model, serial number, and firmware version in the field notebook at the start of each project.

9. References

- Garmin International (2019)
- Avenza Systems (2024)

- U.S. Forest Service (2012)

Avenza Systems. (2024). *Avenza maps user guide*. https://support.avenzamaps.com

Garmin International. (2019). *GPSMAP 66 series owner's manual*. https://www8.garmin.com/manuals/webhelp/gpsmap66st/EN-US/GUID-BF2E4F90-EB9F-4A7D-A94E-52614D3B9D05-homepage.htm

U.S. Forest Service. (2012). *Field data collection with GPS: A best practices guide* (Gen. Tech. Rep. RMRS-GTR-289). USDA Forest Service, Rocky Mountain Research Station. https://www.fs.usda.gov/research/treesearch/43085

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F003	Field Notebook Protocols	Waypoint data recorded in notebook
F005	Random Sampling Design	GPS used to navigate to random points

11. Training Record

Before using GPS equipment independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper waypoint recording (datum check, accuracy verification, naming convention) during a supervised field visit
3. Successfully download and export waypoints to the project repository
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F007

Field Decontamination Protocols

Standard Operating Procedure

SOP Number	SOP-F007
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standardized procedures for decontaminating field equipment between sampling sites and between field outings. Proper decontamination serves two purposes: (1) preventing cross-contamination of samples that would compromise analytical results, and (2) preventing the transfer of invasive species, pathogens, and nuisance organisms between water bodies and field sites (California Department of Fish and Wildlife, 2016; Hosea & Finlayson, 2005). These procedures apply to all equipment that contacts environmental media (water, soil, sediment, biological tissue) or that is transported between sites.

2. Scope

- **Applies to:** All reusable field sampling equipment, wading gear (boots, waders, nets), sampling instruments (sondes, meters), containers, and hand tools used at EA Program field sites
- **Does not apply to:** Disposable, single-use items (e.g., individually packaged sterile Colilert bottles, disposable filter cartridges); laboratory glassware cleaning (see SOP-L101); drone or camera equipment (wipe with dry cloth only unless visibly contaminated)
- **Location:** Performed at field sites (between-site decontamination) and in the lab (post-field cleaning in SGM 131–134)

3. Responsibilities

Role	Responsibility
PI / Field Lead	Ensure decontamination supplies are on the equipment checklist; verify compliance
Trained Personnel	Decontaminate equipment per this SOP before and after each site visit
New Students	Complete training before independent fieldwork

4. Definitions

- **2% bleach solution:** A dilute sodium hypochlorite solution prepared by adding 200 mL of household bleach (5–8% NaOCl) to 10 L of tap water; the standard decontamination solution for wading gear, nets, and hand tools in the EA Program; must be prepared fresh each field day as bleach degrades within hours (California Department of Fish and Wildlife, 2016).
- **Aquatic invasive species (AIS):** Non-native organisms introduced to a water body where they cause ecological or economic harm; common AIS of concern in Southern California include New Zealand mudsnail, quagga mussel, didymo, and chytrid fungus; transferred between water bodies on wet gear (California Department of Fish and Wildlife, 2016).
- **Chytrid fungus (*Batrachochytrium dendrobatidis*, Bd):** A waterborne pathogenic fungus lethal to many amphibian species; transmitted via contaminated water, soil, and equipment; killed by 2% bleach or thermal decontamination at 60 °C (Hosea & Finlayson, 2005).
- **Cross-contamination:** Transfer of analytes, biological material, or organisms from one sample or site to another via inadequately cleaned equipment; can invalidate analytical results and introduce invasive species.
- **Didymo (*Didymosphenia geminata*):** A freshwater diatom that forms dense mats on stream substrates; tolerates damp conditions for many hours; felt-soled wading boots are a primary vector; killed by 2% bleach or drying for at least 48 hours (California Department of Fish and Wildlife, 2016).
- **Equipment blank:** A sample of deionized water rinsed through decontaminated sampling equipment and collected as a QC sample; analyzed alongside field samples to verify that decontamination removes analytes below detection limits.
- **New Zealand mudsnail (*Potamopyrgus antipodarum*):** An invasive snail less than 5 mm long that survives transit on damp wading gear for up to 28 hours; a significant concern

in Southern California streams; killed by 2% bleach immersion for at least 10 minutes or thermal decontamination at 60 °C (Hosea & Finlayson, 2005).

- **Thermal decontamination:** Application of water at 60 °C to kill aquatic invasive species and pathogens; preferred over bleach for equipment sensitive to sodium hypochlorite (rubber gaskets, sensor membranes) (California Department of Fish and Wildlife, 2016; Hosea & Finlayson, 2005).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Dilute bleach (sodium hypochlorite) is a skin and eye irritant and can damage clothing. Handle with gloves and avoid splashing.
- **Physical:** Hot water (60 °C or above) used for thermal decontamination poses a scald risk. Use insulated containers and pour carefully.
- **Biological:** Equipment that has contacted contaminated water or soil may carry pathogens. Wear gloves during decontamination.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses (when using bleach solution)
☐ Lab coat or field clothing you are willing to bleach-stain

5.2. Emergency Procedures

For bleach splashes to skin or eyes, flush with clean water for at least 15 minutes. Refer to SOP-F001 Field Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Decontamination Supplies

Item	Notes
Household bleach (sodium hypochlorite, 5–8%)	For preparing 2% decon solution
Spray bottle (labeled “Decon – Bleach 2%”)	For applying solution in the field
Stiff-bristle scrub brush	For removing visible debris
Bucket (5-gallon, dedicated)	For soaking equipment
Deionized or distilled water	For final rinse of sampling equipment
Tap water (carboy or jug)	For initial rinse in the field
Paper towels or clean rags	For drying

Item	Notes
Garbage bags	For isolating contaminated gear during transport

6.2. Solution Preparation

Prepare a **2% bleach solution** by adding 200 mL of household bleach (5–8% sodium hypochlorite) to 10 L of tap water. Prepare fresh solution each field day; bleach degrades within hours (California Department of Fish and Wildlife, 2016).

For **thermal decontamination** (alternative to bleach), heat water to at least 60 °C. Preferred for equipment that may be damaged by bleach (e.g., rubber gaskets, certain sensor membranes) (Hosea & Finlayson, 2005).

7. Procedure

7.1. Between-Site Decontamination (Field)

Perform the following steps before moving to the next sampling site.

Wading gear (boots, waders, chest packs):

1. Remove from the water body and inspect for visible organisms (snails, plant fragments, mud).
2. Use the scrub brush and tap water to remove all visible debris, paying special attention to boot soles, lace eyelets, and wader seams.
3. Spray all surfaces with 2% bleach solution or immerse boot soles in the bleach bucket for at least one minute (California Department of Fish and Wildlife, 2016).
4. Rinse with tap water to remove bleach residue before entering the next water body.

Nets, sieves, and kick screens:

1. Remove all visible organisms and debris by hand and with the scrub brush.
2. Soak in 2% bleach solution for at least one minute, or pour hot water (60 °C) over all mesh surfaces (Hosea & Finlayson, 2005).
3. Rinse with tap water.

Sampling instruments (sondes, meters, tubing):

1. Rinse the sensor housing and cable with tap water.
2. Wipe external surfaces with a cloth dampened with DI water.
3. Do not immerse sensitive electronics in bleach. If the manufacturer recommends a specific decontamination procedure, follow it instead.

Hand tools (trowels, augers, calipers, measuring tapes):

1. Scrub off visible soil or sediment with tap water and the brush.
2. Spray with 2% bleach solution or rinse with DI water.
3. Dry with paper towels before packing.

7.2. Post-Field Decontamination (Laboratory)

Upon return to the lab, before storing equipment:

1. Unpack all field equipment in the designated cleaning area (SGM 134 sink or outdoor wash area).
2. Repeat the cleaning steps from §6.1, using DI water for the final rinse of any equipment that contacts samples.
3. Allow all equipment to air-dry completely before storing. Incompletely dried equipment promotes mold and bacterial growth.
4. Inspect nets and mesh for tears; report damage to the lab manager.
5. Return all equipment to designated storage locations per SOP-L007.

7.3. Invasive Species of Concern

The following organisms are of particular concern in Southern California watersheds (California Department of Fish and Wildlife, 2016; Hosea & Finlayson, 2005):

Organism	Concern
New Zealand mudsnail (*Potamopyrgus antipodarum*)	< 5 mm; survives on wet gear up to 28 hours
Quagga/zebra mussel (*Dreissena* spp.)	Veligers invisible to the naked eye
Didymo (*Didymosphenia geminata*)	Algal cells survive on damp felt soles
Chytrid fungus (*Batrachochytrium dendrobatidis*)	Lethal to amphibians; transmitted via water and mud
Invasive aquatic plants (e.g., *Arundo donax* fragments)	Fragments root easily at new sites

> **!** Important
>
> If sampling in a watershed known to harbor New Zealand mudsnails or quagga mussels, use thermal decontamination (60 °C) rather than bleach, as hot water is more reliably lethal to these organisms (Hosea & Finlayson, 2005). Allow gear to dry completely (at least 48 hours) before using it at another site.

8. Quality Control

- The field lead verifies that decontamination supplies are packed before departure (include on the SOP-F002 Field Equipment Checklist).
- At the start of each semester, inspect decontamination supplies (bleach expiration, brush condition, spray bottle function) and replace as needed.
- For chemical sampling, submit one equipment blank per sampling event: rinse decontaminated equipment with DI water and collect the rinse as a sample. Analyze alongside field samples to verify that decontamination removes analytes below detection limits.

9. References

- California Department of Fish and Wildlife (2016)
- Hosea & Finlayson (2005)

California Department of Fish and Wildlife. (2016). *Aquatic invasive species decontamination protocol*. California Department of Fish; Wildlife. https://wildlife.ca.gov/Conservation/Invasives/Aquatic

Hosea, R. C., & Finlayson, B. (2005). *Controlling the spread of New Zealand mudsnails on wading gear* (Administrative Report 2005-02). California Department of Fish; Game, Office of Spill Prevention; Response.

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F002	Field Equipment Checklist	Decon supplies on checklist
F304	Stream Physical Habitat	References invasive species prevention
F610	Macroinvertebrate Sampling	Gear decon between streams
L101	Glassware Cleaning	Lab-side cleaning protocols

11. Training Record

Before performing field decontamination independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper preparation of 2% bleach solution
3. Demonstrate between-site decontamination of wading gear and sampling equipment during a supervised field visit
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F008

Sample Labeling, Chain of Custody, and Transport

Standard Operating Procedure

SOP Number	SOP-F008
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Author(s)	Marc Los Huertos
Date	2026-06-26
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1. Purpose

This SOP establishes a single, program-wide standard for labeling field samples, completing chain of custody (COC) documentation, and transporting samples from the field to the laboratory (APHA et al., 2012; U.S. Environmental Protection Agency, 2002). Consistent labeling prevents sample mix-ups, and a complete chain of custody ensures that data are legally defensible and scientifically traceable. These procedures apply to all sample types (water, soil, sediment, biological tissue, air filters) collected by the EA Program.

2. Scope

- **Applies to:** All environmental samples collected during EA Program field activities, whether analyzed in-house or submitted to an external laboratory
- **Does not apply to:** Internal laboratory standards and reagents (labeled per SOP-L005); data files and digital records (see SOP-L801)
- **Location:** All EA Program field sites, vehicles, and laboratory receiving areas (SGM 133, 134)

3. Responsibilities

Role	Responsibility
PI / Project Lead	Approve sample ID scheme for each project; designate receiving personnel
Field Lead	Verify labels and COC forms before leaving the field
Trained Personnel	Label samples and complete COC per this SOP
Receiving Personnel	Accept custody, verify sample condition, log receipt
New Students	Complete training before independent sampling

4. Definitions

- **Chain of custody (COC):** A documented, unbroken record of the collection, transfer, and possession of a sample from the moment of collection through final analysis or disposal; used to demonstrate sample integrity for legal or regulatory purposes (U.S. Environmental Protection Agency, 2002).
- **Holding time:** The maximum elapsed time between sample collection and analysis within which the sample remains chemically stable and analytically valid; analyte- and preservative-specific (e.g., 6 h for E. coli, 28 d for nitrate in H_2SO_4) (APHA et al., 2012).
- **Preservative:** A chemical added to a sample container (e.g., H_2SO_4, HNO_3, $Na_2S_2O_3$) to prevent biological or chemical degradation of target analytes during transport and storage (APHA et al., 2012).
- **QA/QC sample:** A blank, duplicate, spike, or other quality-control sample collected alongside field samples to assess contamination, precision, and accuracy; documented on the COC form.
- **Sample ID:** A unique alphanumeric code assigned to a sample at the moment of collection; the primary key linking field records, labels, COC forms, and laboratory data.
- **Zero headspace:** A sample container filled completely with no air gap; required for volatile organic compound (VOC) samples to prevent loss of analytes to the headspace.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Some sample containers include chemical preservatives (sulfuric acid, nitric acid, sodium thiosulfate). Handle pre-preserved bottles with care. Refer to SOP-L003.
- **Physical:** Coolers loaded with ice and sample bottles can be heavy. Use proper lifting technique.
- **Biological:** Soil and water samples may contain pathogens. Wear nitrile gloves when handling samples.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses (when handling preserved bottles)

5.2. Required Training

- SOP-F001 Field Safety
- SOP-F003 Field Notebook Protocols

6. Equipment and Materials

Item	Storage	Notes
Pre-labeled sample bottles	SGM 133	Correct preservative per method
Waterproof field labels	SGM 133	Rite in the Rain or equivalent
Permanent markers (fine-tip)	SGM 133	Black; waterproof
Chain of custody forms	SGM 133	One form per cooler
Cooler with ice packs	SGM 132	Pre-chilled to 4 °C
Bubble wrap / packing foam	SGM 133	For fragile glass bottles
Zip-lock bags	SGM 133	Secondary containment for preserved bottles
Sample seals / custody tape	SGM 133	For regulatory submissions

7. Procedure

7.1. Sample ID Scheme

The PI or project lead establishes the sample ID scheme before the first field outing. A standard EA Program sample ID has the structure:

```
[ProjectCode]-[SiteCode]-[YYYYMMDD]-[SampleType]-[Replicate]
```

Example: `SGMC-US1-20260415-GW-A`

Record the scheme in the project metadata file and in the field notebook before the first sampling event.

7.2. Labeling Sample Containers

1. Write the sample ID on the pre-printed label or directly on the container using a waterproof marker.
2. Include the following minimum fields on every label:
   - Sample ID
   - Collection date and time (24 h format)
   - Collector initials
   - Matrix (water / soil / sediment / tissue / air filter)
   - Preservative (if present)
3. For pre-preserved bottles, verify the preservative type is printed or written on the container before field use.
4. For biological samples requiring cold preservation, add an ice temperature indicator sticker.

7.3. Completing the COC Form

1. Start a new COC form for each cooler.
2. Fill in all header fields: project name, project number, field lead, date, field site.
3. Enter each sample in the log: sample ID, matrix, collection date and time, preservative, number of containers, and requested analysis.
4. Include QA/QC samples (field blanks, field duplicates, trip blanks) on the same COC form as the associated samples.
5. Sign and date the form before departing the field.

7.4. Sample Transport

1. Place samples in the cooler with sufficient ice to maintain 4 °C.
2. Separate chemically preserved bottles from unpreserved samples using a zip-lock bag as secondary containment.
3. Verify the cooler temperature on departure and on arrival using a thermometer; record both readings on the COC form.
4. Transport samples promptly; do not leave samples in a hot vehicle.

7.5. Receiving Samples in the Laboratory

1. The receiving person checks the sample count against the COC form.
2. Verify that the number of containers matches the COC listing; note any discrepancies.
3. Check sample temperature with a thermometer placed among the bottles and record on the COC form.
4. Transfer samples to appropriate storage:

- Water samples: refrigerator at 4 °C (SOP-L106)
- Soil samples: refrigerator at 4 °C (short-term) or freezer (archiving)
- Biological samples: freezer at -20 °C or -80 °C as required

5. Both the field lead and the receiving person sign and date the COC form to document the transfer of custody.
6. File the signed COC form with the project records.

8. Quality Control

- The field lead reviews all labels and the COC form before leaving the field site.
- Receiving personnel verify sample count, condition, and temperature upon arrival.
- Any broken, leaking, or incorrectly labeled samples are flagged on the COC form and reported to the PI within 24 hours.
- COC forms are retained for the duration of the project plus a minimum of 5 years.

9. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

U.S. Environmental Protection Agency. (2002). *Guidance for quality assurance project plans (EPA QA/G-5)* (EPA/240/R-02/009). U.S. Environmental Protection Agency. https://www.epa.gov/sites/default/files/2015-06/documents/g5-final.pdf

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F002	Field Equipment Checklist	Labeling supplies on checklist
F003	Field Notebook Protocols	COC recorded in notebook
F300	Surface Water Sampling	Sample-specific preservation
F303	Groundwater Sampling	Well-specific labeling
F400	Soil Sampling	Soil-specific labeling
L802	Quality Control and QA	QC sample requirements

11. Training Record

Before labeling and handling samples independently, personnel must:

1. Read this SOP and SOP-F003 Field Notebook Protocols in full
2. Demonstrate correct sample labeling using the project ID scheme
3. Complete a COC form correctly during a supervised field outing
4. Be signed off by the field lead or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F100

EA Program Vehicle Use

Standard Operating Procedure

SOP Number	SOP-F100
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the procedures for reserving, inspecting, operating, and returning EA Program vehicles. Following these procedures ensures that vehicles remain safe, reliable, and available for all program research and teaching activities.

2. Scope

- **Applies to:** All use of EA Program vehicles for field research, class field trips, and program-related transportation
- **Does not apply to:** Personal vehicles used for commuting to field sites; rental vehicles; Facilities and Maintenance fleet vehicles
- **Location:** EA Program vehicle parking (campus garage)

3. Responsibilities

Role	Responsibility
EA Coordinator	Manage reservation calendar, maintain insurance records
PI / Instructor	Approve vehicle requests, ensure drivers are authorized
Authorized Driver	Follow this SOP, complete pre-trip and post-trip checks
New Students	Submit insurance form and complete training before driving

4. Definitions

- **Authorized driver:** A person who holds a valid driver's license and has a completed, approved Pomona College driver insurance form on file with the EA Coordinator; the only persons permitted to operate EA Program vehicles.
- **Drowsy driving:** Operating a motor vehicle while significantly impaired by fatigue; a leading cause of serious crashes on long drives to remote field sites; if any crew member feels impaired by fatigue, pull over safely and rest before continuing.
- **Fuel card:** A fleet credit card stored in the center console of each EA vehicle; used to purchase regular unleaded gasoline (87 octane) at participating stations; if declined, pay out of pocket and submit the receipt to the EA Coordinator for reimbursement.
- **Pre-trip inspection:** A walk-around check of the vehicle before departure to identify safety issues (warning lights, tire damage, fluid leaks) that would make the vehicle unsafe to drive; required before every trip.
- **Post-trip return standard:** The condition in which the vehicle must be returned after every use – clean interior, at least 3/4 tank of fuel, fuel card in the center console, no warning lights, parked in the designated garage spot.
- **Pomona College driver authorization:** The college insurance form required before driving any Pomona College-owned or -leased vehicle; submitted to the EA Coordinator with a copy of a valid driver's license; must be approved before the first trip.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Motor vehicle operation on public roads and unpaved field-site access roads; loading and unloading heavy field equipment.
- **Environmental:** Driving in adverse weather, reduced visibility, or on unfamiliar roads near field sites.
- **Fatigue:** Long drives to remote field sites increase the risk of drowsy driving.

5.1. Requirements

- Valid driver's license (must be carried while driving)
- Completed and approved Pomona College driver insurance form on file with the EA Coordinator
- Familiarity with this SOP and SOP-F001 Field Safety

5.2. Emergency Procedures

In the event of an accident or breakdown:

1. Ensure all occupants are safe; move to a safe location if possible
2. Call 911 if anyone is injured
3. Contact Pomona College Campus Safety: (909) 621-8170
4. Notify the EA Coordinator and PI
5. Document the incident (photos, written description, other party information if applicable)
6. Do not admit fault at the scene; exchange insurance and contact information only
7. Complete a college incident report within 24 hours

Refer to SOP-F001 Field Safety for general emergency response procedures.

6. EA Program Fleet

Vehicle	Make / Model	Seats	Notes
EA-1	Ford Explorer	7	Garage card in center console
EA-2	Ford Explorer	7	Garage card in center console

Both vehicles are parked in the campus garage when not in use. Keys and fuel cards are stored in each vehicle's center console.

7. Procedure

7.1. Before You Drive: Insurance Authorization

Before driving any EA vehicle for the first time, you must:

1. Obtain the Pomona College driver authorization and insurance form from the EA Coordinator.
2. Complete and submit the form with a copy of your valid driver's license.
3. Wait for written approval before operating any EA vehicle.

> **!** No Exceptions
>
> You may not drive an EA vehicle until your insurance form has been approved and is on file with the EA Coordinator. This is a college requirement, not a suggestion. Driving without approval puts you and the program at serious liability risk.

7.2. Reserving a Vehicle

1. Contact the EA Coordinator to check availability.
2. Provide: date(s) and times needed, destination, number of passengers, and the name of the PI or instructor authorizing the trip.
3. Confirm the reservation before your trip; cancellations should be communicated as early as possible so others can use the vehicle.

7.3. Pre-Trip Inspection

Before departing, walk around the vehicle and verify:

- ☐ Fuel gauge reads at least 3/4 full (refuel before departing if below 3/4; see §6.5)
- ☐ Fuel card is present in the center console
- ☐ Tires appear properly inflated with no visible damage
- ☐ All lights (headlights, brake lights, turn signals) are functional
- ☐ Mirrors are adjusted for the driver
- ☐ Windshield is clean and wipers are functional
- ☐ First aid kit is present and stocked (see SOP-F001)
- ☐ No warning lights are illuminated on the dashboard
- ☐ Any pre-existing damage is noted and reported to the EA Coordinator before departing

If any safety issue is found (warning lights, tire damage, brake problems), do not drive the vehicle. Contact the EA Coordinator immediately.

7.4. During the Trip

- Obey all state and local traffic laws at all times.
- All occupants must wear seatbelts (California law).
- The driver must not use a handheld cell phone while driving (California Vehicle Code §23123).
- Do not exceed the vehicle's passenger capacity.
- Secure all field equipment so it cannot shift during transport.
- On unpaved roads, reduce speed and increase following distance.
- If conditions become unsafe (severe weather, road hazards, driver fatigue), pull over and reassess before continuing.

7.5. Refueling

1. Use the fuel card located in the center console.
2. Select regular unleaded gasoline (87 octane).
3. If the fuel card is declined or missing, pay out of pocket and submit the receipt to the EA Coordinator for reimbursement.
4. Replace the fuel card in the center console after refueling.

7.6. Returning the Vehicle

After every trip, complete the following before leaving the vehicle:

1. **Refuel:** Return with at least a 3/4 tank of gas. If you used the vehicle, you are responsible for refueling.
2. **Clean:** Remove all personal items, trash, and field equipment. Brush or vacuum out dirt and mud. Wipe down surfaces if visibly soiled.
3. **Fuel card:** Confirm the fuel card is back in the center console.
4. **Park:** Return the vehicle to its designated spot in the campus garage.
5. **Report problems:** If anything is wrong with the vehicle (warning lights, unusual noises, damage, low tire pressure), notify the EA Coordinator immediately. Do not assume someone else will report it.

> **Return It Ready**
>
> The next person to use the vehicle should find it clean, fueled to at least 3/4 tank, with the fuel card in the center console, parked in the garage, and free of warning lights.

8. Prohibited Uses

- Personal errands unrelated to EA Program activities
- Transporting hazardous materials not approved by the PI (standard field samples and preserved water samples are acceptable)
- Allowing unauthorized or uninsured individuals to drive
- Off-road driving beyond established field-site access roads
- Towing trailers or other vehicles without prior approval from the EA Coordinator

9. References

For driver authorization requirements, consult the EA Coordinator or the Pomona College Office of Risk Management directly. There is no publicly accessible bib entry for this internal policy; confirm current requirements with the EA Coordinator before each field season.

- SOP-F001 Field Safety

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required reading; emergency procedures
F002	Field Equipment Checklist	Vehicle pre-trip checklist

11. Training Record

Before driving EA Program vehicles independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Submit a completed driver insurance authorization form to the EA Coordinator and receive written approval
3. Familiarize themselves with the vehicle (controls, mirrors, fuel card location) before the first trip
4. Be signed off by the PI or EA Coordinator

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F101

Electrical Power in the Field

Standard Operating Procedure

SOP Number	SOP-F101
Revision	0.2
Author(s)	Kay Sterner, Katheryn Kornegay
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

Electrical power in the field can come from infrastructure sources (such as outdoor or indoor outlets), portable generators, or solar power. This SOP outlines the risks associated with using electricity in the field and develops strategies to mitigate those risks.

2. Scope

- **Applies to:** Use of portable generators (Honda EU2000i and Ryobi RYi2200A) and extension cords for powering field equipment
- **Does not apply to:** Permanent electrical installations or solar panel systems (to be covered in a future SOP)
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, perform generator maintenance
New Students	Read and understand this SOP before operating generators; complete SOP-F001 Field Safety training

4. Definitions

- **Carbon monoxide (CO):** A colorless, odorless, tasteless toxic gas produced by incomplete combustion of gasoline and other fuels; displaces oxygen in the bloodstream and is rapidly fatal at elevated concentrations; never operate a generator in any enclosed or semi-enclosed space.
- **Carburetor:** The engine component that mixes air and fuel in the correct ratio for combustion; must be drained before storage of 30 days or more to prevent gum and varnish deposits from clogging fuel passages.
- **Choke:** A valve in the carburetor air intake that restricts airflow to enrich the fuel mixture during cold starts; must be moved to the OPEN or RUN position after the engine warms up to prevent over-fueling.
- **Eco-throttle:** An automatic throttle control (Honda EU2000i) that reduces engine speed when load demand is low, improving fuel economy and reducing noise; must be switched OFF during startup.
- **Ethanol-free gasoline:** Fuel containing no ethanol additive; recommended for small engines because ethanol absorbs water, degrades rubber fuel lines, and causes carburetor deposits during storage.
- **Fuel stabilizer:** A chemical additive mixed into gasoline before storage to prevent oxidation and gum formation; required when the generator will not be used for 30 days or more.
- **Ground fault:** An unintended electrical path between a live conductor and the ground or equipment casing; a leading cause of electrocution; mitigated by using grounded extension cords and GFCI protection.
- **Grounded extension cord:** An extension cord with a three-prong plug that includes a ground conductor; required for all generator connections to provide a safe fault-current return path.
- **Inverter generator:** A generator that produces AC power through an electronic inverter circuit, yielding cleaner, more stable voltage and frequency than a conventional generator;

both the Honda EU2000i and Ryobi RYi2200A are inverter generators, making them suitable for powering sensitive electronic field instruments.

- **Spark arrestor:** A fine wire-mesh screen fitted to the exhaust outlet that prevents hot carbon particles from escaping and igniting surrounding combustible material; required by law in many fire-prone areas; must be inspected and cleaned periodically.
- **Spark plug gap:** The distance between the center and ground electrodes of a spark plug; must be within the manufacturer-specified range (0.024–0.028 in for both units) for reliable ignition.

5. Safety and Hazards

⚠ Hazards

- **Carbon monoxide:** Engines emit carbon monoxide. Never use a generator inside an enclosed area (home, garage, crawl space). Fatal fumes can build up even with a fan or open doors and windows. Only operate generators outdoors, away from open windows, vents, or doors.
- **Fire and explosion:** Gasoline and its vapors are extremely flammable. Allow the engine to cool at least 2 minutes before refueling. Always use fresh gasoline. Never operate the generator near combustible materials.
- **Electrical shock:** Generators produce powerful voltage. Never operate under wet conditions and take precautions to protect the generator from rain and snow.
- **Electrocution / home wiring:** Never plug a generator directly into a home outlet. A qualified electrician must install a Power Transfer Switch for any home connection.
- **Heat from cables:** Ensure cables are rated for the current being carried. Never leave cables coiled, as coiled cords can generate excessive heat.

5.1. Required PPE

☐ Closed-toed shoes
☐ Work gloves (when handling hot engine components or refueling)
☐ Hearing protection (if operating near the generator for extended periods)

5.2. Emergency Procedures

Use a battery-powered carbon monoxide detector in the area where you are running a generator. If the alarm sounds, move to fresh air immediately and shut down the generator. Refer to SOP-F001 Field Safety for general field emergency procedures.

6. Equipment and Materials

Item	Notes
Honda EU2000i generator	Portable inverter generator
Ryobi RYi2200A generator	Portable inverter generator
High-capacity extension cords	Must be grounded type, rated for the application
Spare gas container	Filled with ethanol-free gasoline
Synthetic 10W-30 engine oil	Recommended oil type for both generators
Funnel	For adding oil

7. Procedure

7.1. Pre-Start Checks

1. **Check oil level.** If low, use a funnel to add synthetic 10W-30 oil. This does not need to be done every time; check every few weeks. If the generator shuts off after running for about 30 minutes, low oil is the likely cause.

2. **Check gas level.** If low, add ethanol-free gas from the spare container. On the Ryobi, fill to about an inch below the top of the tank. Both generators can be refilled while running. The Ryobi should be refilled after about 4 hours of operation; the Honda after about 6 hours.

7.2. Starting the Honda EU2000i

1. Turn the fuel tank cap vent (black cap on top of the generator) to the ON position. If this is not done, the generator will shut off after a few minutes.
2. Make sure the eco-throttle switch (on the side panel, below the fuel cap vent) is OFF.
3. For a cold start, move the choke (upper left front, near the engine switch) to the CLOSED position (slide the switch to the right).
4. Turn the engine switch to ON.
5. Pull the starter grip quickly several times until the engine starts.
6. Allow the engine a few minutes to warm up, then slowly move the choke to the OPEN position.
7. **To shut down:** Turn the engine switch to OFF. After the engine has cooled, turn the fuel tank cap vent to OFF.

7.3. Starting the Ryobi RYi2200A

1. Make sure the auto idle switch is OFF.
2. Move the choke to approximately halfway across. The marked cold-start position is slightly less than halfway, but the choke needs to go a bit further; you should feel a slight resistance at the correct position.
3. Prime the system by slowly pulling the starter grip several times.
4. Pull the grip quickly until the engine runs.
5. Allow the engine to run for 15–30 seconds, then move the choke all the way across to the RUN position.
6. **To shut down:** Switch the choke back to the OFF position.

7.4. Storage

If you do not plan to use the generator within 30 days, add fuel stabilizer to the gas and drain the carburetor.

7.5. Extension Cord Safety

- Use only grounded extension cords rated for the intended application.
- Always uncoil cords completely and lay them flat in open locations; coiled cords can generate excessive heat.

8. Maintenance

8.1. General (Both Units)

Keep generators in a clean, dry environment, not exposed to dust, dirt, moisture, or corrosive vapors. To clean, use a soft-bristle brush and/or vacuum cleaner to loosen and remove dirt and debris. Clean air vents with air pressure no more than 25 psi. Wipe exterior surfaces with a damp cloth. Perform maintenance more frequently when the generator is used in dusty areas. If you notice a power loss, the exhaust port and muffler may be blocked with carbon deposits; allow only qualified service technicians to clean these.

8.2. Ryobi RYi2200A Maintenance Schedule

Item	Frequency	Notes
Air filter	Check before each use; clean every 3 months or 50 hours; replace every year or 300 hours	Wash with soapy water, rinse, squeeze dry
Engine lubricant	Check before each use; change after first 20 hours, then every 6 months or 100 hours	Drain while warm for faster flow
Spark plug	Check every 6 months or 100 hours; replace every year or 300 hours	Gap: 0.024–0.028 in (0.60–0.70 mm)

Item	Frequency	Notes
Spark arrestor	Clean periodically	Wire brush; inspect screen for damage
Fuel tank / carburetor	Check fuel hose before each use; inspect fuel filter before each use; have tank and filter cleaned by qualified service center every 6 months or 100 hours	Drain both before storage to prevent gum deposits
Valve clearance	Check every year or 300 hours	Service by qualified technician

8.3. Honda EU2000i Maintenance Schedule

Item	Frequency	Notes
Air filter	Check before each use; clean every 3 months or 50 hours	Wash in detergent and warm water or non-flammable solvent; soak in clean engine oil and squeeze excess before re-installing
Engine lubricant	Check before each use; change after first 20 hours, then every 6 months or 100 hours	Fill to upper limit of oil filler neck

Item	Frequency	Notes
Spark plug	Check every 6 months or 100 hours; replace every year or 200 hours	Gap: 0.024–0.028 in (0.60–0.70 mm); new plug: tighten 1/2 turn after seating; used plug: tighten 1/8–1/4 turn
Spark arrestor	Clean every 6 months or 100 hours	Wire brush; inspect screen for damage
Fuel tank / combustion chamber	Clean fuel tank and filter every 6 months or 100 hours; clean combustion chamber every 300 hours; check fuel line every 2 years	Service by authorized Honda dealer
Valve clearance	Check every year or 200 hours	Service by qualified technician

9. Quality Control

TBD – establish routine checks to verify generator output voltage and frequency are within acceptable ranges for field instruments.

10. References

11. Training Record

Before operating generators independently, personnel must:

1. Read this SOP in full

2. Complete SOP-F001 Field Safety training
3. Review the manufacturer's operating instructions for the generator being used
4. Observe a trained operator start, run, and shut down the generator
5. Perform the startup and shutdown procedure under supervision
6. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F200

Eureka Manta2 Multiprobe for Water Quality Testing

Standard Operating Procedure

SOP Number	SOP-F200
Revision	0.8
Author(s)	Haley Land-Miller
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the procedures for calibrating, operating, and maintaining the Eureka Manta2 Sub2 Multiprobe for water quality measurements including temperature, pH, depth, conductivity, and dissolved oxygen (mg/L and % saturation) (APHA et al., 2012; Eureka Environmental Engineering, 2018).

2. Scope

- **Applies to:** Water quality measurements in lakes, streams, rivers, and other surface water bodies
- **Does not apply to:** Groundwater monitoring wells (requires different deployment procedures); laboratory bench-top water analysis
- **Location:** All EA Program field water sampling sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Field Lead	Verify calibration before deployment, coordinate logistics
Trained Personnel	Calibrate, operate, and clean the probe per this SOP
New Students	Complete training before independent use

3.1. Required Training

Researchers using this SOP should be trained on SOP-F001 Field Safety.

4. Definitions

- **Amphibian 2:** The ruggedized handheld field computer (portable PC) manufactured by Eureka that controls the Manta2 probe, displays real-time readings, stores data snapshots, and connects to the probe via the underwater cable (Eureka Environmental Engineering, 2018).
- **Barometric pressure:** Atmospheric pressure at the measurement location, expressed in mm Hg; required as an input for dissolved oxygen calibration because oxygen solubility decreases with decreasing pressure (increasing altitude) (APHA et al., 2012).
- **Calibration:** The process of adjusting sensor readings to match known reference standards before field use; required before every field outing to ensure data accuracy (Eureka Environmental Engineering, 2018).

- **Conductivity (specific conductance, spCond):** A measure of a water sample's ability to conduct electrical current, expressed in µS/cm or mS/cm; proportional to the concentration of dissolved ions; temperature-corrected to 25 °C for comparability across sites and seasons (APHA et al., 2012).
- **Dissolved oxygen (DO):** The concentration of oxygen dissolved in water, expressed as mg/L or percent saturation (%sat); measured by the HDO sensor using luminescent optical technology; decreases with increasing temperature, salinity, and altitude (APHA et al., 2012).
- **HDO (Handheld Dissolved Oxygen):** The luminescent optical dissolved oxygen sensor on the Manta2; measures both %sat and mg/L; calibrated in water-saturated air or with an air-saturated water standard (Eureka Environmental Engineering, 2018).
- **PDOP (Position Dilution of Precision):** A dimensionless measure of GPS accuracy based on satellite geometry; lower values indicate better positional accuracy; values below 3 are acceptable for most field applications.
- **Reference electrode:** The electrochemical half-cell that provides a stable reference potential for pH measurement; requires periodic refilling with reference electrolyte to maintain accurate pH readings (Eureka Environmental Engineering, 2018).
- **Sensor Response Factor (SRF):** A calibration diagnostic value indicating how the sensor responded to the reference standard; acceptable ranges are sensor-specific and indicate whether calibration was successful (Eureka Environmental Engineering, 2018).
- **Snapshot:** A single instantaneous record of all sensor readings captured and stored by the Amphibian at a specific date, time, and GPS location.
- **Sonde (probe):** The cylindrical instrument body of the Manta2 that houses all sensors (temperature, pH, conductivity, dissolved oxygen, and reference electrode) and is submerged during measurements (Eureka Environmental Engineering, 2018).

5. Safety and Hazards

⚠ Hazards

- **Biological/Chemical:** Water bodies may be contaminated; avoid ingestion and direct skin contact.
- **Physical:** Slippery surfaces near water; risk of submersion; electrical components near water.
- **Chemical:** pH standards (pH 4, 7, 10) and conductivity standard are mild irritants.

5.1. Required PPE

☐ Nitrile or latex gloves
☐ Water shoes or wading boots (if entering the water)
☐ Safety glasses (during calibration with standards)
☐ Sun protection (hat, sunscreen)

Refer to SOP-F001 Field Safety for general field safety, transportation, and emergency response.

6. Equipment and Materials

Item	Notes
Eureka Manta2 Sub2 Sonde	Inspect sensors before each use
Amphibian 2 field computer	Ensure fully charged (Eureka Environmental Engineering, 2018)
Underwater cable	Inspect for damage before use
pH 4, 7, and 10 standard solutions	For pH calibration; check expiration dates
Conductivity standard	Appropriate concentration for expected range
Deionized water	For rinsing sensors between calibration steps
Storage/calibration cup	Filled with tap water for storage
Field notebook	Per SOP-F003

7. Procedure

7.1. Startup

1. Connect the underwater cable to both the sonde and the Amphibian.
2. Power on the Amphibian.
3. Open the **Amp__2__2__6** app (or press **P1** shortcut).
4. Allow sensors to stabilize for 5–10 minutes before calibration.

7.2. Calibration

Calibrate before every field outing (APHA et al., 2012; Eureka Environmental Engineering, 2018). Record all calibration data (SRF values, standard concentrations, date, operator) in the field notebook.

7.2.1. pH

1. Remove the storage cup. Rinse sensors with DI water.
2. Submerge the sonde in pH 7 standard. Select **Calibrate > pH** and follow the on-screen prompts.
3. Rinse with DI water. Submerge in pH 4 standard. Accept the second calibration point.
4. (Optional) Verify with pH 10 standard. Record the SRF (acceptable range: 95–105).

7.2.2. Conductivity

1. Rinse sensors with DI water.
2. Submerge in the appropriate conductivity standard.
3. Select **Calibrate > Conductivity**. Enter the standard value and accept (Eureka Environmental Engineering, 2018).
4. Record the SRF (acceptable range: 110–120).

7.2.3. Dissolved Oxygen (HDO)

1. Remove the sonde from the calibration cup. Allow the HDO sensor to equilibrate in water-saturated air for 2 minutes (sensor face moist but not submerged).
2. Enter the current barometric pressure in mm Hg.
3. Select **Calibrate > DO** and accept the 100% saturation calibration (Eureka Environmental Engineering, 2018).
4. Record the SRF (should equal 100%).

7.3. Field Measurement

1. Lower the sonde to the desired depth. Allow 1–2 minutes for readings to stabilize before recording.
2. Press **Snapshot** on the Amphibian to save readings. Record the snapshot ID, location, and notes in the field notebook.
3. For depth profiles, lower the sonde in 0.5 or 1 m increments, pausing 30–60 s at each depth (Eureka Environmental Engineering, 2018).

7.4. Disconnecting

1. Remove the probe from the water.
2. Disconnect the underwater cable from the probe.
3. Insert the blue storage plug.
4. Cover the cable end with the plastic protector.

8. Post-Field Procedures

8.1. Cleaning the Probe

1. Clean the probe with warm soapy water (liquid dish soap or mild household cleaner).
2. Clean sensor stems with a soft brush.
3. Rinse thoroughly with tap water.
4. Store sensors with tap water inside the cup.

8.2. Downloading Data

1. Connect the Amphibian to your PC using a USB cable.
2. A green window will appear with four options; select **File Management**.
3. In the next window, navigate to the hard drive, then select **My Documents**.
4. Locate the Excel document with your data.
5. Copy the file to your project data folder.

8.3. Charging

Return the Amphibian to its charging station after each field outing.

9. Quality Control

- Calibrate all sensors before every field outing (APHA et al., 2012; Eureka Environmental Engineering, 2018).
- Verify SRF values are within acceptable ranges; do not proceed if any sensor fails calibration.
- Record a post-deployment calibration check on return to the lab; if any sensor has drifted beyond acceptable limits, flag the data.
- Compare spot readings against an independent DO meter or multiprobe where possible; readings should agree within ±0.3 mg/L for DO and ±0.1 units for pH (APHA et al., 2012).

10. Troubleshooting

Problem	Solution
No power	Check the green LED on the Manta2; if off, the voltage is insufficient. Try another cable.
pH SRF out of range	Recalibrate with fresh standards. If still out of range, refill the reference electrolyte and contact the PI.
Conductivity SRF outside 110–120	Recalibrate. If still out of range, contact the PI.
HDO %sat SRF 100	Redo the calibration with freshly aerated water.
GPS not acquiring satellites	Ensure the top cap (antenna) is uncovered; move to an open area.

11. References

- Eureka Environmental Engineering (2018)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Eureka Environmental Engineering. (2018). *Manta2 water quality multiprobe: User manual*. https://www.rshydro.co.uk/PDFs/Eureka/Manta2-manual.pdf

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F003	Field Notebook Protocols	Data recording
F209	Dissolved Oxygen Meter	Standalone DO companion
F307	Lake Profiling	Depth profile application

13. Training Record

Before using this instrument independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator perform calibration and field deployment
4. Perform a supervised calibration of all sensors
5. Perform a supervised field deployment with data collection
6. Be signed off by the field lead or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F201

Pygmy and Type AA Velocity Meters

Standard Operating Procedure

SOP Number	SOP-F201
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the specifications, assembly, maintenance, and ordering of replacement parts for the Price Pygmy and Price Type AA mechanical current meters used by the EA Program. These vertical-axis cup-type meters convert water velocity into rotational counts and are the standard instruments for measuring point velocities during stream discharge measurements (Rantz et al., 1982) (see also SOP-F301 Stream Discharge Measurement).

2. Scope

- **Applies to:** Price Pygmy current meters and Price Type AA current meters owned by the EA Program, including standard cat-whisker and magnetic-head contact chamber variants
- **Does not apply to:** Electronic velocity meters (e.g., Marsh-McBirney, FlowTracker ADV), propeller-type meters (e.g., Ott), or acoustic Doppler instruments
- **Location:** Instruments stored in the EA field equipment room; maintenance performed in SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve purchases, authorize meter retirement
Field Lead	Perform pre-field spin tests, coordinate maintenance
Trained Personnel	Assemble, inspect, and clean meters per this SOP
New Students	Complete training before handling meters

4. Definitions

- **AquaCalc:** A digital flow computer that counts current meter revolutions electronically and converts them to velocity automatically using the stored rating table; eliminates manual timing and reduces transcription error.
- **Cat-whisker contact:** A spring-wire contact element in the Type AA meter's contact chamber; makes electrical contact with a solder bead on the shaft once per revolution (single-revolution contact) or once per five revolutions (penta contact); must be checked for wear and proper contact force before each field season (Rantz et al., 1982).
- **Contact chamber:** The sealed housing on the Type AA meter that contains the electrical contact mechanism (cat-whisker wire and solder bead, or magnetic head); connects to the cable leading to the headset or digital counter.

- **Penta contact:** A gear-reduction contact in the Type AA meter that closes the circuit once every five bucket-wheel revolutions; used at high velocities to reduce the counting rate to a manageable level; not available on the Pygmy meter (Rantz et al., 1982).
- **Pivot:** The tungsten carbide tip at the bottom of the current meter shaft; rides in a hardened bearing cup; the single most wear-sensitive part of a mechanical current meter; must be inspected for burrs, flat spots, and magnetization before each field season (Rantz et al., 1982; U.S. Geological Survey, Office of Surface Water, 2002).
- **Price Pygmy current meter:** A vertical-axis cup-type current meter with a 2-inch (5.1 cm) diameter bucket wheel; designed for shallow streams (depth 1.0 ft); wading rod suspension only; rated for velocities of 0.1–4.9 ft/s (Rantz et al., 1982).
- **Price Type AA current meter:** The standard USGS vertical-axis cup-type current meter with a 5-inch (12.7 cm) diameter bucket wheel; rated for velocities of 0.1–25 ft/s; can be suspended from a wading rod or cable (Rantz et al., 1982).
- **Rating table:** A lookup table, supplied with each meter by the manufacturer and approved by the USGS, that converts bucket-wheel revolutions per second to velocity in ft/s or m/s; laminated copies must be kept in each meter's carrying case (Rantz et al., 1982).
- **Raising nut:** The nut that controls the vertical position of the shaft and pivot within the yoke; must be tightened to the correct end-play setting (~0.001 in) for accurate measurements; never loosen by holding the nut and spinning the bucket wheel.
- **Spin test:** A field quality control check in which the bucket wheel is given a sharp spin by hand and the time for it to stop is measured; a proxy for bearing condition; minimum acceptable spin times are 40 s (Type AA) and 24 s (Pygmy) at room temperature (Rantz et al., 1982).
- **Wading rod (top-setting):** A graduated aluminum rod used to position the current meter at a known depth fraction (0.6 or 0.2/0.8 of total depth) below the water surface; the depth-setting mechanism is located on the handle, enabling depth changes without removing the meter from the water.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Pivot points and shaft tips are sharp; handle with care. The bucket wheel can pinch fingers if spun while adjusting the assembly.
- **Chemical:** Instrument oil is a mild skin irritant. Avoid eye contact.

5.1. Required PPE

☐ Nitrile gloves (when oiling or cleaning with solvents)
☐ Safety glasses (when using compressed air for cleaning)

6. Instrument Specifications

6.1. Price Type AA Current Meter

The Type AA is the standard USGS Price-type current meter for general streamflow measurement (Rantz et al., 1982). The bucket wheel has six conical cups (12.7 cm diameter) rotating on a vertical axis inside a brass yoke. Tungsten carbide bearings are seated in deeply recessed inverted cups that trap air and exclude water and silt. A tailpiece assembly aligns the meter with the current when suspended by cable.

Parameter	Value
Velocity range	0.1–25 ft/s (0.03–7.6 m/s)
Bucket wheel diameter	5 in (12.7 cm)
Number of cups	6
Contact types	Single revolution and penta (every 5 rev)
Suspension	Wading rod or cable with sounding weight
Construction	Brass and stainless steel, chrome plated
Rating	USGS standard rating table (two equations joined at 1 rev/s = 2.2 ft/s) (Rantz et al., 1982)

6.2. Price Pygmy Current Meter

The Pygmy meter is scaled to two-fifths the size of the Type AA, designed for shallow streams (Rantz et al., 1982). The contact chamber is integral to the yoke; single-revolution contact only (no penta gear). The meter has no tailpiece and cannot be used with cable suspension.

Parameter	Value
Velocity range	0.1–4.9 ft/s (0.03–1.5 m/s)
Bucket wheel diameter	2 in (5.1 cm)
Number of cups	6
Contact type	Single revolution only
Suspension	Wading rod only (no cable suspension)
Construction	Brass and stainless steel, chrome plated
Rating	USGS standard rating table (single equation) (Rantz et al., 1982)

6.3. Choosing Between Meters

Condition	Meter to Use
Water depth $>$ 1.0 ft (0.3 m)	Type AA
Water depth 1.0 ft (0.3 m)	Pygmy
Velocities expected $>$ 4.9 ft/s	Type AA
Shallow stream edges (supplemental data)	Pygmy

Condition	Meter to Use
Cable suspension required	Type AA

7. Equipment and Accessories

Item	Used With	Notes
Top-setting wading rod	Both	Graduated in tenths of a foot; depth-setting mechanism on handle
Round wading rod	Both	Simpler design; depth read from rod markings
Headset (earpiece)	Both	For counting clicks manually
AquaCalc or digital flow computer	Both	Automatically counts revolutions and computes velocity
Stopwatch	Both	For timed revolution counts if no digital counter
Sounding weight (Columbus type)	Type AA only	For cable-suspended measurements
Tailpiece assembly	Type AA only	Remove when using wading rod
Electrical cable	Both	Connects meter to counter or headset

7.1. Maintenance Supplies

Item	Notes
Instrument oil (light machine oil)	Supplied with meter; for pivot bearings
Lint-free cloth	For cleaning and drying
Cotton swabs (pointed)	For cleaning pivot bearing recesses
Small flat-head screwdriver	For contact chamber adjustments
Magnifying glass or loupe	For inspecting pivot points for burrs
Paper clip	For checking pivot magnetization
Bulk magnetic tape eraser	For demagnetizing pivots
Soft-bristle brush	For removing debris from cups
Compressed air (filtered)	For drying internal components

8. Procedure

8.1. Assembly

Type AA on a wading rod:

1. Remove the tailpiece assembly (used for cable suspension only).

2. Slide the meter yoke onto the wading rod and secure with the thumbscrew.
3. Connect the electrical cable from the binding posts on the contact chamber to the AquaCalc or headset.
4. For penta contact, connect to the penta binding post; for single-revolution counting, use the single-revolution binding post.

Pygmy on a wading rod:

1. Slide the pygmy yoke directly onto the wading rod and secure with the thumbscrew.
2. Connect the electrical cable to the binding post. The Pygmy has only a single-revolution contact.

8.2. Pre-Field Spin Test

Perform a spin test before every field outing (Rantz et al., 1982). Conduct with the meter level, shaft vertical, in a location with no air currents.

1. Hold the meter steady and use your finger or a pencil to give the bucket wheel a sharp, single spin.
2. Immediately start a stopwatch.
3. Stop the stopwatch when the wheel completely stops rotating.
4. Record the spin time in the field notebook.

Minimum acceptable spin times (Rantz et al., 1982):

Meter	Minimum Spin Time	Optimum Spin Time
Type AA	40 s	60–120 s
Pygmy	24 s	40–80 s

If the spin time falls below the minimum, clean and oil the pivot before proceeding (§7.3). If the spin time remains low after cleaning and oiling, replace the pivot.

8.3. Cleaning and Oiling (Pre-Season and As Needed)

1. **Inspect the pivot.** Remove the raising nut and carefully withdraw the shaft. Examine the pivot tip under a magnifying glass. A good pivot is highly polished, with no flat spots, pits, or scratches. Replace a damaged pivot immediately.
2. **Check for magnetization.** Hold the pivot near a paper clip. If the clip is attracted to the pivot, the pivot is magnetized and will cause erroneously high velocity readings. Demagnetize using a bulk magnetic tape eraser.
3. **Oil the bearings.** Apply one small drop of instrument oil to the upper shaft bearing and one drop to the pivot. Do not over-oil; excess oil attracts grit.
4. **Inspect the bucket wheel.** Spin the wheel slowly and watch for wobble. If the upper hub wobbles more than the lower, the shaft is bent and must be replaced.

5. **Inspect the contact chamber.** For cat-whisker contacts, check that the wire makes clean contact with the solder bead on each revolution. For magnetic-head contacts, verify the counter registers properly during the spin test.

> ⚠ Raising Nut Caution
>
> Never loosen the raising nut by holding the nut and spinning the bucket wheel. This will bend the shaft. Always hold the bucket wheel still and unscrew the nut (U.S. Geological Survey, Office of Surface Water, 2002).

8.4. Annual Maintenance

1. Disassemble the meter completely: remove bucket wheel, shaft, and pivot.
2. Clean all parts with a lint-free cloth dampened with instrument oil.
3. Inspect the shaft for straightness by rolling on a flat surface.
4. Replace worn or damaged parts.
5. Reassemble and adjust the pivot per the manufacturer's instructions.
6. Perform a spin test to verify performance.
7. Record maintenance date, parts replaced, and spin test results in the instrument log (U.S. Geological Survey, Office of Surface Water, 2002).

8.5. Pivot Adjustment

1. Hold the meter inverted with the pivot pointing up.
2. Release the keeper screw and unscrew the pivot-adjusting nut a few turns.
3. Advance the pivot-adjusting nut until it touches the yoke.
4. Set end play to approximately 0.001 in – the shaft should have just barely perceptible vertical play detectable by feel.
5. Tighten the keeper screw to lock the adjustment.

8.6. Storage

- Store meters in their waterproof carrying cases.
- Loosen the raising nut to relieve pressure on the pivot.
- Ensure meters are clean and lightly oiled.
- Store in a dry location away from dust and vibration.
- If a meter will not be used for more than 3 months, remove the bucket wheel assembly and store separately.

9. Rating Tables

Each meter ships with a USGS-approved standard rating table that converts bucket-wheel revolutions per second to velocity in ft/s or m/s (Rantz et al., 1982; Turnipseed & Sauer, 2010). Keep a laminated copy in each meter's carrying case and a digital copy on the lab shared drive.

- Type AA rating table: https://prph2o.com/usgs-type-aa-current-meter/
- Pygmy rating table: https://prph2o.com/usgs-pygmy-current-meter/

9.1. Meter Calibration Verification

USGS studies have found that approximately 35% of Type AA meters and 20% of Pygmy meters in routine field use fail to meet standard rating criteria, typically because of worn pivots, bent shafts, or damaged contacts (Rantz et al., 1982). The spin test (§7.2) is the primary field check.

10. Ordering Parts and Supplies

Primary supplier for USGS-type Price AA and Pygmy current meters and replacement parts:

Performance Results Plus, Inc. Website: https://prph2o.com

10.1. Replacement Parts to Stock

Part	Qty to Stock	Fits
Pivot (tungsten carbide tip)	2	Both
Shaft	1 per type	AA or Pygmy
Bucket wheel assembly	1 per type	AA or Pygmy
Bucket wheel hub nut	2	Both
Raising nut	1 per type	AA or Pygmy
Cat-whisker wire and solder bead	2 sets	AA
Instrument oil	1 bottle	Both
Pivot bearing	2	Both

11. Troubleshooting

Problem	Probable Cause	Corrective Action
Spin time below minimum	Dirty or worn pivot; bent shaft; dry bearings	Clean pivot; inspect for burrs; oil bearings; replace pivot or shaft if damaged
Counter not registering	Loose cable; worn cat-whisker; dead counter battery	Check cable; replace contact wire; replace battery
Bucket wheel wobbles	Bent shaft or frame	Replace shaft; if hub wobbles, replace bucket wheel assembly

Problem	Probable Cause	Corrective Action
Meter reads high	Magnetized pivot; worn bearing	Demagnetize pivot; replace pivot bearing
Meter reads low	Excessive friction; over-oiled	Clean and re-oil; replace pivot if burred

12. References

- Rantz et al. (1982)
- Turnipseed & Sauer (2010)
- U.S. Geological Survey, Office of Surface Water (2002)

Rantz, S. E. et al. (1982). *Measurement and computation of streamflow: Volume 1. Measurement of stage and discharge* (Water-Supply Paper 2175). U.S. Geological Survey. https://pubs.usgs.gov/wsp/wsp2175/

Turnipseed, D. P., & Sauer, V. B. (2010). *Discharge measurements at gaging stations* (Techniques and Methods 3-A8). U.S. Geological Survey. https://doi.org/10.3133/tm3A8

U.S. Geological Survey, Office of Surface Water. (2002). *Maintenance of Price type AA current meters.* U.S. Geological Survey. https://water.usgs.gov/osw/pubs/9906attachments.pdf

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site equipment cleaning
F202	SonTek Acoustic Velocity Meters	Acoustic alternative
F301	Stream Discharge Measurement	Primary application of this instrument

14. Training Record

Before handling or using current meters independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator assemble, spin-test, and clean both meter types
4. Successfully perform assembly and spin test under supervision
5. Read SOP-F301 before using meters in the field
6. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F202

SonTek Acoustic Velocity Meters

Standard Operating Procedure

SOP Number	SOP-F202
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Author(s)	Marc Los Huertos
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1. Purpose

This SOP describes the operation, calibration verification, and maintenance of SonTek acoustic Doppler velocity meters used for point velocity and discharge measurements in wadeable streams. Acoustic Doppler instruments measure water velocity by transmitting ultrasonic pulses and detecting the frequency shift of sound reflected by suspended particles in the water column (Rehmel, 2007). Unlike mechanical current meters (SOP-F201), acoustic meters have no moving parts, respond faster to turbulent fluctuations, and can measure very low velocities.

2. Scope

- **Applies to:** SonTek FlowTracker2 and FlowTracker (original model) handheld acoustic Doppler velocimeters (ADVs) used for wading discharge measurements in streams and channels
- **Does not apply to:** SonTek ADP/ADCP profiling instruments for non-wadeable rivers or boat-mounted surveys; mechanical current meters (see SOP-F201); fixed-installation acoustic flow meters in pipes or culverts
- **Location:** All EA Program wadeable stream sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve instrument deployment; ensure firmware is current
Trained Personnel	Operate instrument per this SOP; download and archive data
New Students	Complete training before independent use

4. Definitions

- **ADV (acoustic Doppler velocimeter):** An instrument that measures water velocity at a single point by transmitting ultrasonic pulses and measuring the Doppler frequency shift of sound scattered back from suspended particles in a small sampling volume located at a fixed distance from the transducer head (Rehmel, 2007).
- **Averaging time:** The duration over which velocity samples are collected and averaged at each measurement vertical; the FlowTracker default is 40 seconds; increase to 60 seconds in low-velocity or highly turbulent flows to improve measurement stability (SonTek/Xylem, 2019).
- **Beam check:** A pre-measurement quality check in which the FlowTracker2 tests all three acoustic beams and reports signal-to- noise ratio (SNR) for each; all beams must return SNR > 4 dB before a measurement can proceed (SonTek/Xylem, 2019).
- **Boundary flag:** An automated QC flag set by the FlowTracker when the probe is too close to the streambed or water surface for the sampling volume to be fully within the water column; corrected by adjusting the depth setting.

- **Doppler effect:** The change in frequency of a wave (sound or light) observed when the source or the reflector is in motion relative to the observer; the physical basis of acoustic Doppler velocity measurement – the frequency shift of reflected ultrasound is proportional to the component of particle velocity along the acoustic beam axis (Rehmel, 2007).
- **FlowTracker2:** The current production SonTek handheld ADV for wading discharge measurements; uses three acoustic beams and integrated pressure depth sensor; stores data internally and transfers via USB; includes automated QA/QC flagging (SonTek/Xylem, 2019).
- **Mid-section method:** The standard USGS discharge calculation method in which each vertical's discharge is the product of measured velocity, depth, and the horizontal width extending halfway to the adjacent verticals on each side; computed automatically by the FlowTracker (Turnipseed & Sauer, 2010).
- **MMT file:** The proprietary binary data file format produced by the SonTek FlowTracker; stores raw velocity data, QC flags, site information, and the computed discharge summary; must be archived before exporting to CSV.
- **Sampling volume:** The small water volume approximately 10 cm from the FlowTracker transducer head from which velocity data are collected; must be free of boundary interference (streambed, water surface, or large obstacles) for valid measurements (SonTek/Xylem, 2019).
- **SNR (signal-to-noise ratio):** The ratio of acoustic signal strength returned from suspended particles to background noise; expressed in dB; the FlowTracker requires SNR > 4 dB on all three beams for a valid measurement; low SNR typically indicates insufficient suspended particles in very clear water (SonTek/Xylem, 2019).
- **Spike flag:** An automated QC flag set by the FlowTracker when a single velocity sample deviates significantly from the running mean, indicating a transient disturbance (bubble, debris, wading disturbance); the affected sample is excluded from the average.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Wading in flowing water carries drowning risk. Always work in pairs. Do not wade in flows deeper than waist height or where footing is unstable.
- **Electrical:** The FlowTracker uses internal rechargeable batteries. Do not open the sealed housing in the field.
- **Biological:** Waterborne pathogens; decontaminate equipment between sites per SOP-F007.

5.1. Required PPE

☐ Chest or hip waders with felt or studded soles
☐ Closed-toed shoes or wading boots
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

A minimum of two people must be present for all in-stream work. Refer to SOP-F001 Field Safety for general emergency procedures.

6. Instrument Specifications

6.1. FlowTracker2

Parameter	Value
Measurement principle	Acoustic Doppler velocimetry (ADV)
Velocity range	0.001–4.0 m/s (0.003–13 ft/s)
Velocity accuracy	±1% of measured velocity
Velocity resolution	0.0001 m/s
Sampling volume	Located 10 cm from the transducer
Depth sensor	Integrated pressure sensor, 0–5 m range
Data storage	Internal memory; USB download
Battery	Rechargeable lithium-ion; ~12 hr continuous
QC diagnostics	Automated QA/QC (SNR, boundary, flow angle, spike detection)

(SonTek/Xylem, 2019)

6.2. FlowTracker (Original)

Parameter	Value
Measurement principle	Acoustic Doppler velocimetry (ADV)
Velocity range	0.001–4.5 m/s
Velocity accuracy	±1% of measured velocity
Sampling volume	Located 10 cm from the transducer
Data storage	Internal memory; RS-232 download
Battery	3 C-cell alkaline batteries

7. Equipment and Materials

Item	Notes
SonTek FlowTracker2 (or original)	Charged and firmware updated (SonTek/Xylem, 2019)
Top-setting wading rod	Standard USGS-type; FlowTracker mounts on the rod

Item	Notes
Mounting bracket and thumbscrew	Specific to SonTek; included with instrument
USB cable	For data download (FlowTracker2)
RS-232 cable and adapter (original model)	For data download
Measuring tape (50 m)	For establishing the tag line
Tape stakes (2)	For anchoring the tag line
Laptop or tablet with SonTek software	FlowTracker2 uses SonTek's desktop software
Field data sheet (backup)	Printed on waterproof paper
Spare batteries	C-cells for original model

8. Procedure

8.1. Pre-Field Setup

1. Charge the FlowTracker2 battery fully before each field day (SonTek/Xylem, 2019). For the original model, install fresh batteries.
2. Power on the instrument and verify that the firmware version is current. Update if prompted.
3. Set the site information: site name, station ID, date, and operator initials.
4. Select the measurement method:
   - **Mid-section** (default, consistent with USGS practice (Turnipseed & Sauer, 2010))
   - **Mean-section** (if required by the project)
5. Set the measurement parameters:
   - Averaging time: 40 seconds (default; increase to 60 seconds for low or turbulent flows) (SonTek/Xylem, 2019)
   - Number of measurements per vertical: 1 (six-tenths method) or 2 (two-point method at 0.2 and 0.8 depth) (Turnipseed & Sauer, 2010)

8.2. Beam Check

Perform a beam check before each measurement (SonTek/Xylem, 2019).

1. Submerge the probe head at least 15 cm below the water surface in a location with minimal turbulence.
2. On the FlowTracker2, select **QC > Beam Check** from the menu.
3. The instrument tests all three acoustic beams and reports SNR for each beam.
4. All beams must report SNR > 4 dB. If any beam fails, clean the transducer faces and retest. If the beam check still fails, do not proceed with the measurement.
5. Record the beam check result in the field notebook.

8.3. Site Selection and Tag Line

Follow the site selection and tag line procedures described in SOP-F301 Stream Discharge Measurement, Sections 7.1–7.3 (Turnipseed & Sauer, 2010). The same cross-section criteria apply to acoustic and mechanical meters.

8.4. Making the Measurement

1. Mount the FlowTracker probe on the wading rod at the correct depth setting:
   - **Six-tenths method:** Set the probe at 0.6 of the total depth below the water surface (same as for mechanical meters)
   - **Two-point method:** Measure at 0.2 and 0.8 of the depth
2. At each vertical, the instrument automatically records velocity (Vx, Vy components) and runs QC checks (SNR, spike detection, boundary check, flow angle).
3. If a measurement is flagged, the instrument displays the reason. Common flags and responses:

Flag	Meaning	Response
Low SNR	Insufficient suspended particles	Increase averaging time; stir bed gently upstream
Boundary	Probe too close to bed or surface	Adjust depth setting
High flow angle	Flow not perpendicular to tag line	Reposition tag line or note angle
Spike	Velocity spike detected	Repeat measurement

(SonTek/Xylem, 2019)

4. After completing all verticals, the instrument computes total discharge automatically using the mid-section method (Turnipseed & Sauer, 2010).
5. Review the on-screen discharge summary before leaving the site.

8.5. Post-Measurement

1. Save the measurement file on the instrument.
2. Record a backup note in the field notebook: site, date, total discharge, number of verticals, and any flagged stations.
3. Rinse the probe with clean water. Do not use solvents or abrasives on the transducer faces.
4. Decontaminate per SOP-F007 before moving to the next site.

8.6. Data Download

1. Connect the FlowTracker2 to a laptop via USB.
2. Open the SonTek desktop software and download all measurement files (SonTek/Xylem, 2019).
3. Export each measurement as a `.csv` and a `.dis` (discharge summary) file.
4. Save files using the naming convention: `YYYY-MM-DD_SiteID_discharge_FT2.csv`
5. Upload to the project GitHub repository under `data/discharge/` within 48 hours.
6. Archive the raw `.mmt` file to the lab shared drive.

9. Maintenance

9.1. After Each Field Day

1. Rinse the entire probe head and cable with clean fresh water.
2. Dry with a lint-free cloth.
3. Inspect the transducer faces for scratches, algae buildup, or debris. Clean gently with a soft brush if needed.
4. Charge the battery (FlowTracker2) or remove batteries (original model) if storing for more than one week.

9.2. Periodic Maintenance

- **Monthly (active use):** Inspect the wading rod mounting bracket for wear. Verify firmware version.
- **Annually:** Contact SonTek for factory calibration verification if the instrument is used for regulatory or compliance monitoring (Rehmel, 2007).

10. Quality Control

- Beam check before every measurement (§7.2) (SonTek/Xylem, 2019).
- If two measurements are made at the same cross-section, they should agree within 5%; if they differ by more than 5%, make a third measurement and use the mean of the two closest values (Turnipseed & Sauer, 2010).
- Compare FlowTracker results with mechanical meter results at least once per year; results should agree within 5–8% (Rehmel, 2007).
- Review all automated QC flags during data processing. Do not discard flagged data without documentation.

11. References

- SonTek/Xylem (2019)
- Turnipseed & Sauer (2010)
- Rehmel (2007)

Rehmel, M. S. (2007). *Application of acoustic Doppler velocimeters for streamflow measurements* (Scientific Investigations Report 2007-5091). U.S. Geological Survey. https://doi.org/10.3133/sir20075091

SonTek/Xylem. (2019). *FlowTracker2 handheld ADV technical manual*. https://www.sontek.com/flowtracker2

Turnipseed, D. P., & Sauer, V. B. (2010). *Discharge measurements at gaging stations* (Techniques and Methods 3-A8). U.S. Geological Survey. https://doi.org/10.3133/tm3A8

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site cleaning
F201	Pygmy and Type AA Velocity Meters	Mechanical alternative
F301	Stream Discharge Measurement	Cross-section procedures

13. Training Record

Before using SonTek velocity meters independently, personnel must:

1. Read this SOP and SOP-F301 in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator perform a complete discharge measurement including beam check, measurement, and data download
4. Perform a supervised discharge measurement with results that agree within 5% of the trainer's independent measurement
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F203

HOBO Water Level Pressure Transducers

Standard Operating Procedure

SOP Number	SOP-F203
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment, data retrieval, and barometric compensation of Onset HOBO water level loggers for continuous monitoring of water surface elevation in streams, lakes, wetlands, and groundwater wells (Freeman et al., 2004; Onset Computer Corporation, 2020). Continuous stage records are essential for developing stage-discharge rating curves, characterizing hydrologic response to storms, and documenting seasonal water table fluctuations.

2. Scope

- **Applies to:** Onset HOBO U20, U20L, and MX2001 water level loggers deployed at EA Program surface water and groundwater monitoring sites
- **Does not apply to:** Real-time telemetered stage recorders; acoustic or radar level sensors; staff gauge readings (visual observations recorded in the field notebook)
- **Location:** All EA Program hydrologic monitoring sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve deployment locations and logging intervals
Trained Personnel	Deploy, retrieve, and download loggers per this SOP
New Students	Complete training before independent use

4. Definitions

- **Absolute pressure:** Pressure measured relative to a perfect vacuum; the HOBO U20L measures absolute pressure (water column plus atmospheric), which must be compensated to isolate water-column pressure (Freeman et al., 2004).
- **Barometric compensation:** Post-processing step in HOBOware that subtracts a concurrent barometric pressure record (from a reference logger deployed in air) from the absolute pressure record to yield true water depth (Onset Computer Corporation, 2021).
- **Datum:** A fixed reference elevation (e.g., NAVD88, local benchmark) to which stage measurements are referenced.
- **Deployment depth:** The vertical distance from the water surface to the sensor face at the time of deployment; used as the initial reference for water-level calculations.
- **HOBO U20L:** Onset Computer Corporation submersible water-level logger recording absolute pressure and temperature at user-defined intervals; rated to a maximum depth of 9 m (model U20L-01) or 4 m (model U20L-04 barometric) (Onset Computer Corporation, 2020).
- **HOBOconnect / HOBOware:** Onset software (mobile app and desktop, respectively) used to program loggers, download data, apply barometric compensation, and export time-series data (Onset Computer Corporation, 2021).
- **Stage:** Water-surface elevation at a monitoring location, expressed relative to a datum; the primary hydrologic quantity derived from pressure transducer records.

- **Stage-discharge rating curve:** An empirical relationship between stream stage and discharge (Q) at a gauging cross-section; enables conversion of continuous stage records into continuous discharge records (Freeman et al., 2004).
- **Stilling well:** A perforated pipe anchored in the stream or lake bed that protects the logger from direct current, debris, and disturbance while allowing free water exchange.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Deployment sites may involve steep banks, slippery surfaces, swift water, or confined spaces (well casings). Work in pairs at all stream and well sites.
- **Drowning:** Do not deploy loggers during high-flow events. Never wade in flows deeper than waist height.
- **Biological:** Waterborne pathogens; ticks and snakes in riparian areas.

5.1. Required PPE

☐ Wading boots or chest waders (stream deployments)
☐ Closed-toed shoes (well deployments)
☐ Nitrile gloves
☐ Sun protection (hat, sunscreen)

5.2. Required Training

- SOP-F001 Field Safety
- Work with a trained partner for all in-stream deployments

6. Instrument Specifications

6.1. HOBO U20L Water Level Logger

Parameter	Value
Measurement principle	Absolute pressure (water + atmosphere)
Pressure range	0–9 m (U20L-01) or 0–4 m barometric (U20L-04)
Accuracy	+/- 0.1% full scale (~0.5 cm water)
Logging interval	1 second to 18 hours (user-configurable)
Memory	~21,700 combined readings
Battery life	~5 years at 1-minute logging
Communication	Optical USB coupler (U20L)

6.2. HOBO MX2001 Water Level Logger

Parameter	Value
Communication	Bluetooth Low Energy; download via HOBOconnect app
Pressure range	0–9 m or 0–4 m models
Other specs	Similar to U20L

6.3. Barometric Reference Logger

Because the water level loggers measure absolute pressure, a companion barometric pressure logger must be deployed above the water surface at or near the site. An Onset HOBO U20L-04 barometric model or HOBO MX barometric logger serves this purpose.

7. Equipment and Materials

Item	Storage	Notes
HOBO U20L or MX2001 loggers	SGM 133	Check battery before deployment
Optical USB coupler (U20L)	SGM 133	For HOBOware download
HOBOconnect app (MX2001)	Phone	Requires Bluetooth pairing
Stilling well (PVC, 2 in)	SGM 132	With end cap and perforations
Stainless steel cable / line	SGM 131	For securing logger in well
Staff gauge (if not installed)	SGM 131	Painted, graduated to 0.01 m
HOBOware laptop	SGM 133	Pre-loaded with HOBOware Pro
GPS unit	SGM 133	See SOP-F006
Field data sheet	SGM 133	

8. Estimated Time

Task	Time
Logger programming (HOBOware)	10–15 min
Stilling well installation (new)	30–90 min
Logger deployment (existing site)	15–30 min
Data retrieval and export	15–30 min
Barometric compensation (HOBOware)	15–30 min

9. Procedure

9.1. Logger Programming

1. Connect the logger to HOBOware using the optical USB coupler or open HOBOconnect via Bluetooth.

2. Select **Launch** (HOBOware) or **Configure** (HOBOconnect).
3. Set the logging interval appropriate to the project (5 min is typical for hydrologic studies; 15 min for long-term deployment).
4. Enable a delayed start if synchronizing with another logger at the site.
5. Record the logger serial number, launch time, and interval in the field notebook and in the deployment spreadsheet.

9.2. Stream Deployment (Stilling Well)

1. Choose a stable bank location away from direct turbulence but representative of the cross-section stage.
2. Install the stilling well (2-inch PVC with perforations) vertically with the bottom anchored and the top above expected high-water level. Ensure the perforations face upstream and downstream.
3. Lower the logger into the stilling well and secure it with stainless steel wire or cable tie. The logger should hang freely.
4. Record the deployment elevation:
    - Distance from the top of the stilling well to the water surface and to the logger sensor
    - Staff gauge reading (if installed)
    - GPS coordinates per SOP-F006
5. Deploy the barometric reference logger nearby, above the high-water line, in a shaded and ventilated location (e.g., inside a radiation shield attached to a tree or post). Do not seal it in an airtight container.
6. Photograph the deployment.

9.3. Well Deployment

1. Remove the well cap and measure depth to water using an e-tape. Record the measurement.
2. Lower the logger into the well on stainless steel cable or braided fishing line. Position below the lowest expected water level but above the well screen bottom.
3. Secure the suspension cable to the well cap or casing.
4. Record the logger depth below the top of casing (TOC) and the TOC elevation if surveyed.
5. Replace the well cap.
6. Deploy the barometric reference logger at the wellhead or nearby.

9.4. Data Retrieval

Retrieve data at intervals appropriate for the project (typically monthly or quarterly).

U20L (optical coupler):

1. Remove the logger from the stilling well.
2. Attach the optical USB coupler and connect to the laptop.
3. In HOBOware, select **Readout**. The logger continues recording after readout.
4. Return the logger to its deployment position.

MX2001 (Bluetooth):

1. Open the HOBOconnect app on a smartphone within ~30 m line of sight.
2. Select the logger and tap **Readout**.
3. The logger does not need to be removed from the water.

9.5. Barometric Compensation

1. In HOBOware, open the water level logger file and the barometric reference logger file.
2. Select **Data Assistant > Barometric Compensation**.
3. Specify the reference water level: enter a staff gauge reading or a known water surface elevation at a specific date and time.
4. HOBOware subtracts the barometric pressure from the absolute pressure to yield the water column height.
5. Export the compensated data as `.csv`.
6. Save using the convention: `YYYY-MM-DD_SiteID_waterlevel_compensated.csv`.
7. Commit data to the project repository per SOP-L801.

10. Quality Control

- Verify that the barometric logger file overlaps the full duration of the water level record. Gaps in barometric data prevent compensation.
- Compare the compensated stage at the download visit against a simultaneous staff gauge reading. Acceptable agreement is within 1 cm.
- Flag and investigate any sudden offsets or drift in the compensated record (may indicate logger movement, sedimentation, or battery failure).

11. Data Management

1. Save all raw logger files (`.hobo`) to `data/raw/loggers/` in the project repository.
2. Save compensated `.csv` files to `data/processed/stage/`.
3. Commit all files per SOP-L801.

12. References

Freeman, L. A., Carpenter, M. C., Rosenberry, D. O., Rousseau, J. P., Unger, R., & McLean, J. S. (2004). *Use of submersible pressure transducers in water-resources investigations* (Techniques of Water-Resources Investigations Book 8, Chapter A3). U.S. Geological Survey. https://pubs.usgs.gov/twri/twri8a3/

Onset Computer Corporation. (2020). *HOBO U20L water level logger user's manual*. https://www.onsetcomp.com/files/manual_pdfs/14366-F-MAN-U20L.pdf

Onset Computer Corporation. (2021). *HOBOware user's guide*. https://www.onsetcomp.com/files/manual_pdfs/10246-K-MAN-HOBOware.pdf

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Deployment coordinates
F007	Field Decontamination	Between-site cleaning
F301	Stream Discharge Measurement	Stage-discharge rating curves
F303	Groundwater Sampling	Well deployment context
L801	RStudio and GitHub	Data version control

14. Training Record

Before deploying water level loggers independently, personnel must:

1. Read this SOP in full
2. Demonstrate logger programming (launch, interval, delayed start) using HOBOware or HOBOconnect
3. Participate in a supervised stream or well deployment
4. Demonstrate barometric compensation in HOBOware and export of compensated data
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F204

HOBO Weather Station Deployment

Standard Operating Procedure

SOP Number	SOP-F204
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment, programming, data retrieval, and maintenance of Onset HOBO pendant and MX-series data loggers used for unattended monitoring of air temperature, relative humidity, and light intensity at EA Program field sites. These compact loggers provide continuous microclimatic data for characterizing site conditions, supporting ecological studies, and supplementing manual weather observations (Onset Computer Corporation, 2023; World Meteorological Organization, 2018).

2. Scope

- **Applies to:** HOBO Pendant MX2201, MX2202 (temperature/light), HOBO MX2301 (temperature/RH), HOBO Pendant UA-002 (temperature/ light, legacy), and similar Onset loggers deployed at EA Program field sites
- **Does not apply to:** HOBO water level loggers (see SOP-F203); fixed, permanently installed weather stations managed by other departments; air quality sensors (see SOP-F502)
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve deployment plan and logging interval
Trained Personnel	Deploy, retrieve, and download loggers per this SOP
New Students	Complete training before independent use

4. Definitions

- **Bluetooth logging:** A wireless communication protocol used by MX-series HOBO loggers; enables programming and data download via the HOBOconnect smartphone app without cables; requires proximity (typically within 15 m of the logger) (Onset Computer Corporation, 2023).
- **Data gap:** A period within a logger deployment during which no data were recorded; caused by battery failure, logger reset, or memory overflow; must be documented in the project data log.
- **HOBOconnect:** The Onset smartphone app (iOS and Android) used to program, launch, and download data from Bluetooth-enabled MX-series HOBO loggers; requires the logger to be within Bluetooth range (Onset Computer Corporation, 2021).
- **HOBOware:** The Onset desktop software used to program and download data from legacy UA-series HOBO loggers via optical USB coupler; also supports barometric compensation for water level loggers (Onset Computer Corporation, 2021).
- **Logging interval:** The time between successive measurements stored to the logger memory; typical intervals for EA Program deployments are 5 minutes (microclimatic studies) or 15 minutes (long-term monitoring); shorter intervals fill memory faster and require more frequent downloads (Onset Computer Corporation, 2023).
- **MX2301:** The Onset HOBO MX temperature and relative humidity logger; Bluetooth-enabled; ±0.2 °C temperature accuracy and ±2.5% RH accuracy; internal memory stores 500,000 readings (Onset Computer Corporation, 2023).
- **Radiation shield:** A ventilated, white polypropylene housing that surrounds a temperature or RH logger and prevents direct solar radiation from inflating the measured air temperature; a radiation shield is mandatory for any logger exposed to sunlight (World Meteorological Organization, 2018).
- **Relative humidity (RH):** The ratio of the partial pressure of water vapor in air to the saturation vapor pressure at the same temperature, expressed as a percentage; values above

approximately 70% promote sensor drift in some sensor types (World Meteorological Organization, 2018).

- **Sensor drift:** A gradual, systematic change in sensor readings over time not attributable to true environmental change; detected by co-deployment with a reference sensor or comparison with a nearby weather station.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Deployment may involve climbing, ladder use, or working on uneven terrain. Use a spotter when mounting loggers at height.
- **Environmental:** Heat exposure during summer deployments in Southern California. Carry adequate water and sun protection.
- **Wildlife:** Insects (bees, wasps), ticks, and snakes may be present at deployment sites.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Sun protection (hat, sunscreen)
☐ Nitrile gloves (when handling loggers at contaminated sites)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Instrument Specifications

Model	Parameters	Range	Accuracy	Memory	Communication
MX2201	Temp, light	-20 to 70 °C; 0–320,000 lux	±0.5 °C	96,000 readings	Bluetooth
MX2202	Temp, light (waterproof)	-20 to 70 °C	±0.5 °C	96,000 readings	Bluetooth
MX2301	Temp, RH	-40 to 70 °C; 0–100% RH	±0.2 °C; ±2.5% RH	500,000 readings	Bluetooth
UA-002-64	Temp, light (legacy)	-20 to 70 °C	±0.53 °C	64,000 readings	Optical USB

(Onset Computer Corporation, 2023)

7. Equipment and Materials

Item	Notes
HOBO logger(s)	Confirm model and battery status
Radiation shield (RS3 or similar)	Required for temperature loggers exposed to direct sunlight (World Meteorological Organization, 2018)
Mounting hardware (cable ties, U-bolts, brackets)	For securing shield/logger to posts, trees, or tripods
Tripod or mounting post	If no suitable tree or structure available
Smartphone with HOBOconnect app	For Bluetooth-enabled MX loggers (Onset Computer Corporation, 2021)
Laptop with HOBOware software	For legacy UA-series loggers (Onset Computer Corporation, 2021)
Optical USB coupler (for UA loggers)	For launching and downloading
Field notebook	For recording deployment metadata
GPS unit or smartphone	Per SOP-F006
Spare batteries	CR2032 (MX pendant) or 3V lithium (varies by model)

8. Procedure

8.1. Pre-Deployment Programming

MX-series (Bluetooth):

1. Open the HOBOconnect app and connect to the logger via Bluetooth (Onset Computer Corporation, 2021).
2. Configure:
   - Logging interval: 5 or 15 minutes
   - Start mode: delayed start (set to a round time, e.g., top of the next hour)
   - Logger name: use the convention `SiteID_Parameter_LoggerSN` (e.g., `BFS_TempRH_20456789`)
3. Verify the battery level. Replace if below 20%.

UA-series (legacy):

1. Connect the logger via the optical USB coupler and open HOBOware.
2. Select **Launch** and configure the logging interval and start time (Onset Computer Corporation, 2021).
3. Click **Start** to begin logging.

8.2. Deployment

1. Mount the radiation shield at 1.5 m above ground on a post, tree, or tripod in a location representative of the site. Avoid positions within 3 m of heat-absorbing surfaces (pavement, metal walls) (World Meteorological Organization, 2018).
2. Insert the logger into the shield. Ensure the sensor faces are unobstructed and the shield ventilation holes are unblocked.
3. Secure the shield to the mounting structure with cable ties.
4. Record in the field notebook:
   - Logger serial number
   - Deployment coordinates per SOP-F006
   - Date and time of deployment
   - Logging interval and start time
   - Sensor height above ground
5. Photograph the deployed logger with surrounding context visible.

8.3. Downloading Data at Regular Intervals

MX-series (Bluetooth):

1. Open HOBOconnect app and connect to the logger.
2. Select **Download** to transfer all stored data to the smartphone (Onset Computer Corporation, 2021).
3. Export the data as a CSV file.
4. Save using the naming convention: `YYYY-MM-DD_SiteID_Parameter_LoggerSN.csv`
5. Upload to the project GitHub repository under `data/microclimate/` within 48 hours.

UA-series (legacy):

Connect via optical USB coupler and use HOBOware to read out data.

8.4. Logger Retrieval (End of Deployment)

1. Download all remaining data.
2. Remove the logger and mounting hardware.
3. Clean the logger housing with a damp cloth. For waterproof models, rinse with fresh water.
4. Inspect the radiation shield for damage; replace desiccant packs if applicable.
5. Store loggers in a dry, room-temperature location.

9. Quality Control

- At each download visit, compare the logger temperature reading against a handheld thermometer. Discrepancies greater than 1 °C should be investigated (World Meteorological Organization, 2018).

- At least once per year, co-deploy two loggers of the same model at the same location for a minimum of 48 hours to check inter-unit agreement. Temperature should agree within 0.5 °C; RH within 5%.
- Inspect data for signs of sensor failure: constant readings, sudden offsets, or readings pegged at the sensor limit.
- Check battery level at every download. Replace batteries proactively if below 20%.
- For light loggers, verify that the sensor face has not shifted orientation by comparing the deployment photograph to current position.

10. References

- Onset Computer Corporation (2023)
- Onset Computer Corporation (2021)
- World Meteorological Organization (2018)

Onset Computer Corporation. (2021). *HOBOware user's guide*. https://www.onsetcomp.com/files/manual_pdfs/10246-K-MAN-HOBOware.pdf

Onset Computer Corporation. (2023). *HOBO U23 Pro v2 external temperature data logger: User manual*. https://www.onsetcomp.com/products/data-loggers/u23-004

World Meteorological Organization. (2018). *Guide to meteorological instruments and methods of observation (WMO-no. 8)* (2018th ed.). World Meteorological Organization. https://library.wmo.int/idurl/4/41650

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Deployment coordinates
F203	HOBO Water Level Loggers	Companion instrument SOP
F510	Weather Monitoring	Full meteorological context
L801	RStudio and GitHub	Data version control

12. Training Record

Before deploying HOBO loggers independently, personnel must:

1. Read this SOP in full
2. Demonstrate logger programming via HOBOconnect or HOBOware
3. Participate in a supervised deployment including shield mounting and metadata recording
4. Demonstrate data download, export, and upload to the repository
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F205

Handheld Turbidity Meter

Standard Operating Procedure

SOP Number	SOP-F205
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the calibration, operation, and maintenance of handheld nephelometric turbidity meters for field measurement of water clarity. Turbidity is a standard water quality parameter required by the Surface Water Ambient Monitoring Program (SWAMP), NPDES permits, and many ecological studies (APHA et al., 2012). Field measurements complement laboratory analysis by providing an immediate on-site result.

2. Scope

- **Applies to:** Handheld nephelometric turbidity meters (e.g., Hach 2100Q, LaMotte 2020we, Oakton T-100) used for in situ and grab-sample turbidity measurements at EA Program field sites
- **Does not apply to:** Laboratory bench-top turbidimeters; Secchi disk transparency measurements (see SOP-F207); continuous turbidity sensors on multiparameter sondes (see SOP-F200)
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve instrument selection; review QC data
Trained Personnel	Calibrate and operate the meter per this SOP
New Students	Complete training before independent use

4. Definitions

- **Formazin:** A synthetic polymer suspension used as the primary turbidity calibration standard; traceable to NIST; typically supplied as a 4000 NTU stock that is diluted to working standards; contains hydrazine and must be handled as hazardous waste (APHA et al., 2012).
- **Nephelometric turbidity:** Turbidity measured by the intensity of light scattered at 90° to an incident beam; the standard method per APHA Method 2130B; appropriate for samples with NTU < 40; for higher values a ratio or attenuation mode may be used (APHA et al., 2012).
- **NTU (Nephelometric Turbidity Units):** The unit of turbidity measured by a 90° nephelometer; numerically equivalent to FNU (Formazin Nephelometric Units) and approximately equivalent to FAU (Formazin Attenuation Units) at low concentrations (APHA et al., 2012).
- **RPD (relative percent difference):** $|A - B| / ((A + B) / 2) \times 100$; used to assess duplicate precision; acceptance criterion for field turbidity duplicates is RPD $< 20\%$ for readings above 10 NTU.
- **Silicone oil treatment:** Application of a thin film of silicone oil to the outside of a glass sample vial before measurement to fill surface scratches and reduce spurious light scattering; standard practice for glass vial meters (Hach Company, 2022).
- **StablCal:** A sealed formazin-based calibration standard supplied by Hach; stable and ready to use without dilution; available in $<$ 0.1, 20, 100, and 800 NTU vials.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Sampling near water involves slip and fall hazards.
- **Biological:** Water samples may contain pathogens. Wear gloves.
- **Chemical:** Formazin calibration standards contain hydrazine, a suspected carcinogen. Handle with gloves; dispose of as hazardous waste. StablCal sealed standards eliminate direct hydrazine exposure.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses (during calibration with bulk formazin)
☐ Closed-toed shoes or wading boots

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. For formazin contact with skin or eyes, flush with water for at least 15 minutes and consult the SDS.

6. Equipment and Materials

Item	Notes
Handheld turbidity meter	Confirm model and battery level
Calibration standards	Hach StablCal sealed vials: < 0.1, 20, 100, 800 NTU; or prepared formazin dilution series
Sample vials (matched to instrument)	Glass or optical-grade; use only with the meter they were supplied with
Silicone oil and applicator cloth	For glass vials (Hach Company, 2022)
Lint-free wipes (Kimwipes)	For cleaning vials
DI water (squeeze bottle)	For rinsing vials and blanks
Waste container (labeled hazardous)	For used formazin waste
Field notebook	Per SOP-F003
Spare batteries	Matched to meter model

7. Procedure

7.1. Calibration

Calibrate at the start of each field day or after storage of more than one week (APHA et al., 2012).

1. Turn on the meter and allow it to warm up (approximately 1 minute).
2. Clean calibration vial exteriors with a lint-free wipe. Apply a thin, even film of silicone oil for glass vials.
3. Insert the lowest standard (< 0.1 NTU) into the sample chamber with the orientation mark aligned. Close the lid.
4. Press **Cal** and follow the on-screen prompts to read the standard.
5. Repeat with each subsequent standard in ascending order (e.g., 20, 100, 800 NTU).
6. When all standards have been read, the meter confirms a successful calibration. Record the date, standard lot number, and any correction factors in the field notebook.
7. Verify calibration by re-reading the 20 NTU standard; the reading must be within ±10% of 20 NTU. If not, repeat the full calibration.

7.2. Sample Collection

1. Collect a grab sample per SOP-F300 Surface Water Sampling.
2. Avoid disturbing bottom sediments during collection.
3. Analyze as soon as possible; if delayed, keep the sample in the dark and analyze within 24 hours.

7.3. Measurement

1. Gently invert the sample container 3–4 times to resuspend particles uniformly. Do not shake vigorously (creates air bubbles).
2. Pour the sample into a clean sample vial to the fill line.
3. Cap and let sit for 1 minute to allow large air bubbles to rise.
4. Wipe the outside of the vial with a lint-free cloth. Apply silicone oil for glass vials.
5. Insert the vial with the orientation mark aligned. Close the lid.
6. Press **Read** and wait for the display to stabilize.
7. Record the result (NTU), sample ID, time, and site in the field notebook.
8. For samples above the meter range (typically > 1000 NTU), dilute with DI water, measure, and multiply by the dilution factor. Record both the diluted reading and the corrected result.
9. Rinse the vial three times with DI water between samples.

7.4. Post-Field Care

1. Rinse all sample vials with DI water; air-dry inverted.
2. Wipe silicone oil residue from vials before storage.
3. Store calibration standards in their original case, away from direct sunlight, at room temperature.
4. Turn off the meter; remove batteries if storing for more than one month.

8. Quality Control

- Calibrate at the start of each field day (§6.1) (APHA et al., 2012).

- Measure a DI water blank at the start and end of each sampling event; the blank must read < 1 NTU.
- Measure one field duplicate per 10 samples (minimum one per event); duplicate RPD must be $< 20\%$ for readings above 10 NTU.
- If the DI blank drifts above 1 NTU during the day, recalibrate and re-measure the most recent samples.
- Inspect sample vials for scratches before each use; scratches scatter light and produce false-high readings.

9. References

- APHA et al. (2012)
- Hach Company (2022)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2022). *2100Q portable turbidimeter: User manual*. https://www.hach.com/2100q-portable-turbidimeter/product?id=7640196322

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F207	Secchi Disk	Alternative transparency measure
F300	Surface Water Sampling	Sample collection procedures
L303	Hach Colorimetric Analysis	Lab-based Hach methods

11. Training Record

Before using turbidity meters independently, personnel must:

1. Read this SOP in full
2. Demonstrate a complete calibration using StablCal or formazin standards
3. Perform supervised field measurements at one site
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F207

Secchi Disk and Secchi Disk Tube

Standard Operating Procedure

SOP Number	SOP-F207
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the use of the standard Secchi disk and the Secchi disk tube (transparency tube) for measuring water transparency in lakes, reservoirs, and streams. Secchi depth is one of the oldest and simplest limnological measurements, providing a rapid estimate of the photic zone depth and an index of suspended particle concentration (Carlson, 1977; Wetzel, 2001).

2. Scope

- **Applies to:** Visual transparency measurements in lakes, ponds, and reservoirs (standard Secchi disk) and in streams, shallow wetlands, and stormwater basins (Secchi tube)
- **Does not apply to:** Quantitative turbidity measurements (see SOP-F205); laboratory transmissometry; PAR light profiles (requires an underwater quantum sensor)
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Specify measurement protocol for the project
Trained Personnel	Perform measurements per this SOP
New Students	Complete training before independent use

4. Definitions

- **Euphotic zone depth (z_{eu}):** The depth receiving at least 1% of surface irradiance; approximately 2–3 times the Secchi depth in most lakes; defines the lower boundary of net photosynthesis (Wetzel, 2001).
- **Photic zone:** The surface layer of a water body receiving sufficient light to support photosynthesis; its depth is inversely related to turbidity and Secchi depth.
- **Secchi depth (z_{SD}):** The depth at which a standard Secchi disk becomes just invisible when lowered, averaged with the depth at which it becomes visible again when raised; $z_{SD} = (D_1 + D_2)/2$; an index of water transparency inversely related to suspended particle concentration and dissolved color (Carlson, 1977).
- **Secchi tube (transparency tube):** A clear acrylic tube, typically 60 or 120 cm long, with a Secchi disk pattern printed at the bottom and a drain valve at the base; filled with sample water and drained until the pattern disappears; used in shallow or turbid waters where a standard disk cannot be deployed vertically.
- **Trophic state index (TSI):** A numerical scale relating Secchi depth, chlorophyll a, and total phosphorus to lake trophic status; Carlson's TSI for Secchi depth: $\mathrm{TSI}(SD) = 60 - 14.41 \ln(z_{SD})$ (Carlson, 1977).
- **Viewscope:** A tube, box, or bucket with a transparent bottom used to eliminate surface glare when reading Secchi depth; improves consistency between observers but must be noted and applied consistently within a dataset.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Secchi disk measurements from boats, docks, or bridges require caution to prevent falls. Wear a personal flotation device (PFD) when sampling from boats.
- **Biological:** Surface water may contain pathogens. Avoid ingestion; wear gloves if hands contact water.
- **Sun glare:** Measuring Secchi depth requires looking directly into the water; use polarized sunglasses or a viewscope to reduce glare (note use in the field notebook for consistency).

5.1. Required PPE

- ☐ Closed-toed shoes or wading boots
- ☐ Personal flotation device (boat sampling)
- ☐ Nitrile gloves (recommended for impaired waters)
- ☐ Sun protection (hat, sunglasses)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. If sampling from a boat, ensure all occupants know the location of PFDs and throwable flotation devices.

6. Equipment and Materials

6.1. Standard Secchi Disk

Item	Notes
Standard Secchi disk (20 cm diameter)	Alternating black and white quadrants; weighted to sink horizontally
Graduated line (marked every 0.1 m)	Non-stretch braided cord or chain
Viewscope (optional)	Eliminates surface glare; a capped PVC section with a clear bottom works well

6.2. Secchi Tube (Transparency Tube)

Item	Notes
Secchi tube (60 or 120 cm)	Clear acrylic tube; Secchi disk pattern at bottom; cm scale on side; drain valve at base
Sample bucket or wide-mouth bottle	For collecting water to fill the tube

6.3. General

Item	Notes
Field notebook	Per SOP-F003
Watch	For recording time of measurement
Camera	For documenting site conditions

7. Procedure

7.1. Standard Secchi Disk (Lakes, Reservoirs, Deep Pools)

Take measurements on the shaded side of the boat or dock, between 10:00 and 14:00 local time for consistency. Avoid measurements during heavy cloud cover, rain, or high wind (Wetzel, 2001).

1. Lower the Secchi disk on the shaded side until it just disappears from view. Record this depth as **D1** (disappearance depth).
2. Slowly raise the disk until it just reappears. Record this depth as **D2** (reappearance depth).
3. Calculate Secchi depth:
$$\text{Secchi depth} = \frac{D_1 + D_2}{2}$$
4. Record in the field notebook:
   - Secchi depth (m) and D1, D2 individually
   - Date and time
   - Sky conditions (clear, partly cloudy, overcast)
   - Wind and water surface conditions
   - Whether a viewscope was used
   - Observer name (readings vary between observers)
5. If the disk is visible on the bottom, record "Secchi depth > [bottom depth]" and note the bottom depth.
6. Repeat the measurement a second time; the two readings should agree within 0.5 m. Record both and report the mean.

7.2. Secchi Tube (Streams, Shallow Waters)

1. Collect a water sample from mid-depth using a bucket or wide-mouth bottle; avoid disturbing sediment.
2. Pour the sample into the Secchi tube until full.
3. Hold the tube vertically and look down through the water column at the Secchi pattern on the bottom.
4. Open the drain valve slowly. Watch the Secchi pattern.
5. When the pattern just disappears, close the valve and read the water level on the graduated scale. Record as **Secchi tube depth** (cm).
6. Refill the tube and repeat; the two readings should agree within 2 cm. Record both and report the mean.
7. Rinse the tube with DI water between sites to prevent cross-contamination.

8. Data Interpretation

Secchi depth is inversely related to turbidity and suspended particle concentration (Carlson, 1977; Wetzel, 2001). Approximate trophic-state guidelines for lakes:

Secchi Depth (m)	Trophic Class
> 5	Oligotrophic
2–5	Mesotrophic
1–2	Eutrophic
< 1	Hypereutrophic

> **Note**
>
> These categories are approximate and vary with dissolved color, algal species, and suspended sediment type. Do not use Secchi depth alone to classify trophic state; combine with nutrient and chlorophyll data (Carlson, 1977).

9. Quality Control

- Use the same observer for repeated measurements at a monitoring site whenever possible; inter-observer variability is the largest source of error (Wetzel, 2001).
- Always take duplicate measurements and report the mean.
- Record consistently whether sunglasses or a viewscope were used.
- Verify Secchi tube graduation markings against a ruler at least once per year.
- When both a Secchi disk and a turbidity meter are available, pair the measurements to develop a site-specific Secchi-turbidity regression.

10. References

- Wetzel (2001)
- Carlson (1977)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Carlson, R. E. (1977). A trophic state index for lakes. *Limnology and Oceanography*, *22*(2), 361–369. https://doi.org/10.4319/lo.1977.22.2.0361

Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems* (3rd ed.). Academic Press.

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F205	Handheld Turbidity Meter	Quantitative turbidity companion
F300	Surface Water Sampling	Sample collection context
L310	Chlorophyll Extraction	Trophic state companion

12. Training Record

Before performing Secchi measurements independently, personnel must:

1. Read this SOP in full
2. Perform supervised duplicate Secchi disk or Secchi tube measurements at one site
3. Demonstrate agreement within the QC criteria (0.5 m for disk, 2 cm for tube)
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F208

Portable Spectrophotometer / Colorimeter (Field Use)

Standard Operating Procedure

SOP Number	SOP-F208
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the field operation of the Hach DR900 portable colorimeter for on-site water quality analysis (Hach Company, 2024a, 2024b). The DR900 enables rapid, field-based measurement of nutrients, metals, and other parameters using Hach pre-packaged reagent kits. Field colorimetry provides same-day results when samples cannot be transported to the lab within required hold times and supports real-time decisions during stormwater events, spill response, or reconnaissance sampling (APHA et al., 2012).

This SOP focuses on field-specific procedures (transport, outdoor operation, field QC). For detailed method chemistry, reagent handling, the Method Registry, and laboratory use of the DR3900 and DR900, see SOP-L303 Hach Colorimetric Analysis.

2. Scope

- **Applies to:** Field use of the Hach DR900 portable colorimeter at EA Program field sites for grab-sample analysis of nutrients (PO_4, NO_3, NH_3), metals (Fe, Mn, Cu), and general chemistry (hardness, chloride, silica, chlorine)
- **Does not apply to:** Laboratory bench-top analysis (see SOP-L303); field turbidity (see SOP-F205); in situ sonde measurements (see SOP-F200)
- **Location:** All EA Program surface water, groundwater, and stormwater field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Select analytes and approve field methods
Trained Personnel	Operate the DR900 per this SOP; record results
New Students	Complete training on both this SOP and SOP-L303 before independent field use

4. Definitions

- **Beer-Lambert law:** The linear relationship between the absorbance of a solution and the concentration of the absorbing analyte, expressed as $A = \varepsilon c l$, where ε is the molar absorptivity, c is concentration, and l is path length; the physical basis of colorimetric analysis (APHA et al., 2012).
- **Blank (reagent blank / DI blank):** A deionized water sample carried through the complete analytical procedure; used to zero the instrument and establish the baseline absorbance of the reagent system; must read at or near zero before sample measurements begin.
- **Detection limit (MDL):** The minimum concentration reliably distinguishable from the blank; Hach method-specific MDLs are listed in the Method Registry in SOP-L303 (Hach Company, 2024b).
- **Field duplicate:** A second independent measurement of the same sample, prepared through the full analytical procedure, to assess precision; RPD must be $< 20\%$ for concentrations above 5 times the detection limit.
- **Reaction time:** The incubation period specified in the Hach method protocol after reagent addition during which the color-forming reaction proceeds to completion; reading before reaction time has elapsed gives systematically low results (Hach Company, 2024a).
- **RPD (relative percent difference):** |A - B| / ((A + B) / 2) × 100; used to assess duplicate precision.
- **Stored program:** A factory-programmed method on the DR900 that specifies wavelength, calibration curve, and units for a specific Hach reagent kit; accessed by program number on the instrument (Hach Company, 2024a).
- **Zeroing:** The process of inserting a DI water blank into the DR900 sample chamber and pressing Zero to set the baseline absorbance to zero for the selected program; required before reading samples.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Hach reagent powder pillows and liquid reagents may contain acids, oxidizers, or heavy metal indicators. Review the SDS for each kit before field use. Cadmium-containing reagents (older NitraVer 5 formulation) are hazardous waste.
- **Physical:** Glass sample vials can break during transport; pack in padded cases.
- **Biological:** Water samples may contain pathogens. Wear gloves.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses (required for all reagent handling)
☐ Closed-toed shoes or wading boots

5.2. Emergency Procedures

For reagent splashes to skin or eyes, flush with clean water for at least 15 minutes. Carry a squeeze bottle of DI water for emergency rinsing. Refer to SOP-F001 Field Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

Item	Notes
Hach DR900 Portable Colorimeter	Charge before field day (Hach Company, 2024a)
Sample vials (10 mL, 25 mL, 1-inch round)	Matched to the methods being run
Hach reagent kits	Check expiration dates; select analytes per project
DI water (squeeze bottle, 500 mL)	For blanks, rinsing, and dilutions
Lint-free wipes (Kimwipes)	For cleaning vial exteriors
Silicone oil and applicator cloth	For 1-inch round vials
Timer	Phone timer or stopwatch for reaction times
Waste container (sealable)	For used reagent vials and waste solution
Padded carrying case	For protecting vials during transport
Field notebook	Per SOP-F003

7. Procedure

7.1. Pre-Field Preparation

1. Identify the analytes to be measured; pack the corresponding reagent kits and verify none are expired.

2. Pack at least 500 mL DI water and two extra clean sample vials per method.
3. Charge the DR900 battery and confirm stored programs for your methods are accessible (Hach Company, 2024a).
4. Print or photograph method procedure cards for each analyte (from the kit box or SOP-L303 Method Registry).

7.2. Field Setup

1. Place the DR900 on a flat, stable surface **out of direct sunlight**. Direct sunlight on the sample chamber causes erroneous readings.
2. If no shade is available, shade the instrument with a jacket or tarp during each reading.
3. Turn on the DR900 and allow it to complete its self-check.
4. Select the stored program for your analyte.

7.3. Blank and Calibration Check

1. Fill a clean sample vial with DI water to the fill line.
2. Wipe the vial exterior with a lint-free cloth.
3. Insert the vial, close the lid, and press **Zero**.
4. The display should read 0.00 (within the method detection limit); if not, clean the vial and re-zero.
5. If a known mid-range standard is available, read it immediately after zeroing; the result must be within ±10% of the stated value. Record the result in the field notebook (Hach Company, 2024b).

7.4. Sample Analysis

Follow the method-specific procedure from the reagent kit or SOP-L303 Method Registry:

1. Collect a grab sample per SOP-F300.
2. Fill the sample vial to the fill line with sample.
3. Add the reagent per method instructions.
4. Cap, invert, and mix as directed. Start the timer for the required reaction time.
5. When the reaction time is complete, wipe the vial exterior (apply silicone oil for round vials).
6. Insert the vial into the DR900, close the lid (shield from sunlight), and press **Read**.
7. Record the result, analyte, Hach method number, sample ID, units, date, and time in the field notebook.
8. Rinse the vial three times with DI water before the next sample.

7.5. Waste Management

1. Collect all used reagent packets, vials, and waste solution in a sealable container.
2. Do not discard reagent waste in the field.
3. Return waste to the lab and dispose of per SOP-L003.

8. Quality Control

- Zero the instrument with DI water before each batch of samples.
- Run one DI blank and one mid-range standard at the start of each field session; record results in the field notebook (Hach Company, 2024b).
- Analyze one field duplicate per 10 samples (minimum one per event); RPD must be $< 20\%$ for concentrations above $5 \times$ the detection limit.
- If the field blank reads above the MDL, investigate contamination (dirty vials, contaminated DI water) and re-run.
- Note ambient temperature; some Hach methods are temperature-sensitive. If the sample is outside the method-specified range (typically 20–25 °C), note the potential bias (Hach Company, 2024a).

9. References

- Hach Company (2024a)
- Hach Company (2024b)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2024a). *DR900 portable colorimeter: Procedures manual*. https://www.hach.com/dr900-portable-colorimeter/product?id=56440352476

Hach Company. (2024b). *Water analysis guide* (7th ed.). https://www.hach.com/wag

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F300	Surface Water Sampling	Sample collection procedures
L003	Hazardous Materials	Reagent waste disposal
L303	Hach Colorimetric Analysis	Full method registry and lab procedures

11. Training Record

Before using the DR900 in the field independently, personnel must:

1. Read this SOP and SOP-L303 in full
2. Demonstrate a complete field analysis (zero, standard check, sample analysis, waste collection) under supervision
3. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F209

Dissolved Oxygen Meter (Field)

Standard Operating Procedure

SOP Number	SOP-F209
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the calibration, field operation, and maintenance of standalone handheld dissolved oxygen (DO) meters for in situ measurement of dissolved oxygen concentration and percent saturation in surface waters. Dissolved oxygen is a fundamental indicator of water quality and

aquatic habitat condition (APHA et al., 2012). This SOP covers both optical (luminescent) and electrochemical (polarographic and galvanic) DO probes operated as standalone instruments, separate from multiparameter sondes.

2. Scope

- **Applies to:** Handheld DO meters (e.g., YSI Pro20i, YSI ProSolo, Hach HQ40d with LDO probe, Extech DO600) used for in situ DO measurements at EA Program surface water field sites
- **Does not apply to:** DO measured by the Eureka Manta2 multiprobe (see SOP-F200); Winkler titration (laboratory method); BOD_5 analysis (see SOP-L306); continuously deployed sondes
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve instrument selection; review QC data
Trained Personnel	Calibrate and operate the meter per this SOP
New Students	Complete training before independent use

4. Definitions

- **Barometric pressure correction:** Adjustment of the 100% saturation calibration point for the ambient atmospheric pressure; oxygen solubility decreases with decreasing pressure (increasing altitude); entered in mmHg or hPa at the time of calibration (APHA et al., 2012).
- **DO percent saturation (%sat):** The measured DO concentration expressed as a percentage of the equilibrium saturation concentration at the same temperature and pressure; 100% indicates equilibrium with the atmosphere; supersaturation ($> 100\%$) indicates net photosynthesis; values well below 100% indicate high oxygen demand or poor reaeration (APHA et al., 2012).
- **Electrochemical (polarographic/galvanic) probe:** A DO sensor in which oxygen diffuses through a gas-permeable membrane and is reduced at the cathode, generating a current proportional to DO concentration; requires periodic membrane and electrolyte replacement and is flow- dependent (needs water movement past the membrane) (APHA et al., 2012).
- **KCl electrolyte:** The potassium chloride solution filling the electrode cavity of polarographic DO probes; must be replaced when the membrane is changed or when readings become erratic.
- **LDO (luminescent dissolved oxygen) probe:** An optical sensor that measures DO by the quenching of luminescence from a ruthenium-dye sensing element by oxygen molecules; requires no electrolyte or membrane, no warm-up time, and no stirring (Hach Company, 2023).

- **Membrane (DO probe):** The gas-permeable Teflon or polyethylene film covering the electrode of an electrochemical DO probe; must be free of bubbles, tears, or debris; replace if damaged or if readings are erratic.
- **Sensing cap:** The replaceable luminescent element on an optical DO probe; replace when the cap expires (typically 1–2 years) or when readings become erratic (YSI Inc., 2022).
- **Winkler titration:** The reference wet-chemical method for DO determination; used to verify DO meter accuracy and required for calibration validation in some regulatory programs (APHA et al., 2012).
- **Zero-oxygen calibration:** A secondary calibration point using an oxygen-free solution (sodium sulfite with cobalt chloride catalyst) to verify the zero response of the DO probe; recommended when expected DO values are below 2 mg/L.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Measuring DO requires placing the probe in or near water. Slip, fall, and drowning hazards apply. Work in pairs at stream sites.
- **Chemical:** Polarographic DO probes use KCl electrolyte solution, a mild irritant. Sodium sulfite (used for zero-oxygen calibration) is a mild irritant and reducing agent.
- **Biological:** Water may contain pathogens. Avoid ingestion; wash hands after field work.

5.1. Required PPE

☐ Closed-toed shoes or wading boots
☐ Nitrile gloves (especially for impaired waters)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Instrument Types

6.1. Optical (Luminescent) DO Probes

Optical probes (e.g., Hach HQ40d with LDO101 probe, YSI ProSolo ODO) measure DO by detecting luminescence quenching. They require no electrolyte, no warm-up time, no stirring, and no membrane replacement (Hach Company, 2023).

Advantage	Limitation
No membrane or electrolyte	Higher initial cost

Advantage	Limitation
No stirring required Fast stabilization Lower maintenance	Sensing cap has finite lifespan (1–2 yr)

6.2. Electrochemical (Polarographic / Galvanic) DO Probes

Electrochemical probes (e.g., YSI Pro20i) measure DO through an oxygen-permeable membrane (YSI Inc., 2022).

Advantage	Limitation
Lower initial cost	Requires membrane and electrolyte replacement
Well-established method	Needs 5–15 min warm-up
Widely available	Flow-dependent (stirring or moving water needed)

7. Equipment and Materials

Item	Notes
Handheld DO meter with probe	Fully charged; confirm calibration supplies
Water-saturated air calibration chamber	Wet sponge in probe storage cap
Barometric pressure reference	Built into some meters; otherwise Kestrel or phone app
Spare membranes and electrolyte	For electrochemical probes
Spare sensing cap	For optical probes; replace when expired or erratic
Thermometer (for verification)	Most DO probes include temperature sensor
DI water (squeeze bottle)	For rinsing the probe
Lint-free wipes	For drying the probe before calibration
Field notebook	Per SOP-F003
Spare batteries	Matched to meter model

8. Procedure

8.1. Calibration

Calibrate the DO meter before each field day using the **water-saturated air** method.

Water-saturated air calibration:

1. Ensure the calibration chamber (probe storage cap with damp sponge) is moist but not dripping; the probe tip must not touch the sponge.

2. Turn on the meter. Allow electrochemical probes to warm up for 5–15 minutes (see instrument manual) (YSI Inc., 2022).
3. Enter the current barometric pressure (mmHg or hPa). Verify the displayed value against a Kestrel or weather station.
4. Place the probe in the calibration chamber; wait for the reading to stabilize (1–3 min for optical; 5–10 min for electrochemical).
5. Press **Cal** per the instrument prompts; the meter sets 100% saturation at the current temperature and pressure.
6. Record in the field notebook: calibration date, time, barometric pressure, temperature, and whether calibration was accepted.

Zero-oxygen calibration (optional – low-DO environments):

1. Dissolve excess sodium sulfite in DI water with a pinch of cobalt chloride catalyst.
2. Immerse the probe and wait for the reading to stabilize.
3. Calibrate the zero point per meter instructions.

8.2. Field Measurement

1. Immerse the probe to mid-depth at the sampling location. For electrochemical probes, maintain a minimum flow of 0.3 m/s past the membrane (gently move the probe in still water). Optical probes do not require flow (Hach Company, 2023).
2. Wait for the reading to stabilize.
3. Record in the field notebook:
   - DO concentration (mg/L) and percent saturation (%)
   - Water temperature (°C) as reported by the probe
   - Date, time, sample ID, and measurement depth
   - Flow conditions (riffle, pool, still water with manual stirring)
4. For depth profiles, lower the probe in 0.5 or 1.0 m increments and allow stabilization at each depth.

8.3. Post-Field Care

1. Rinse the probe with DI water after each use.
2. For electrochemical probes, check the membrane for tears, bubbles, or deposits; replace membrane and electrolyte if damaged (YSI Inc., 2022).
3. For optical probes, gently wipe the sensing cap with a soft, damp cloth if biofouling is visible; do not use solvents.
4. Store the probe in the calibration chamber with a moist sponge (not submerged).
5. Turn off the meter; remove batteries if storing for more than one month.

9. Data Interpretation

DO concentrations are influenced by temperature, atmospheric pressure, salinity, photosynthesis, respiration, and reaeration (APHA et al., 2012).

DO (mg/L)	Condition
> 8	Well-oxygenated; suitable for most aquatic life
5–8	Adequate for many species; marginal for cold-water fish
2–5	Stressful for most fish; hypoxic conditions
< 2	Severe hypoxia; lethal to most aquatic organisms

10. Quality Control

- Calibrate at the start of each field day.
- At the end of the field day, check calibration by returning the probe to the water-saturated air chamber; reading must be within ±2% of 100% saturation. If drift exceeds 5%, note it and recalibrate.
- When possible, verify against a second DO meter or the Eureka Manta2; readings should agree within ±0.5 mg/L.
- For long field days (> 6 hours), perform a mid-day calibration check.
- Record all QC results in the field notebook (APHA et al., 2012).

11. References

- APHA et al. (2012)
- Hach Company (2023)
- YSI Inc. (2022)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2023). *HQ40d portable multi-parameter meter: User manual*. https://www.hach.com/hq40d-portable-multi-parameter-meter/product?id=7640196365

YSI Inc. (2022). *Pro20i portable dissolved oxygen instrument: User manual*. https://www.ysi.com/pro20i

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F200	Eureka Manta2 Multiprobe	Multiprobe includes DO sensor
F300	Surface Water Sampling	Sampling context
F304	Stream Physical Habitat	Habitat characterization

13. Training Record

Before using DO meters independently, personnel must:

1. Read this SOP in full
2. Demonstrate a complete water-saturated air calibration
3. Perform supervised DO measurements at one field site
4. Demonstrate membrane inspection and replacement (electrochemical probes) or sensing cap inspection (optical probes)
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F300

Surface Water Sampling

Standard Operating Procedure

SOP Number	SOP-F300
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for the manual collection, preservation, and handling of surface water samples. Representative samples are required for defensible water quality data (APHA et al., 2012; US Environmental Protection Agency, Region 4, 2014).

2. Scope

- **Applies to:** Manual collection of surface water samples from streams, rivers, lakes, ponds, lagoons, surface impoundments, wastewater access points, and outfalls
- **Does not apply to:** Automated sampling devices, pelagic marine sampling, or groundwater sampling
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate logistics, verify equipment and supplies
Trained Personnel	Collect and handle samples per this SOP
New Students	Complete training before independent sampling

3.1. Required Training

- SOP-F001 Field Safety
- SOP-L003 Hazardous Materials Handling (for sample preservation chemicals)

4. Definitions

- **Bailer:** A cylindrical sampling device lowered on a line to collect water at a specific depth; commonly used in wells and deep water; can be disposable (polyethylene) or reusable (stainless steel).
- **Bottle blank:** A sample container filled with laboratory-pure water, preserved identically to field samples, and submitted to the laboratory as a QC sample to detect contamination from the container or washing process (APHA et al., 2012).

- **Chain of custody (COC):** A legal record of sample possession at every transfer point from collection to analysis; must accompany all samples; see SOP-F008 for the full procedure (US Environmental Protection Agency, Region 4, 2014).
- **Discrete-depth sampler:** A device (Van Dorn, Kemmerer bottle) that collects a water sample from a specific depth without mixing with overlying or underlying water; tripped by a messenger weight.
- **Equipment blank:** Laboratory-pure water poured through or into sampling equipment in the field, collected and analyzed as a QC sample to verify equipment cleanliness.
- **Field duplicate:** A second sample collected at the same time and location as a primary sample and submitted blind to the laboratory; used to assess field and laboratory precision.
- **Grab sample:** A single-point, instantaneous water sample collected at one time and place; represents conditions at that moment only; the simplest and most common field collection method (APHA et al., 2012).
- **Holding time:** The maximum elapsed time between sample collection and analysis within which the sample remains analytically valid; analyte- and preservative-specific (e.g., 6 h for E. coli, 28 d for nitrate preserved with H_2SO_4) (APHA et al., 2012).
- **Peristaltic pump:** A battery-operated field sampling pump that moves sample water through disposable tubing by compressing the tube with rollers; used to collect samples from depths or locations inaccessible by hand.
- **Preservative:** A chemical added to a sample container to retard biological or chemical degradation of target analytes during transport and storage (e.g., H_2SO_4 for nutrients, HNO_3 for metals, $Na_2S_2O_3$ for chlorine) (APHA et al., 2012).
- **Sampler blank:** Laboratory-pure water passed through or into a sampling device (bailer, peristaltic pump) and submitted as a QC sample to verify the device is clean.
- **Zero headspace:** A sample container filled completely with no air gap; required for volatile organic compound (VOC) samples to prevent analyte loss to the headspace.

5. Safety and Hazards

⚠ Hazards

- **Biological/Chemical:** Water bodies may be contaminated; avoid ingestion and direct skin contact.
- **Physical:** Drowning risk, slippery surfaces, swift currents, unstable banks, and bridge traffic.
- **Chemical:** Sample preservatives (H_2SO_4, HNO_3, NaOH) in pre-preserved bottles are corrosive.

5.1. Required PPE

☐ Latex or nitrile gloves
☐ Leather gloves (for handling ropes and cables)
☐ Antibacterial hand sanitizer or soap
☐ Personal flotation device (PFD) when sampling from boats or in deep/swift water
☐ Safety vest and lines (as appropriate for the site)
☐ Traffic control signs and cones (for bridge sampling)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety for emergency response procedures, including medical emergencies near water.

6. Summary of Collection Methods

Sampling situations vary widely and no universal procedure applies. Representative samples from most surface waters can be collected by one or more of the following:

- Bailers
- Dip samplers
- Direct method (hand collection)
- Discrete depth samplers (Kemmerer or Van Dorn bottles)
- Peristaltic pumps
- Stormwater collection devices

(APHA et al., 2012; US Environmental Protection Agency, Region 4, 2014)

7. Required Materials

7.1. Field Documentation

- Field notebook and pens (per SOP-F003)
- Chain of custody forms (per SOP-F008)
- Sample tags with assigned sample numbers
- Field data sheets

7.2. Sampling Equipment

Item	Notes
500 or 1000 mL sampling bottles	Polypropylene or glass, as required by the analyte
Syringe filters (0.45 µm)	For dissolved analyte samples
Stainless steel or Teflon dipper	For surface grab samples
Depth-integrated sampler	For vertically integrated samples
Van Dorn or Kemmerer sampler	For discrete depth samples
Extension pole with container holder	For sampling from piers, docks, or banks
Deionized / ultrapure distilled water	For blanks and equipment rinsing
Decontamination supplies	To prevent invasive species transfer

7.3. Transport and Storage

Item	Notes
Coolers with ice	Pre-chilled to 4 °C
Pre-labeled sample containers	From the analytical lab, with preservatives
Custody seals	For sealing coolers before shipment

8. Interferences and Contamination Prevention

> **!** Critical
>
> The inner surfaces of sample bottles and caps must not be touched with bare hands, gloves, or any other material. Keep sample bottles in a clean environment away from dust, dirt, fumes, and vehicle exhaust (APHA et al., 2012).

- Collect samples upstream of your position and upstream of any sampling-related disturbance.
- Sample away from areas influenced by tributaries, outfalls, or other point sources unless those features are the target.
- Change gloves between sample sites to prevent cross-contamination.

9. Procedure

9.1. Field Collection – Direct Method (Grab Sample)

1. Remove the stopper or lid from the container just before sampling.
2. Do not contaminate the cap, neck, or inside of the bottle.
3. **If no preservative is present:** Face upstream in flowing water (or upwind in still water). Hold the container near its base, reach out in front of your body, and plunge it mouth-down below the surface to approximately mid-water column.
4. If the water is too shallow for this technique (risk of disturbing sediment), collect a surface water sample and note the change of method in the field notebook.
5. Fill the bottle to the appropriate level for the target analyte.
6. Leave a small headspace for mixing, **except** for volatile organics and alkalinity samples (no headspace).

9.2. Field Collection – Extension Pole

1. Securely attach the sample container (lid in place) to the pole holder using clamps.
2. Remove the lid carefully to avoid contamination.
3. Follow the direct method procedure above.
4. Do not use this method for pre-preserved containers; use intermediate devices instead.

9.3. Field Collection – Pre-Preserved Containers

1. Hold the container upright.
2. Place the lid over the mouth to create a small opening.
3. Submerge and fill carefully to avoid spilling or diluting the preservative.

9.4. Field Collection – Intermediate Devices

For samples collected via Van Dorn, Kemmerer, bailer, or peristaltic pump, follow the device operating instructions (see SOP-F202 for pump procedures). Transfer collected water to appropriate labeled sample containers promptly (US Environmental Protection Agency, Region 4, 2014).

Sampler Blanks

Prepare periodic sampler blanks by pouring ultrapure distilled water through or into the sampler and submitting for laboratory analysis to verify cleanliness (APHA et al., 2012).

10. Sample Preservation, Handling, and Storage

Once samples are collected:

1. Transfer samples to correctly labeled containers.
2. Preserve immediately, or verify that pre-preserved bottles were used (see holding-time requirements, Table 9.1 in APHA et al. (2012)).
3. Record all pertinent data in the field notebook and on the COC form.
4. Attach custody seals to the cooler before transport.
5. Decontaminate all sampling equipment before collecting additional samples (see SOP-F007).

Important

Samples must never stand in the sun. Store on ice in coolers immediately after collection and ship to the laboratory without delay (US Environmental Protection Agency, Region 4, 2014).

11. Quality Control

11.1. Bottle Blanks

Before each field trip, select one sample bottle per ten of each container type, fill with ultrapure distilled water, preserve identically to field samples, and submit for analysis as a “bottle blank” (APHA et al., 2012).

11.2. Field Duplicates

Collect one field duplicate per 10 samples (minimum one per event). Duplicates are collected from the same location at the same time and submitted blind to the laboratory.

11.3. Equipment Blanks

When non-dedicated sampling equipment is used, collect an equipment blank (ultrapure distilled water through the equipment) and submit for analysis.

11.4. Documentation

- Complete the COC form before samples leave the field.
- Record all deviations from this SOP in the field notebook.
- Photograph site conditions at each sampling location.

12. References

- APHA et al. (2012)
- US Environmental Protection Agency, Region 4 (2014)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

US Environmental Protection Agency, Region 4. (2014). *Operating procedure for sediment sampling* (SESDPROC-200-R4). Science; Ecosystem Support Division, US EPA. https://www.epa.gov/quality/sediment-sampling

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Equipment cleaning between sites
F008	Sample Labeling and COC	Chain of custody
F205	Handheld Turbidity Meter	Field turbidity
F208	Portable Colorimeter	Field nutrients/metals
F209	Dissolved Oxygen Meter	Field DO measurement

14. Training Record

Before collecting surface water samples independently, personnel must:

1. Read this SOP, SOP-F001, and SOP-L003 in full
2. Demonstrate correct grab sampling technique (contamination prevention, bottle handling)
3. Demonstrate proper use of pre-preserved containers and at least one intermediate device (bailer, Van Dorn, or pump)
4. Complete at least one supervised sampling event including COC completion and sample cooler packaging
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F301

Stream Discharge Measurement – Wadable Streams

Standard Operating Procedure

SOP Number	SOP-F301
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the procedure for measuring stream discharge (volumetric flow rate) in wadable streams using the USGS midsection velocity-area method with Price Pygmy or Price Type AA mechanical current meters. Discharge data are fundamental to characterizing watershed hydrology, calculating pollutant loads, and supporting water quality assessments (Rantz et al., 1982; Turnipseed & Sauer, 2010).

2. Scope

- **Applies to:** Wadable streams and channels where personnel can safely stand in the flow (generally depths < 3–4 ft and velocities < approximately 4 ft/s)
- **Does not apply to:** Non-wadable rivers requiring bridge, cableway, or boat measurements; acoustic Doppler instruments (see SOP-F202); or flume and weir installations
- **Location:** All EA Program stream monitoring sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve measurement sites and protocols
Field Lead	Select cross-section locations, verify equipment
Trained Personnel	Perform measurements and record data per this SOP
New Students	Complete training before making independent measurements

4. Definitions

- **AquaCalc:** A digital flow computer that counts current meter revolutions and computes velocity automatically; eliminates manual timing with a headset and stopwatch and reduces transcription errors.
- **Cross-section:** The plane perpendicular to the direction of flow at which discharge is measured; ideally straight, uniform, and free of eddies or dead zones (Rantz et al., 1982).
- **Discharge (Q):** The volumetric flow rate of a stream, expressed in cubic feet per second (cfs) or liters per second (L/s); computed as the sum of the products of water velocity, depth, and width across all subsections of the cross-section (Rantz et al., 1982).
- **Midsection method:** The standard USGS computational approach in which the discharge of each subsection is calculated as the product of the measured velocity, the depth at that vertical, and the horizontal distance to the midpoints on either side of the station (Rantz et al., 1982; Turnipseed & Sauer, 2010).

- **Price Pygmy meter:** A small-cup mechanical current meter (5 cm diameter) used in shallow water (< 1.5 ft depth) or when velocities are low; 1 revolution = 1 click per second at approximately 0.1 ft/s (Rantz et al., 1982).
- **Price Type AA meter:** The standard USGS mechanical cup-type current meter (12.5 cm diameter) used in deeper flows; more accurate than the Pygmy for velocities above approximately 0.2 ft/s (Rantz et al., 1982).
- **Six-tenths-depth method:** Placement of the current meter at 0.6 of the total depth below the water surface; yields the mean vertical velocity in most natural channels; the standard method for shallow profiles (depth 2.5 ft) (Rantz et al., 1982).
- **Spin test:** A quality control check in which the current meter bucket wheel is spun by hand and the time for it to stop is measured; the wheel should spin freely for a minimum number of seconds (see SOP-F201); indicates bearing condition and mechanical integrity.
- **Tag line:** A measuring tape stretched taut across the stream perpendicular to the flow; anchored at both banks; used to measure horizontal distances to each measurement station (Rantz et al., 1982).
- **Thalweg:** The line of deepest flow along the channel axis; usually the zone of highest velocity and the target for closely spaced measurement stations.
- **Two-point method:** Placement of the current meter at 0.2 and 0.8 of the total depth; the arithmetic mean of the two velocities gives the mean vertical velocity; preferred when depth > 2.5 ft (Rantz et al., 1982).
- **Wetted width:** The horizontal distance across the stream from water's edge to water's edge at the measurement cross-section.

5. Safety and Hazards

⚠ Hazards

- **Drowning and swift water:** Do not wade in flows deeper than approximately waist height or where you feel unstable. Use a wading rod as a support. Always face upstream while wading.
- **Slippery substrates:** Cobble, gravel, and algae-covered rocks are extremely slippery. Wear felt-soled or studded wading boots.
- **Hypothermia:** Prolonged immersion in cold water can cause hypothermia. Wear insulated waders in cold conditions.
- **Biological:** Waterborne pathogens may be present. Avoid swallowing water. Wash hands and equipment after each site visit.

5.1. Required PPE

☐ Chest or hip waders (neoprene or breathable)
☐ Wading boots with felt or studded soles
☐ PFD if flow conditions are marginal
☐ Sun protection (hat, sunscreen)
☐ Nitrile gloves (optional, for concurrent water quality sampling)

5.2. Emergency Procedures

If a team member loses footing, release the wading rod and equipment and focus on self-rescue by floating feet-first downstream. Never attempt a discharge measurement alone; a minimum of two people must be present. Refer to SOP-F001 Field Safety.

6. Equipment and Materials

Item	Notes
Price Pygmy or Type AA current meter	See SOP-F201
Top-setting wading rod	Graduated in tenths of a foot
AquaCalc digital flow computer or headset and stopwatch	For counting revolutions
Measuring tape (50 m / 150 ft)	Fiberglass; for establishing the tag line
Tape stakes or rebar pins	Two; for anchoring the tag line
Mallet	For driving stakes
Field data sheet (Appendix A)	Printed on waterproof paper
Clipboard and pencils	
GPS unit or smartphone	For recording cross-section coordinates
Thermometer	For recording water temperature
Camera	For photo-documenting the cross-section

7. Estimated Time

Task	Time Required
Site selection and tag line setup	10–20 minutes
Spin test	5 minutes
Discharge measurement (20–30 verticals)	30–60 minutes
Data recording and site breakdown	10–15 minutes

8. Procedure

8.1. Site Selection

Select a measurement cross-section with as many of the following characteristics as possible (Rantz et al., 1982):

1. Straight channel reach for at least 3–5 channel widths upstream and downstream
2. Uniform flow – no eddies, backwater, or dead zones
3. Stable streambed (no excessive soft sediment or mobile cobbles)
4. Flow perpendicular to the tag line

5. Avoid locations immediately downstream of bridges, confluences, debris jams, or sharp bends
6. Depth sufficient to submerge the current meter cups; shallow enough to wade safely

> **Tip**
>
> Ideal cross-sections are trapezoidal with gradually varying depth. Avoid abrupt depth changes, undercut banks, and boulders that create turbulence (Rantz et al., 1982).

8.2. Establishing the Tag Line

1. Stretch the measuring tape across the stream perpendicular to the flow direction.
2. Anchor each end with a tape stake driven into the bank.
3. Pull the tape taut so it does not sag into the water.
4. Record the distance at the left water's edge (LEW) and the right water's edge (REW), looking downstream. All station distances are measured from the same reference bank (typically the left bank, looking downstream).

8.3. Determining Station Spacing

Divide the wetted width into subsections. The goal is 20–30 subsections; no single subsection should carry more than 5–10% of total estimated discharge (Rantz et al., 1982).

Wetted Width	Recommended Station Spacing
$<$ 5 ft (1.5 m)	0.5 ft (0.15 m)
5–15 ft (1.5–4.5 m)	1.0 ft (0.3 m)
$>$ 15 ft (4.5 m)	Divide into 20–30 equal intervals

Space stations more closely near the thalweg where depth or velocity changes rapidly.

8.4. Assembling and Testing the Meter

1. Mount the current meter on the wading rod per SOP-F201, Section 7.1.
2. Connect the cable to the AquaCalc or headset.
3. Perform a spin test and record the result on the data sheet.

8.5. Making the Measurement

Work from one bank to the other. At each station:

1. **Position yourself downstream** of the wading rod; your body must not disturb the flow at the measurement point.
2. Record the **station distance** from the tag line.
3. Measure and record the **water depth** to the nearest 0.01 ft.

8.5.1. Setting the Meter Depth

Method	Meter Position	When to Use
Six-tenths	0.6 × depth from the surface	Depth 2.5 ft or Pygmy
Two-point	0.2 and 0.8 × depth (average the two)	Depth > 2.5 ft
Surface	0.2 × depth; apply coefficient 0.85–0.90	Very shallow or weedy

(Rantz et al., 1982; Turnipseed & Sauer, 2010)

4. **Count revolutions** for a minimum of 40 seconds (up to 70 s at low or unsteady velocities). Record revolutions and elapsed time (or velocity if using the AquaCalc).
5. Move to the next station and repeat.

> **!** Important
>
> When counting revolutions manually, look up velocity in the rating table for your meter model (see SOP-F201, Section 9.3).

8.6. Edge Measurements

At the left and right water's edges, record depth = 0 and velocity = 0. These close the cross-section.

8.7. Post-Measurement Spin Test

Repeat the spin test after completing the measurement. A reduced spin time indicates potential bearing damage; the measurement quality is suspect (Rantz et al., 1982).

9. Discharge Calculation – Midsection Method

Total discharge is the sum of discharge through each subsection (Rantz et al., 1982; Turnipseed & Sauer, 2010):

$$Q = \sum_{i=1}^{n} V_i \cdot d_i \cdot w_i$$

where for station i:

- V_i = mean vertical velocity (ft/s or m/s)
- d_i = water depth (ft or m)
- w_i = width of the subsection = distance from the midpoint to the adjacent station on each side (ft or m)

Report discharge in cfs or L/s, rounded to three significant figures following USGS conventions (Turnipseed & Sauer, 2010):

Discharge Range	Rounding
< 1 cfs	Nearest 0.01 cfs
1–10 cfs	Nearest 0.1 cfs
10–100 cfs	Nearest 1 cfs

10. Data Processing

1. Enter field data into the lab's standard discharge spreadsheet template on the same day as the measurement.
2. Verify the total discharge calculation matches the field computation.
3. Flag any station carrying more than 10% of total discharge in a single subsection – this indicates station spacing was too wide.
4. Archive the original field data sheet (scan if paper).

11. Quality Control

- No single subsection should carry more than 5–10% of total discharge (Rantz et al., 1982).
- Pre- and post-measurement spin tests must meet minimum thresholds (see SOP-F201).
- If two measurements are made at the same cross-section, they should agree within 5%; if they differ by more than 5%, make a third measurement and use the mean of the two closest values (Turnipseed & Sauer, 2010).
- **Oblique flow:** If current is not perpendicular to the tag line, velocity readings underestimate true velocity. Reposition or apply a cosine correction.
- **Wading disturbance:** Stand downstream of the meter; avoid shuffling feet near the measurement point.
- **Unsteady flow:** In flashy streams, stage may change during measurement. Record the time at each station.

12. References

- Rantz et al. (1982)
- Turnipseed & Sauer (2010)

Rantz, S. E. et al. (1982). *Measurement and computation of streamflow: Volume 1. Measurement of stage and discharge* (Water-Supply Paper 2175). U.S. Geological Survey. https://pubs.usgs.gov/wsp/wsp2175/

Turnipseed, D. P., & Sauer, V. B. (2010). *Discharge measurements at gaging stations* (Techniques and Methods 3-A8). U.S. Geological Survey. https://doi.org/10.3133/tm3A8

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Equipment cleaning between sites
F201	Pygmy and Type AA Velocity Meters	Meter selection and operation
F202	SonTek Acoustic Velocity Meters	Acoustic alternative
F300	Surface Water Sampling	Water quality sampling at discharge stations

14. Training Record

Before making discharge measurements independently, personnel must:

1. Read this SOP and SOP-F201 in full
2. Demonstrate safe wading technique and proper use of the wading rod
3. Successfully set up a tag line and conduct a pre-measurement spin test
4. Measure a discharge under supervision (minimum one complete cross-section)
5. Demonstrate correct midsection calculation
6. Be signed off by the PI or field lead

15. Appendix A: Field Data Sheet

Print on waterproof paper (Rite in the Rain). One sheet per measurement.

Site: _____ **Date:** _____ **Time:** _____

Operator(s): _____ **Meter type:** _____ **Meter #:** _____

Pre-measurement spin time: _____ s **Post-measurement spin time:** _____ s

Water temperature: _____ °C **Weather:** _____

Station (ft)	Depth (ft)	Meter depth setting (ft)	Revolutions	Time (s)	Velocity (ft/s)	Width (ft)	Q_sub (cfs)

Station (ft)	Depth (ft)	Meter depth setting (ft)	Revolutions	Time (s)	Velocity (ft/s)	Width (ft)	Q_sub (cfs)

Total Q: _____ cfs **Notes:** _____

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F302

Stormwater Sampling

Standard Operating Procedure

SOP Number	SOP-F302
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1. Purpose

This SOP describes procedures for collecting stormwater runoff samples from urban and peri-urban watersheds during wet-weather events. Storm runoff is a primary pathway by which sediments, nutrients, metals, bacteria, and other pollutants enter receiving waters. Characterizing stormwater quality is essential for assessing nonpoint source pollution, evaluating the effectiveness of best management practices (BMPs), and supporting compliance with National Pollutant Discharge Elimination System (NPDES) Municipal Separate Storm Sewer System (MS4) permit requirements (APHA et al., 2012; California State Water Resources Control Board, 2018).

The procedures described here are consistent with the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP) guidance and the State Water Board's Stormwater Sampling Guidance Document for construction and industrial general permits.

2. Scope

- **Applies to:** Manual grab and time-composite sampling of stormwater runoff from outfalls, storm drains, open channels, sheet flow, and receiving streams during and immediately following precipitation events
- **Does not apply to:** Dry-weather urban runoff (use SOP-F300 Surface Water Sampling); automated composite sampling with programmed autosamplers (requires a separate instrument-specific SOP); industrial facility compliance sampling governed by site-specific SWPPPs
- **Location:** All EA Program stormwater monitoring sites in the greater Los Angeles basin and Inland Empire watersheds

3. Responsibilities

Role	Responsibility
PI / Project Lead	Approve sampling plan and storm-event criteria
Field Lead	Monitor weather forecasts, mobilize crew, verify equipment
Trained Personnel	Collect and handle samples per this SOP
New Students	Complete training before participating in storm sampling

4. Definitions

- **Antecedent dry period:** The number of consecutive dry days before a storm event; typically a minimum of 72 hours is required for a storm to qualify as an independent event that will produce a representative first flush; shorter antecedent periods may indicate ongoing, non-independent runoff (California State Water Resources Control Board, 2018).
- **BMP (best management practice):** A structural or operational practice designed to reduce the volume or concentration of pollutants in stormwater runoff; includes detention basins, bioswales, permeable pavement, inlet filters, and street sweeping.

- **Event mean concentration (EMC):** The flow-weighted average concentration of an analyte across the entire storm event; calculated as total analyte load divided by total event runoff volume; the standard metric for characterizing stormwater quality and comparing across sites and storms (U.S. Environmental Protection Agency, 2002).
- **First flush:** The initial pulse of stormwater runoff, which typically carries a disproportionately high load of accumulated pollutants relative to later portions of the storm; occurs most prominently in highly impervious urban catchments.
- **Flow-weighted composite sample:** A sample assembled by combining aliquots that are proportional to instantaneous flow rate at each collection interval; produces a sample whose concentration approximates the event mean concentration (California State Water Resources Control Board, 2018).
- **MS4 (Municipal Separate Storm Sewer System):** A conveyance system owned and operated by a municipality that is designed to collect and convey stormwater separately from sanitary sewage; regulated under NPDES permits.
- **NPDES (National Pollutant Discharge Elimination System):** The federal permit program under the Clean Water Act that regulates point-source discharges of pollutants, including stormwater from MS4s, construction sites, and industrial facilities.
- **Outfall:** A pipe, channel, or other structure through which stormwater is discharged to a receiving water body; a standard sampling location for MS4 compliance monitoring.
- **SWPPP (Stormwater Pollution Prevention Plan):** A site-specific plan required for construction sites and certain industrial facilities under NPDES permits; documents BMPs, monitoring requirements, and inspection schedules.
- **Time-composite sample:** A sample assembled by combining equal aliquots collected at fixed time intervals throughout the storm event; simpler than flow-weighted compositing but does not account for flow variability.

5. Safety and Hazards

⚠ Hazards

- **Drowning and flash flooding:** Stormwater channels and storm drains can fill rapidly during precipitation. Water levels can rise without warning. Never enter a storm drain, culvert, or engineered channel during active flow.
- **Slippery surfaces:** Wet concrete, asphalt, and channel banks are extremely slippery.
- **Electrical hazard:** Avoid sampling near downed power lines during storms.
- **Traffic:** Storm sampling often occurs at night or in low visibility. Wear high-visibility vests and use traffic cones. Park away from traffic lanes.
- **Establish an escape route** before approaching any sampling location.
- **Monitor upstream conditions.** If water is rising, retreat immediately.
- If lightning is observed, cease sampling and move to a vehicle or enclosed structure. Wait 30 minutes after the last observed lightning before resuming.

5.1. Emergency Procedures

Refer to SOP-F001 Field Safety for general emergency response. If a team member is swept into a channel, call 911 immediately. Do not attempt an in-water rescue unless you have swift-water rescue training and equipment.

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Pre-labeled sample bottles	Type and preservative per analyte (see §9)
Polypropylene grab-sample bottles (1 L)	For general chemistry and nutrients
Sterile 100 mL Colilert bottles	With sodium thiosulfate for bacteria
Extension pole with bottle clamp	For sampling from bridges, banks, or above outfalls
Stainless steel or HDPE bucket	For flow-weighted composites
Graduated cylinder or measuring cup	For volumetric aliquots into composites
Deionized water	For equipment blanks and rinsing
Syringe and 0.45 µm filters	For dissolved analyte samples

6.2. Field Documentation and Transport

Item	Notes
Field data sheets (waterproof paper)	Record time, sample ID, conditions
Chain of custody (COC) forms	Required for lab submission
Camera (waterproof)	Photo-document site conditions
Rain gauge (manual or tipping bucket)	For recording on-site precipitation
Coolers with ice	Maintain samples at 4 °C during transport
Custody seals	For sealing coolers before shipment

7. Storm Event Criteria

7.1. Qualifying Storm Events

A qualifying storm event must meet all of the following criteria unless the project sampling plan specifies otherwise (California State Water Resources Control Board, 2018):

- **Antecedent dry period:** At least 72 hours (3 days) of dry weather preceding the storm
- **Minimum rainfall:** At least 2.5 mm (0.1 inches) of total precipitation predicted or observed
- **Duration:** Sufficient to produce runoff at the sampling location

7.2. Storm Tracking and Mobilization

1. Monitor the 3–7 day National Weather Service (NWS) forecast and local radar (e.g., Weather.gov, NWS Doppler) during the October– March wet season.
2. When a qualifying storm is forecast, notify the team at least 24 hours in advance if possible.
3. Pre-position coolers, ice, and sampling supplies in vehicles. Confirm all sample bottles are correct and labeled.
4. Confirm sampling site access (locked gates, private property) before departure.

8. Sample Collection Procedures

8.1. Sampling Time

Attempt to collect at least three samples per event when possible (APHA et al., 2012; California State Water Resources Control Board, 2018):

1. **First flush:** As early as practical after runoff reaches the sampling point; ideally within 30 minutes of runoff initiation
2. **Mid-storm:** Approximately at peak flow
3. **Recession:** During the falling limb, at least 2 hours after peak flow

Record the time, rainfall amount (if a gauge is present), and an estimate of flow conditions (trickle, moderate, high) at each sampling time.

8.2. Outfall and Stormwater Channel Sampling

1. Approach the outfall or channel sampling point from upstream or from above. Never stand downstream of an active outfall.
2. Using the extension pole, position the bottle mouth facing upstream at mid-depth (or as close to mid-depth as safely possible).
3. Fill to the appropriate volume for the analytes being collected.
4. Cap immediately, label, and place in the cooler with ice.

8.3. Sheet Flow Sampling

1. Identify a low point or convergence zone where sheet flow concentrates.
2. Place a clean stainless steel or HDPE tray or shallow pan at ground level to intercept the flow.
3. Transfer collected water to sample bottles using a clean syringe or by pouring directly.
4. Record the contributing area and surface type (impervious, pervious, mixed).

8.4. Time-Composite Sampling

1. At each time interval (e.g., every 30 minutes), collect a grab sample per §6.1 or §6.2.

2. Measure a fixed aliquot volume (e.g., 50–100 mL) using a graduated cylinder.
3. Add the aliquot to the composite bottle. Mix gently.
4. Record the time, flow estimate, and aliquot volume in the field notebook.
5. At the end of the event, transfer the composite to appropriately preserved bottles for each analyte.

9. Sample Preservation and Holding Times

Follow SOP-F300 Surface Water Sampling §9 for general preservation (APHA et al., 2012). Common stormwater analytes:

Analyte	Container	Preservative	Holding Time
pH	Poly, 250 mL	None; analyze in field	15 minutes
Conductivity	Poly, 250 mL	Refrigerate 4 °C	28 days
TSS / SSC	Poly, 1 L	Refrigerate 4 °C	7 days
Turbidity	Poly, 250 mL	Refrigerate 4 °C	48 hours
Nitrate + nitrite	Poly, 250 mL	H_2SO_4 to pH $<$ 2; 4 °C	28 days
Ammonia	Poly, 250 mL	H_2SO_4 to pH $<$ 2; 4 °C	28 days
Total phosphorus	Poly, 250 mL	H_2SO_4 to pH $<$ 2; 4 °C	28 days
Ortho-phosphate	Poly, 250 mL	Filter 0.45 µm; 4 °C	48 hours
Total metals	Poly, 500 mL	HNO_3 to pH $<$ 2; 4 °C	6 months
Dissolved metals	Poly, 500 mL	Filter 0.45 µm; HNO_3 to pH $<$ 2; 4 °C	6 months
Total coliforms / *E. coli*	Sterile, 100 mL	$Na_2S_2O_3$; 4 °C	6 hours
Oil and grease	Glass, 1 L	HCl to pH $<$ 2; 4 °C	28 days

10. Quality Control

- **Field blank:** At least one per event – fill a sample bottle with deionized water at the site and handle identically to field samples (APHA et al., 2012).
- **Field duplicates:** Collect one per 10 samples (or at least one per event).
- **Equipment blanks:** If non-dedicated equipment is used, collect a deionized water rinse blank.
- **Documentation:** Complete the COC form before samples leave the field; photograph site conditions at each sampling location.

11. Data Management

1. Enter field data (times, conditions, flow estimates, sample IDs) into the project database within 48 hours.
2. When laboratory results are received, merge with field data and apply QC flags per SOP-L802 Quality Control.
3. Archive original field data sheets (scan if paper).
4. Version-control all analysis scripts per SOP-L801 RStudio and GitHub.

12. References

- California State Water Resources Control Board (2018)
- California State Water Resources Control Board (2017)
- APHA et al. (2012)
- U.S. Environmental Protection Agency (2002)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

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13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F300	Surface Water Sampling	Sample collection and preservation
F008	Sample Labeling and COC	Chain of custody
L802	Quality Control and QA	QC flags and data review

14. Training Record

Before collecting stormwater samples independently, personnel must:

1. Read this SOP, SOP-F001, and SOP-F300 in full
2. Review the safety rules in §4 with the field lead
3. Participate in at least one supervised storm sampling event, including mobilization, sample collection, preservation, and COC completion
4. Demonstrate proper grab and composite sampling techniques
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F303

Groundwater Monitoring Well Sampling

Standard Operating Procedure

SOP Number	SOP-F303
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Author(s)	Marc Los Huertos
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1. Purpose

This SOP describes procedures for collecting representative groundwater samples from monitoring wells using the low-flow (minimal drawdown) purging and sampling method. Low-flow sampling minimizes disturbance to the well and surrounding formation, reduces turbidity artifacts, and yields samples that are more representative of in-situ groundwater chemistry than traditional high-volume purge methods (Puls & Barcelona, 1996).

The procedures follow EPA low-flow guidance (Puls & Barcelona, 1996), EPA Region 4 groundwater sampling procedures (U.S. Environmental Protection Agency, Region 4, Science and Ecosystem Support Division, 2017), and Cal-EPA DTSC guidance on representative groundwater sampling.

2. Scope

- **Applies to:** Sampling of existing groundwater monitoring wells with an inside diameter of 2 inches (5 cm) or greater, screened intervals of 3 m (10 ft) or less, and a water level above the top of the screen
- **Does not apply to:** Well installation or development; direct-push (e.g., Geoprobe) sampling; spring or seep sampling; wells requiring high-volume purging for regulatory compliance; wells with water levels below the top of the screen (consult the PI for alternative procedures)
- **Location:** All EA Program groundwater monitoring sites

3. Responsibilities

Role	Responsibility
PI / Project Lead	Approve sampling plan and well selection
Field Lead	Coordinate logistics, maintain well access agreements
Trained Personnel	Purge and sample wells per this SOP
New Students	Complete training before participating in well sampling

4. Definitions

- **Dedicated pump:** A pump permanently installed in a single well and not removed between sampling events; eliminates inter-well cross-contamination and decontamination burden; preferred for long- term monitoring programs.
- **Drawdown:** The decline in water level within a well caused by pumping; in low-flow sampling, drawdown must remain at or below 10 cm to minimize vertical mixing within the screened interval and preserve the representativeness of the sample (Puls & Barcelona, 1996).
- **E-tape (electric tape, water level indicator):** An electronic device consisting of a two-conductor cable with a sensing probe at the tip; when the probe contacts the water surface, an audible alarm sounds; used to measure depth to water from a reference point (typically the top of casing, TOC).

- **Flow-through cell:** A sealed flow cell into which purge water is routed so that a multiparameter probe can measure field parameters continuously without exposure to the atmosphere; prevents degassing of CO_2 and O_2 that would bias pH, DO, and ORP readings.
- **High-volume purge:** The traditional well purging approach in which 3–5 well volumes (or more) are removed before sampling; causes high drawdown, turbulence, and resuspension of fine particles; generally produces less representative samples than low-flow methods (Puls & Barcelona, 1996).
- **Low-flow (minimal-drawdown) sampling:** A groundwater sampling technique in which the pump is set at the mid-screen interval and the flow rate is adjusted to maintain drawdown of 10 cm or less; sampling begins only after field parameters have stabilized (Puls & Barcelona, 1996; Standard Practice for Low-Flow Purging and Sampling for Wells and Devices Used for Ground-Water Quality Investigations, 2021).
- **ORP (oxidation-reduction potential):** A measure of the electron-donating or -accepting capacity of a groundwater sample; expressed in millivolts; sensitive to exposure to atmospheric oxygen; must be measured in a flow-through cell without headspace.
- **Parameter stabilization:** The criterion indicating that the water being pumped from the well is representative of the aquifer rather than stagnant casing water; defined as three consecutive readings within specified limits for pH, temperature, specific conductance, DO, ORP, and turbidity (see §7.3 criteria table).
- **Screen interval (screened interval):** The perforated section of the well casing through which groundwater enters the well from the surrounding aquifer; the pump intake should be positioned at the center of this interval during low-flow sampling.
- **Standing water volume:** The volume of water within the well casing above the bottom of the well screen; calculated as $V = \pi r^2 h$, where r is the inside casing radius and h is the height of the water column; used to determine whether enough water exists for low-flow sampling.
- **TOC (top of casing):** The surveyed reference elevation from which all depth-to-water measurements are taken; must be marked on the well casing with a permanent reference point.
- **Trip blank:** A set of 40 mL VOC sample vials filled with laboratory-grade water in the lab, transported to the field in a sealed cooler alongside field samples, and returned to the lab unopened; detects contamination introduced during transport (APHA et al., 2012).
- **Well volume:** The volume of water stored in the well casing above the screen; typically calculated from well construction records; one well volume is the minimum purge volume before parameter monitoring in high-volume purge methods.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Heavy well caps and locks; lifting pumps and equipment; uneven terrain around well pads; potential for back strain when bending over flush-mount wells
- **Chemical:** Groundwater may contain VOCs, metals, or other contaminants. Purge water may require containerization as hazardous waste. Preservative acids in pre-preserved bottles are corrosive.
- **Biological:** Standing water in well vaults may harbor mosquitoes or other vectors
- **Confined spaces:** Flush-mount well vaults may be classified as confined spaces. Do

not enter vaults.

5.1. Required PPE

- ☐ Nitrile gloves (double-glove when handling suspected hazardous groundwater)
- ☐ Safety glasses or chemical splash goggles
- ☐ Closed-toed shoes or field boots

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Low-flow peristaltic pump or bladder pump	Programmable flow rate; 0.1–0.5 L/min
Pump tubing (dedicated or new)	PTFE or polyethylene; appropriate ID for pump
Flow-through cell	With ports for multiparameter probe
Multiparameter probe	pH, temperature, SpC, DO, ORP, turbidity; calibrated per instrument SOP
E-tape (water level indicator)	Metric and/or English gradations
Pre-labeled sample bottles	Per analyte; type and preservative per project QA plan (APHA et al., 2012)
Deionized water	For equipment blanks and rinsing
Well keys	For locked well caps

6.2. Field Documentation

Item	Notes
Well construction logs	Casing depth, screen interval, diameter
Groundwater purge log (Appendix A)	For recording stabilization parameters
Chain of custody (COC) forms	Required for lab submission
Field notebook	
Camera	Photo-document well condition
GPS unit or smartphone	For confirming well locations

7. Procedure

7.1. Pre-Sampling Preparation

1. Review well construction logs for each well: casing material, diameter, total depth, screen interval, and previous water level data.
2. Calculate the volume of standing water in the well casing above the screen using $V = \pi r^2 h$.
3. Calibrate the multiparameter probe per the manufacturer's instructions. Record calibration data on the purge log.
4. Assemble pump, tubing, and flow-through cell. For non-dedicated equipment, decontaminate with deionized water before use at each well.

7.2. Water Level Measurement

1. Remove the well cap. Note the condition of the well (damage, standing water in vault, obstructions).
2. Wipe the e-tape sensor with a clean paper towel.
3. Lower the e-tape slowly until the alarm sounds at the water surface.
4. Record depth to water from the TOC to the nearest 0.3 cm (0.01 ft).
5. Measure well depth from the same reference point (after sampling, to avoid resuspending settled solids).
6. Calculate the water column height and standing water volume.

> **Tip**
>
> Always measure water level before inserting the pump. Lowering the pump displaces water and changes the apparent water level.

7.3. Pump Placement and Low-Flow Purging

1. Slowly lower the pump intake to the center of the screened interval. Avoid dropping the pump or allowing it to contact the bottom of the well.
2. Start the pump at the lowest speed setting and slowly increase until discharge begins. Target a flow rate of 0.1–0.5 L/min (Puls & Barcelona, 1996; Standard Practice for Low-Flow Purging and Sampling for Wells and Devices Used for Ground-Water Quality Investigations, 2021).
3. Check the water level. Drawdown must not exceed 10 cm. If drawdown exceeds 10 cm, reduce the pumping rate.
4. Route discharge through the flow-through cell for continuous monitoring.
5. Record field parameters and cumulative purge volume on the purge log at 3–5 minute intervals.
6. Continue purging until field parameters have stabilized (Puls & Barcelona, 1996):

Parameter	Stabilization Criterion
pH	±0.1 units over three consecutive readings
Temperature	±0.2 °C over three consecutive readings

Parameter	Stabilization Criterion
Specific conductance	±3% over three consecutive readings
Dissolved oxygen	±0.3 mg/L over three consecutive readings
ORP	±10 mV over three consecutive readings
Turbidity	±10% over three readings (or < 10 NTU)

7. Once all parameters are stable, proceed immediately to sample collection. Do not turn off the pump between purging and sampling.

! If the Well Purges Dry

If the well dewaters before stabilization, stop pumping and allow the well to recover. Sample as soon as adequate volume has recovered. Record the dewatering event and recovery time on the purge log. Note that samples from wells purged to dryness may not be representative.

7.4. Sample Collection

1. Disconnect the flow-through cell from the discharge line. Collect samples directly from the pump tubing (U.S. Environmental Protection Agency, Region 4, Science and Ecosystem Support Division, 2017).
2. Maintain the same low flow rate used during purging.
3. Collect samples in this order to minimize bias:
    a. VOCs (if applicable) – 40 mL glass vials, zero headspace
    b. Dissolved gases (if applicable)
    c. Total metals and other unfiltered analytes
    d. Filtered (dissolved) analytes – attach a 0.45 µm in-line filter
    e. General chemistry (pH, conductivity, alkalinity, nutrients)
    f. Bacteria (use sterile bottles)
4. Fill bottles slowly to minimize aeration and turbulence.
5. Cap immediately, label, and place in ice.

7.5. Post-Sampling Procedures

1. Measure and record the final depth to water.
2. Measure the total well depth.
3. Remove the pump and tubing. Replace the well cap and lock.
4. Containerize all purge water per the site waste management plan.
5. Decontaminate non-dedicated equipment and collect an equipment blank.
6. Complete the chain of custody form.

7.6. Decontamination Between Wells

1. Flush the pump and tubing with 1 L of laboratory-grade detergent solution (Alconox or equivalent).

2. Flush with 3 L of deionized water.
3. Change nitrile gloves between wells.
4. Collect one equipment blank per sampling event (APHA et al., 2012).

8. Sample Preservation and Holding Times

Follow preservation requirements specified by your analytical laboratory (APHA et al., 2012). Common groundwater analytes:

Analyte	Container	Preservative	Holding Time
pH	Poly, 250 mL	None; measure in field	15 minutes
Specific conductance	Poly, 250 mL	Refrigerate 4 °C	28 days
Alkalinity	Poly, 250 mL	Refrigerate 4 °C	14 days
Nitrate + nitrite	Poly, 250 mL	H_2SO_4 to pH < 2; 4 °C	28 days
Total metals	Poly, 500 mL	HNO_3 to pH < 2; 4 °C	6 months
Dissolved metals	Poly, 500 mL	Filter 0.45 µm; HNO_3 to pH < 2; 4 °C	6 months
VOCs	Glass, 40 mL (×3)	HCl to pH < 2; 4 °C	14 days

9. Quality Control

- **Field blank:** One per event; deionized water at the well site (APHA et al., 2012).
- **Equipment blank:** One per event from the final deionized rinse.
- **Field duplicates:** One per 10 wells sampled.
- **Trip blanks (VOCs only):** One per cooler.

10. References

- Puls & Barcelona (1996)
- U.S. Environmental Protection Agency, Region 4, Science and Ecosystem Support Division (2017)
- (Standard Practice for Low-Flow Purging and Sampling for Wells and Devices Used for Ground-Water Quality Investigations, 2021)
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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-well equipment cleaning
F008	Sample Labeling and COC	Chain of custody
F300	Surface Water Sampling	Preservation and handling
L802	Quality Control and QA	QC requirements

12. Training Record

Before sampling groundwater monitoring wells independently, personnel must:

1. Read this SOP, SOP-F001, and SOP-F300 in full
2. Review well construction logs for the wells to be sampled
3. Demonstrate proper water level measurement technique
4. Observe a trained operator complete a full low-flow purge and sample collection at one well
5. Perform a supervised purge and sampling event achieving stabilization within the criteria in §7.3
6. Demonstrate proper decontamination and chain of custody procedures
7. Be signed off by the PI or field lead

13. Appendix A: Groundwater Purge Log

Print on waterproof paper and complete one form per well.

Site: _____ **Well ID:** _____ **Date:** _____ **Sampler:** _____

Well Construction: Casing diameter: _____ in | Total depth: _____ ft | Screen: _____ to _____ ft below TOC

Pre-sampling water level: _____ ft from TOC **Calculated standing water volume:** _____ L

Time	Elapsed (min)	Cum. Vol. (L)	Flow (L/min)	Draw-down (cm)	pH	Temp (°C)	SpC (µS/cm)	DO (mg/L)	ORP (mV)	Turbidity (NTU)

Stabilization achieved? Y / N **Time:** _____

Post-sampling water level: _____ ft from TOC **Total well depth (post-sampling):** _____ ft from TOC

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F304

Stream Physical Habitat Assessment – SWAMP Protocol

Standard Operating Procedure

SOP Number	SOP-F304
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1. Purpose

This SOP describes procedures for characterizing the physical habitat of wadeable streams using the quantitative transect-based protocol developed by the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). Physical habitat data document the structural condition of streams – channel dimensions, substrate composition, bank stability, riparian vegetation, instream cover, and human influence – and are essential for interpreting bioassessment results and diagnosing the causes of biological impairment (Barbour et al., 1999; Ode et al., 2016).

The protocol produces data compatible with the California Index of Physical-habitat Integrity (IPI), the California Stream Condition Index (CSCI), and the Algal Stream Condition Index (ASCI), enabling direct comparison with statewide reference data (Mazor et al., 2016; Rehn et al., 2018).

2. Scope

- **Applies to:** Wadeable perennial and intermittent streams and rivers where personnel can safely wade (generally depths less than approximately 1 m and velocities less than approximately 1.2 m/s)
- **Does not apply to:** Non-wadeable rivers requiring boat-based protocols; estuarine or tidal reaches; depressional wetlands (use CRAM instead); lake or reservoir shorelines
- **Location:** All EA Program stream monitoring sites, including tributaries to the Santa Ana River watershed and other southern California waterbodies

3. Responsibilities

Role	Responsibility
PI / Project Lead	Approve site selection and sampling design
Field Lead	Coordinate logistics, lead reach delineation, verify equipment
Trained Personnel	Collect physical habitat data per this SOP

Role	Responsibility
New Students	Complete training before participating in field crews

4. Definitions

- **b-axis (intermediate axis):** The intermediate dimension of a sediment particle (not the longest or shortest axis); the standard measurement used to classify particle size in pebble counts; measured with a metric ruler to the nearest mm (Ode et al., 2016).
- **Bankfull channel:** The stage at which water just begins to overflow the channel banks into the floodplain; the reference condition for channel geometry measurements in SWAMP; typically evidenced by a change in bank slope, a break in vegetation, or the top of the point bar (Ode et al., 2016; Peck et al., 2006).
- **Cobble embeddedness:** The percentage of the surface area of gravel, cobble, and boulder particles that is surrounded and buried by fine sediment (sand, silt, clay); an indicator of fine sediment loading and hyporheic connectivity (Ode et al., 2016).
- **CPOM (coarse particulate organic matter):** Organic material (leaves, needles, twigs, bark) with a largest dimension between 1 mm and 10 cm; recorded at substrate measurement points as an indicator of allochthonous organic input to the stream.
- **D16, D50, D84:** Percentile statistics of the particle-size distribution from a pebble count; the particle size at which 16%, 50%, or 84% of particles are smaller; used to characterize substrate texture; D50 is the median particle diameter.
- **Embeddedness:** See cobble embeddedness.
- **IPI (Index of Physical-habitat Integrity):** The California statewide index of stream physical condition calculated from SWAMP physical habitat data; scores from 0 (most disturbed) to 1 (least disturbed); used alongside the CSCI for reach-level condition assessment (Rehn et al., 2018).
- **Inter-transect:** A transect located halfway between two main transects; used for substrate, embeddedness, and organic matter measurements but not for cross-section geometry; see Appendix B for inter-transect locations (Ode et al., 2016).
- **Main transect:** One of 11 equally spaced transects (A through K) across the full sampling reach; the primary sampling unit for all SWAMP physical habitat modules including cross-section geometry, bankfull identification, substrate, canopy cover, bank stability, human influence, and riparian vegetation.
- **OHW (ordinary high-water mark):** The line on the bank formed by the action of water in its ordinary course; used as an alternative bankfull indicator where clear geomorphic evidence is absent.
- **Pebble count:** A randomized survey of substrate particle sizes at systematically distributed points along transects; each particle is measured at the toe of the measurer's boot or wading rod tip to ensure objectivity; produces the particle-size distribution used to calculate IPI substrate metrics (Ode et al., 2016; Peck et al., 2006).
- **Reach:** A representative stream segment typically 150 m in length (or 37.5× the mean wetted width for wider streams), within which all SWAMP physical habitat measurements are collected; selected to encompass representative channel features and minimize confounding influences of point sources or major confluences (Ode et al., 2016).
- **Riparian zone:** The terrestrial area adjacent to the stream channel that is influenced by

periodic flooding and that contributes organic material, shade, and bank stability to the aquatic system; assessed in SWAMP modules I (riparian vegetation) and G (bank stability).
- **Sinuosity:** The ratio of the actual stream channel length to the straight-line valley length over the reach; values greater than 1.5 indicate a meandering channel; a component of the SWAMP reach characterization.
- **Thalweg:** The line of deepest flow along the channel axis; followed during the longitudinal profile module to characterize channel bed elevation changes.
- **Wetted width:** The horizontal distance across the stream from water's edge to water's edge at a transect; used to determine reach length (37.5× mean wetted width) and to scale substrate sampling interval.

5. Safety and Hazards

⚠ Hazards

- **Drowning and swift water:** Do not wade in flows deeper than approximately waist height or where footing is unstable. Always face upstream. Use a wading rod for support.
- **Slippery substrates:** Algae-covered cobble and bedrock are extremely slippery. Wear felt-soled or studded wading boots.
- **Biological:** Waterborne pathogens, poison oak, ticks, snakes, and stinging insects. Check for ticks after every field day.
- **Heat:** Southern California field sites can exceed 40 °C during summer. Carry adequate water and take shade breaks.
- **Sensitive species:** California red-legged frog, arroyo toad, southwestern willow flycatcher, and steelhead may be present at some sites. Consult permit requirements before sampling (see SOP-F004).

5.1. Required PPE

☐ Chest or hip waders (neoprene or breathable)
☐ Wading boots with felt or studded soles
☐ Sun protection (hat, sunscreen, long sleeves)
☐ Nitrile gloves (when handling samples)

5.2. Permits

Sampling in or near habitat for listed species may require a CDFW Scientific Collecting Permit and/or a USFWS Section 10 permit. The PI must confirm permit status before fieldwork at any new site.

5.3. Invasive Species Prevention

Decontaminate all wading gear between sites per SOP-F007.

5.4. Emergency Procedures

A minimum of two people must be present for any in-stream work. Refer to SOP-F001 Field Safety.

6. Equipment and Materials

Item	Notes
Fiberglass measuring tape (50 m)	For establishing transect tag lines
Surveyor's rod or stadia rod	For measuring depth at cross-sections
Metric ruler (30 cm, mm gradations)	For pebble count (b-axis measurement)
Convex spherical densiometer	For canopy cover measurements
Clinometer (Suunto or equivalent)	For channel slope
Compass	For bearing and sinuosity
GPS unit or smartphone	For recording coordinates at each transect
Wading rod	For support and depth reference
SWAMP Physical Habitat Field Data Sheets	See Appendix A; print on waterproof paper
Clipboard and mechanical pencils	
Camera (waterproof or in dry bag)	Photo-document each transect
Flagging tape	For marking transect locations

7. Estimated Time

Task	Time (2-person crew)
Reach delineation and transect setup	20–30 minutes
Full physical habitat assessment (Modules C–M)	1.5–2.5 hours
Slope and sinuosity (Module L)	20–40 minutes
Photographs (Module M)	10–15 minutes
Total	2–3.5 hours per reach

8. Procedure

8.1. Reach Delineation

The sampling reach defines the stream segment over which all measurements are collected (Ode et al., 2016).

1. Select a representative reach of appropriate length (150 m for streams with mean wetted width 10 m; 250 m for wider streams).
2. Walk the reach before beginning measurements to identify the thalweg, bankfull indicators, and any safety hazards.

3. Mark the downstream boundary (Transect A) and record GPS coordinates.
4. Stretch the measuring tape upstream along the thalweg (not the bank) to the upstream boundary (Transect K).
5. Set transect locations at equal intervals (e.g., every 15 m for a 150 m reach), placing chaining pins or flagging at each transect.

> **Tip**
>
> Select reaches that are representative of the stream segment. Avoid reaches with immediate upstream or downstream disturbances (bridges, dam tailwaters, outfalls) unless those features are the sampling target (Ode et al., 2016).

8.2. Module A: Reach-Level Information

Record at the start of each visit:

- GPS coordinates at Transects A (downstream) and K (upstream)
- Date, time, crew members
- General weather and flow conditions (normal, high, low, dry)
- Any unusual conditions (algal blooms, trash accumulation, visible pollutants, recent disturbance)

8.3. Module B: Bankfull Identification Calibration

Before beginning measurements, the full crew must agree on bankfull indicators (Ode et al., 2016):

1. Identify at least two independent lines of evidence for bankfull (e.g., break in bank slope, top of point bar, change in vegetation, flood debris line).
2. Walk the first 2–3 transects together and calibrate agreement on bankfull height.
3. Differences in bankfull identification between crew members are the largest source of inter-observer variability in SWAMP physical habitat data.

8.4. Module C: Cross-Section Geometry (Main Transects)

At each main transect (A through K) (Ode et al., 2016; Peck et al., 2006):

1. Stretch the measuring tape across the channel from left to right bank at the bankfull boundary (looking downstream).
2. Record the total bankfull width to the nearest 0.1 m.
3. Record the wetted width to the nearest 0.1 m.
4. At 5 equally spaced points across the wetted channel, measure water depth to the nearest cm using the surveyor's rod.
5. Record the thalweg depth (deepest point).
6. Estimate the dominant bank angle (undercut, vertical, steep, gradual, flat) for left and right banks.

8.5. Module D: Substrate (Pebble Count)

At each of the 21 transects (main and inter-transects), at 5 equally spaced points across the wetted channel (Ode et al., 2016):

1. Measure **water depth** to the nearest cm.
2. Pick up the particle at the toe of your boot (or wading rod tip) and measure the **b-axis** to the nearest mm. Record on the field form.
3. If the particle cannot be picked up (bedrock, hardpan), record the appropriate size class code (see Appendix C).
4. Record presence or absence of **CPOM** at each point.

This produces 105 substrate measurements per reach.

8.6. Module E: Cobble Embeddedness

At each substrate measurement point on main transects, if the particle is gravel, cobble, or boulder:

1. Estimate the **percentage of the particle surface buried** by fine sediment to the nearest 5% or 10%.
2. Record sand and finer substrate as 100% embedded by convention.
3. Do not assess embeddedness for bedrock.

8.7. Module F: Algal and Macrophyte Cover

At each of the five substrate measurement points (main and inter-transects):

1. Record presence/absence of **macroalgae** on the substrate particle.
2. Record presence/absence of **macrophytes** (rooted vascular aquatic plants; mosses excluded).
3. At main transects, estimate percent cover within a 1 m radius.

8.8. Module G: Bank Stability

At each main transect, for both left and right banks (Ode et al., 2016):

1. Estimate the **percent of bank covered by erosion** (exposed bare soil, slumping, undercutting) to the nearest 5%.
2. Estimate the **angle of the bank** (undercut, vertical, steep, gradual, flat).

8.9. Module H: Human Influence

At each main transect, record the proximity of human influences within a 5 m upstream/downstream window and 10 m from each bank. Record each feature as 0 (absent), P (present $>$ 10 m), C (within 10 m), or B (on bank). Features include: walls/rip-rap/dams, buildings, pavement, roads/railroads, pipes, landfill/trash, park/lawn, row crops, pasture/hay, logging, and mining.

8.10. Module I: Riparian Vegetation

At each main transect, for both left and right banks, estimate areal cover (0–4 scale: 0 absent, 1 < 10%, 2 = 10–40%, 3 = 40–75%, 4 > 75%) in three layers:

- Canopy (> 5 m)
- Understory (0.5–5 m)
- Ground cover (< 0.5 m)

Record the dominant vegetation type for each layer.

8.11. Module J: Instream Cover

At each main transect, estimate percent cover of instream features within one channel width upstream and downstream of the transect line:

Feature	Definition
Boulder (> 25 cm)	Visual estimate of surface coverage
Cobble (6.4–25 cm)	Visual estimate
Wood (snag, rootwad)	Visual estimate
Overhanging vegetation	Bank overhang within 0.5 m of water surface
Undercut bank	Bank extending over water
Macroalgae	Attached filamentous or mat-forming algae
Macrophytes	Rooted vascular aquatic plants

8.12. Module L: Channel Slope and Sinuosity

Measure channel slope using a clinometer at a minimum of three measurement intervals along the reach (between Transects A–C, E–G, and I–K) or more frequently if slope is variable.

Measure sinuosity by comparing total thalweg length (measured with the tape along the thalweg) to the straight-line valley distance (measured with a compass and tape) (Ode et al., 2016).

8.13. Module M: Photographs

At each main transect, take the following photographs:

1. Looking upstream along the thalweg
2. Looking downstream along the thalweg
3. Left bank at the transect line
4. Right bank at the transect line

At Transects A and K, also photograph the downstream and upstream reach boundaries. All photographs should include the transect label (flagging or field data sheet) in the frame for orientation.

Record photo file names in the field data sheet.

9. Quality Control

9.1. Inter-Observer Variability

For substrate measurements, the precision targets are (Ode et al., 2016):

- Median substrate size class (D50): agreement within one Wentworth size class between independent observers
- Embeddedness: agreement within 15 percentage points

9.2. Repeat Measurements

For periodic quality assurance, conduct a repeat measurement by an independent crew within 48 hours. Both crews must achieve agreement within the precision targets above.

9.3. Common Errors to Avoid

- Measuring transects out of sequence (always work upstream)
- Inconsistent bankfull identification between crew members (calibrate at the first transect)
- Forgetting to record GPS coordinates at Transects A and K
- Omitting inter-transect measurements (required for full IPI calculation)
- Failing to record the measurement units (mm vs. size class code) for substrate

10. References

- Ode et al. (2016)
- Rehn et al. (2018)
- Peck et al. (2006)
- Barbour et al. (1999)
- Mazor et al. (2016)

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish* (2nd ed.). U.S. Environmental Protection Agency, Office of Water. https://www.epa.gov/cwa-methods/rapid-bioassessment

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F004	Site Access and Permits	Permits for listed species
F007	Field Decontamination	Between-site decontamination
F301	Stream Discharge	Concurrent discharge measurement
F606	Canopy Cover	Densiometer technique
F610	BMI Sampling	Co-collected bioassessment data

12. Training Record

Before conducting physical habitat assessments independently, personnel must:

1. Read this SOP, SOP-F001, and the SWAMP Supplemental Guidance Document (Ode et al., 2016) in full
2. Review the SWAMP physical habitat field data sheets (Appendix A)
3. Participate in a calibration exercise for bankfull identification and substrate measurement with an experienced crew leader
4. Complete at least one full reach assessment under supervision, with substrate and embeddedness estimates agreeing with the trainer within the precision targets in §9
5. Demonstrate proper densiometer technique and slope measurement
6. Be signed off by the PI or field lead

13. Appendix A: Field Data Sheets

SWAMP Bioassessment Field Data Sheets (including physical habitat forms) are available from SWAMP@waterboards.ca.gov or at: https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.html

Print on waterproof paper (Rite in the Rain) for field use. Forms needed:

1. Station Information and Notable Field Conditions
2. Channel/Riparian Cross-Section and Thalweg Profile (one per transect)
3. Slope and Bearing
4. Post-Sampling Qualitative Observations
5. Discharge Measurement (if applicable)

14. Appendix B: Quick Reference – Transect Layout

For a 150 m reach (average wetted width 10 m):

Transect	Station (m)	Type
A	0	Main
AB	7.5	Inter
B	15	Main
BC	22.5	Inter
C	30	Main
…	…	…
K	150	Main

For a 250 m reach, multiply all station distances by 250/150.

15. Appendix C: Substrate Size Class Quick Reference

Code	Class	Size Range (mm)
RS	Bedrock smooth	Continuous
RR	Bedrock rough	Continuous
HP	Hardpan	Continuous
BL	Boulder	> 250
CB	Cobble	64–250
GC	Coarse gravel	16–64
GF	Fine gravel	2–16
SA	Sand	0.06–2
FN	Fines	< 0.06
WD	Wood	–
OT	Other	–

Always measure the **b-axis** (intermediate axis) of the particle.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F305

Sediment Sampling – Streams and Lakes

Standard Operating Procedure

SOP Number	SOP-F305
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the collection, handling, and field documentation of sediment samples from streams, rivers, lakes, and reservoirs. Sediment is a major sink and source of nutrients, metals, organic contaminants, and fine particles in aquatic systems. The procedures in this SOP are designed to produce samples suitable for physical characterization (grain size, organic matter content), chemical analysis (metals, nutrients, organic contaminants), and biological assessment (benthic invertebrate habitat substrate) (Mudroch et al., 1999; US Environmental Protection Agency, 2001).

2. Scope

- **Applies to:** Surficial sediment sampling (top 0–10 cm) in wadeable streams, non-wadeable rivers (from bridges or boats), and lentic systems (lakes, ponds, reservoirs). Deeper cores for stratigraphic or paleolimnological work follow the same safety and documentation requirements but use specialized coring equipment not covered in detail here.
- **Does not apply to:** Soil sampling on upland or riparian surfaces (see SOP-F400); suspended sediment sampling by filtration (see future SOP-L312); bedload transport measurements; marine sediment sampling
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling plan, coordinate permits
Trained Personnel	Collect and handle samples per this SOP
New Students	Complete training before independent sampling

4. Definitions

- **Benthic sediment:** Material that has settled and accumulated on the bed of a stream, lake, or estuary; composed of mineral particles, organic matter, and biological material; the primary matrix for sampling of trace metals, nutrients, and benthic organisms.
- **Core sample:** A cylindrical sediment sample extracted with a tube corer or freeze corer that preserves the vertical stratigraphy and depth profile of deposited material; used for porewater analysis, dating, and historical pollutant records.
- **Disturbed sample:** A bulk sediment sample collected without preserving internal structure (e.g., by grab sampler or trowel); used for bulk chemical analysis, particle size, and loss on ignition when stratigraphy is not required.
- **Ekman grab:** A spring-loaded, box-shaped sampler lowered on a cable that closes on impact with the sediment bed; collects an undisturbed surface sample from soft, fine-grained sediments in lakes and slow-moving water.

- **Fine earth fraction:** The portion of sediment passing through a 2 mm sieve (sand, silt, and clay); the standard fraction for chemical analysis; gravel and coarser material are excluded.
- **Grab sample:** A surface sediment sample collected from the top 1–5 cm of the bed using a grab sampler, trowel, or scoop; represents recently deposited material and is appropriate for screening contamination in surface sediments.
- **Porewater:** Water occupying the spaces between sediment particles; often enriched in dissolved nutrients, metals, and gases released by diagenetic processes; collected by in situ peepers or by centrifugation of sediment cores.
- **Sediment trap:** A cylindrical or funnel-shaped container deployed in the water column to intercept settling particles; used to measure particle flux rates and collect settling material for chemical characterization.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Wading in streams and working from boats carry slip, fall, and drowning hazards. Sediment sampling often involves bending, lifting, and handling heavy wet material.
- **Biological:** Sediment may harbor pathogens, parasites (e.g., *Schistosoma*, *Naegleria*), and sharp debris (glass, metal).
- **Chemical:** Sediment from urban or industrial sites may contain heavy metals, hydrocarbons, or pesticides. Avoid skin contact and do not ingest.

5.1. Required PPE

☐ Nitrile gloves (doubled for contaminated sites)
☐ Wading boots or hip waders
☐ Personal flotation device (boat sampling)
☐ Safety glasses
☐ Sun protection

5.2. Emergency Procedures

A minimum of two people must be present for any in-water work. Refer to SOP-F001 Field Safety.

6. Equipment and Materials

6.1. Sampling Devices

Device	Application	Notes
Stainless steel scoop or trowel	Wadeable stream surficial sediment	Simplest; suitable for coarse to fine sediment
Ponar grab sampler (standard or petite)	Lakes, deep pools, non-wadeable rivers	Collects approximately 0.023 m^2 (petite) to 0.052 m^2 (standard)
Ekman grab sampler	Soft lake-bottom sediment (silt, clay, organic muck)	Lighter than Ponar; not suitable for sand or gravel
Hand corer (acrylic tube, 5–7 cm ID)	Intact surficial cores in soft sediment	Preserves vertical stratigraphy
Gravity corer or piston corer	Deep cores for paleolimnological work	Specialized; consult PI

6.2. Support Equipment

Item	Notes
Stainless steel bowls and spoons	For homogenizing grab samples
Pre-labeled sample bags (Whirl-Pak) or jars	Appropriate to analyte (see Section 7)
Cooler with ice packs	Maintain samples at 4 C
Deionized water (squeeze bottle)	For rinsing equipment
Measuring tape and depth rod	For recording water depth at sample location
GPS unit	For recording coordinates per SOP-F006
Field notebook and data sheets	Waterproof (Rite in the Rain)
Camera	For photo-documenting sample location and sediment appearance
Chain of custody forms	Per SOP-F008

7. Procedure

7.1. Site Selection and Documentation

1. Identify the sampling location based on the study design (e.g., depositional zones for contaminant assessment, riffles for substrate characterization)
2. Record GPS coordinates, date, time, water depth, and a visual description of the sediment surface (color, texture, odor, presence of debris or biota) in the field notebook
3. Photograph the site and the sediment surface before disturbing it

7.2. Stream Sediment (Wadeable)

1. Approach the sampling location from downstream to avoid disturbing the target area
2. Using a stainless steel scoop or trowel, carefully skim the top 5–10 cm of sediment. Avoid collecting underlying undisturbed substrate unless the study requires a depth profile.

3. Transfer the sediment into a stainless steel bowl
4. If a composite sample is required, collect sediment from 3–5 locations within the reach (e.g., across the channel width at the designated transect) and combine in the bowl
5. Homogenize using a stainless steel spoon; remove large rocks, sticks, and debris
6. Transfer the homogenized sample into the appropriate container(s) (see Section 7)

7.3. Lake and Reservoir Sediment (Grab Sampler)

1. Lower the Ponar or Ekman grab sampler to the bottom on a measured line from a boat or dock. Record the water depth.
2. Trip the sampler per the manufacturer's instructions
3. Raise the sampler slowly to avoid losing material; keep it horizontal as it breaks the water surface
4. Inspect the sample: a successful grab is at least half full with an undisturbed sediment surface and overlying water. Reject and re- sample if the grab is less than one-third full, tilted, or shows evidence of washout (US Environmental Protection Agency, 2001).
5. Carefully drain overlying water without disturbing the sediment surface
6. For surficial samples, subsample the top 2–5 cm using a stainless steel spoon
7. Homogenize and containerize as in Section 6.2

7.4. Intact Cores (Hand Corer)

1. Push the acrylic core tube vertically into soft sediment to the desired depth (typically 10–30 cm)
2. Cap the top of the tube (creating suction) before pulling the core from the sediment
3. Once extracted, cap the bottom immediately
4. Label the core tube with sample ID, date, depth, and orientation (top/bottom)
5. Keep the core vertical during transport and store at 4 C

7.5. Decontamination Between Sites

1. Scrub all stainless steel equipment with a brush and rinse with site water
2. Follow with a deionized water rinse
3. For trace metal work, perform an additional acid rinse (10% HNO_3) followed by a triple DI water rinse
4. See SOP-F007 Field Decontamination

8. Sample Containers and Preservation

Analyte	Container	Preservative
Grain size / texture	Whirl-Pak or poly bag	Refrigerate 4 C
Organic matter (LOI)	Whirl-Pak or poly bag	Refrigerate 4 C
Total metals	Glass jar (acid-washed)	Refrigerate 4 C; freeze if holding $>$ 7 days

Analyte	Container	Preservative
Nutrients (N, P)	Whirl-Pak or poly bag	Refrigerate 4 C; freeze if holding > 48 hours
Organic contaminants	Glass jar (solvent-rinsed)	Refrigerate 4 C
Benthic macroinvertebrates	Separate container	95% ethanol or 10% formalin

> **Note**
>
> Confirm specific container requirements and holding times with the analytical laboratory before each sampling event.

9. Quality Control

- Collect field duplicates at a rate of at least 1 per 10 samples (or 1 per sampling event, whichever is greater)
- Collect one equipment blank per sampling event: rinse decontaminated equipment with DI water and submit the rinse as a sample
- Record all deviations from this SOP in the field notebook
- Complete the chain of custody form per SOP-F008
- Photograph each grab or core before subsampling

10. Data Management

1. Enter field data into the project database within 48 hours
2. Archive original field data sheets (scan if paper)
3. When laboratory results are received, merge with field data and apply QC flags per SOP-L802

11. References

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12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite

SOP	Title	Relationship
F007	Field Decontamination	Between-site decontamination
F008	Sample Labeling and COC	Sample ID and chain of custody
F300	Surface Water Sampling	Companion water collection
F400	Soil Sampling	Upland soil sampling
L406	Loss on Ignition	Sediment organic matter analysis
L410	Acid Digestion	Sediment metals preparation

13. Training Record

Before collecting sediment samples independently, personnel must:

1. Read this SOP, SOP-F001, and SOP-F007 in full
2. Observe a trained operator demonstrate grab sampler deployment, core collection, and decontamination
3. Practice deploying the Ponar or Ekman sampler (or hand corer) under supervision
4. Demonstrate proper homogenization, containerization, and labeling
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F306

Wetland Hydrology Monitoring

Standard Operating Procedure

SOP Number	SOP-F306
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes field methods for monitoring wetland hydrology, including measurement of water table depth, surface inundation extent, and hydroperiod documentation. Hydrology is the primary driver of wetland ecosystem structure and function: it controls soil saturation, redox

chemistry, plant community composition, and jurisdictional wetland status under the Clean Water Act (Mitsch & Gosselink, 2015; US Army Corps of Engineers, 1987).

Wetland hydrology monitoring supports baseline characterization, impact assessment, restoration monitoring, and regulatory compliance (e.g., Army Corps of Engineers wetland delineation criteria).

2. Scope

- **Applies to:** Freshwater wetlands (marshes, swamps, riparian wetlands, vernal pools, constructed wetlands) at EA Program field sites. Methods include manual water-level measurements in monitoring wells and piezometers, crest gauges, staff gauges, and automated water level loggers.
- **Does not apply to:** Tidal wetland hydrology (requires tide- corrected methods); stream stage-discharge relationships (see SOP-F301); groundwater monitoring in non-wetland wells (see SOP-F303)
- **Location:** All EA Program wetland field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve monitoring design, coordinate permits
Trained Personnel	Install equipment, collect data per this SOP
New Students	Complete training before independent use

4. Definitions

- **Hydroperiod:** The duration and timing of surface water or near-surface saturation in a wetland during a given year; the most important determinant of wetland vegetation, soil development, and biogeochemical function.
- **Hydric soil:** A soil that formed under conditions of saturation, flooding, or ponding long enough to develop anaerobic conditions in the upper part; identified by redoximorphic features and low-chroma colors; a required criterion for jurisdictional wetland delineation.
- **Ordinary high water mark (OHWM):** The line on the shore established by the fluctuations of water leaving physical characteristics such as a clear, natural line impressed on the bank, shelving, destruction of terrestrial vegetation, or presence of litter and debris; used to define the lateral extent of jurisdictional waters.
- **Piezometer:** A small-diameter tube installed in the soil with a screened section at a defined depth; measures hydraulic head at that depth; used to determine the direction and magnitude of groundwater flow and to detect perched water tables.
- **Redox potential (Eh):** A measure of the oxidizing or reducing conditions in soil or water, expressed in millivolts; positive values indicate oxidizing conditions; negative values indicate reducing conditions under which Fe, Mn, and sulfate are reduced and redoximorphic features develop.

- **Staff gauge:** A graduated rod installed vertically in a water body or wetland to allow direct visual reading of water surface elevation; used to record surface water stage and to relate water level logger records to a local datum.
- **Water table:** The upper surface of the zone of saturation in the soil; measured as the depth to free-standing water in a well or piezometer; fluctuates seasonally and in response to precipitation events.
- **Wetland indicator status:** A US Army Corps of Engineers classification of plant species by their probability of occurrence in wetlands: OBL (obligate wetland), FACW (facultative wetland), FAC (facultative), FACU (facultative upland), UPL (upland); used in vegetation-based wetland delineation.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Wetland substrates are unstable; risk of sinking in soft muck, tripping on submerged debris, or falling. Standing water may conceal holes and channels.
- **Biological:** Mosquitoes (disease vectors), ticks, venomous snakes, poison oak/ivy, and waterborne pathogens. Avoid direct skin contact with standing water.
- **Environmental:** Heat stress in summer; hypothermia in winter. Wetlands offer little shade.

5.1. Required PPE

☐ Rubber boots or chest waders
☐ Nitrile gloves
☐ Long sleeves and pants (tick and mosquito protection)
☐ Insect repellent (DEET or picaridin)
☐ Sun protection (hat, sunscreen)
☐ Personal flotation device (if water depth exceeds knee level)

5.2. Emergency Procedures

Work in pairs. If a team member becomes stuck in soft substrate, distribute weight on a board or backpack and pull laterally rather than vertically. Refer to SOP-F001 Field Safety.

6. Equipment and Materials

6.1. Manual Measurement

Item	Notes
Water level indicator (electric tape, e-line)	For measuring depth to water in wells and piezometers
Staff gauge (graduated, metric)	For measuring surface water stage
Crest gauge	Records peak water level between visits
Meter stick or folding rule	Backup; for shallow inundation depth
Field notebook and data sheets	Waterproof (Rite in the Rain)
GPS unit	Per SOP-F006
Camera	For photo-documenting site conditions

6.2. Automated Monitoring

Item	Notes
Pressure transducer water level logger (e.g., HOBO U20L, Solinst Levelogger, In-Situ Level TROLL)	Deployed in wells or stilling wells
Barometric pressure logger	Required for compensating non-vented transducers; install above the high water line at or near the site
PVC monitoring wells (2 in or 4 in diameter, slotted screen)	Installed per Section 6.1
Well caps and locks	To prevent tampering and wildlife entry
Well keys or locks	For secured installations
Laptop or shuttle for data download	With manufacturer software

7. Procedure

7.1. Monitoring Well Installation

> **Note**
>
> Well installation may require permits (e.g., well permits, Section 404 permits for work in wetlands). Confirm requirements with the PI before installation.

1. Select well locations based on the study design (e.g., transect from upland to wetland center, paired wells at different depths)
2. Auger a borehole to the target depth (typically 1–2 m for shallow water table monitoring)
3. Install a 2-inch or 4-inch PVC well with a slotted screen in the saturated zone and a solid riser above the ground surface
4. Backfill the annular space around the screen with clean silica sand; seal the upper portion with bentonite to prevent surface water infiltration along the well casing
5. Install a protective well cap; record the top-of-casing (TOC) elevation relative to a local benchmark using a survey level

6. Develop the well by surging and bailing to remove drilling mud and fine sediment. Allow the well to equilibrate for at least 24 hours before the first measurement.
7. Record the well construction details (total depth, screen interval, stick-up height, TOC elevation, GPS coordinates) on the well installation log

7.2. Manual Water Level Measurement

1. Remove the well cap. If the well is locked, note the time and condition of the lock.
2. Lower the water level indicator (e-line) slowly into the well. When the probe contacts the water surface, the indicator will sound or light up.
3. Read the depth to water from the top of casing (TOC) to the nearest 0.01 ft or 0.5 cm. Record this value.
4. Calculate the water table elevation:

$$\text{WTE} = \text{TOC elevation} - \text{depth to water}$$

5. If the water table is above the ground surface (artesian or flooded conditions), measure the height of water above the TOC or use a staff gauge for ponded water.
6. Record date, time, depth to water, and any observations (e.g., well condition, nearby disturbance, wildlife activity) in the field notebook.
7. Replace and secure the well cap.

7.3. Staff Gauge and Crest Gauge Reading

1. Read the water level on the staff gauge to the nearest 0.5 cm. Read at eye level to avoid parallax.
2. Record the reading, date, time, and any obstructions (debris on the gauge, algae).
3. For crest gauges: record the peak water mark left by granular cork or paint inside the gauge since the last visit. Reset the gauge per the manufacturer's instructions.

7.4. Automated Logger Deployment and Download

1. Program the logger for the desired recording interval (typically 15 minutes to 1 hour for wetland hydrology)
2. Deploy the logger in the well, suspended at a known depth below the TOC using a stainless steel cable or direct-read cable
3. Deploy a barometric logger above the expected high water line (for non-vented transducers)
4. Record the deployment details: logger serial number, deployment depth, start time, recording interval
5. On each visit, download data using the manufacturer's software. Take a manual water level measurement at the time of download for calibration verification.
6. Compensate pressure transducer data for barometric pressure changes using the barometric logger data. Most manufacturer software includes an automated compensation routine.

7.5. Inundation Mapping

1. During each visit, note the spatial extent of surface water or saturated soil at the site
2. Record the inundation boundary on a site map or using GPS waypoints
3. Photograph fixed photo points to document seasonal changes in inundation
4. Record the presence of wetland hydrology indicators (oxidized rhizospheres, water-stained leaves, drift lines, sediment deposits, water marks on trees) per the Army Corps of Engineers wetland delineation manual (US Army Corps of Engineers, 1987)

8. Quality Control

- Take a manual water level reading concurrent with every logger download to verify logger accuracy. If the manual reading and logger reading differ by more than 2 cm, troubleshoot the logger (drift, fouling, cable slip).
- Record duplicate manual readings at 10% of wells per visit (read, remove the tape, re-read). Duplicates should agree within 0.5 cm.
- Check e-line calibration against a measured standard (e.g., a surveyed depth) at least once per season.
- Maintain a well construction log with TOC elevations; re-survey TOC elevations annually or after ground disturbance (frost heave, animal activity, mowing).
- Archive raw logger files in the project data folder before any processing. Version-control all compensation and correction scripts per SOP-L801.

9. Data Management

1. Enter manual water level data into the project database within 48 hours of collection
2. Download, compensate, and archive logger data within 7 days of retrieval
3. Convert all water level data to a common datum (e.g., NAVD88 or a local benchmark) before analysis
4. Flag and document any data gaps, logger malfunctions, or well damage

10. References

Mitsch, W. J., & Gosselink, J. G. (2015). *Wetlands* (5th ed.). John Wiley & Sons.

US Army Corps of Engineers. (1987). *Corps of engineers wetlands delineation manual* (Technical Report Y-87-1). Waterways Experiment Station, US Army Corps of Engineers. https://www.lrl.usace.army.mil/Portals/64/docs/regulatory/Wetland-Delineation-Manual.pdf

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite

SOP	Title	Relationship
F006	GPS and Site Navigation	Well and gauge locations
F200	Eureka Manta2 Multiprobe	Water quality at wetland sites
F301	Stream Discharge	Surface water flow context
F303	Groundwater Sampling	Well sampling companion
F400	Soil Sampling	Wetland soil characterization

12. Training Record

Before performing wetland hydrology monitoring independently, personnel must:

1. Read this SOP and SOP-F001 in full
2. Observe a trained operator demonstrate manual water level measurement and logger download at an active monitoring site
3. Practice using the water level indicator (e-line) under supervision and demonstrate agreement within 0.5 cm on duplicate readings
4. Demonstrate competence in logger programming and data download
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F307

Lake and Reservoir Profiling

Standard Operating Procedure

SOP Number	SOP-F307
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Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the collection of vertical depth profiles of water quality parameters (temperature, dissolved oxygen, pH, conductivity, turbidity, and chlorophyll fluorescence) in lakes, reservoirs, and deep ponds. Depth profiling characterizes thermal stratification, the location and extent of the thermocline and oxycline, and vertical gradients in water chemistry that control nutrient cycling, habitat availability, and water treatment operations (Kalff, 2002; Wetzel, 2001).

2. Scope

- **Applies to:** Vertical profiling from boats, docks, or platforms in lentic water bodies (lakes, reservoirs, ponds) and slow-moving deep river pools. Covers both manual sonde profiles and Secchi depth measurements.
- **Does not apply to:** Continuous deployed sonde monitoring (see future SOP); horizontal spatial surveys; stream cross-section water quality (see SOP-F200 for general sonde operation); bathymetric mapping
- **Location:** All EA Program lake and reservoir field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve profiling plan, coordinate boat access and permits
Trained Personnel	Calibrate sonde, perform profiling per this SOP
New Students	Complete training and boat safety orientation before participating

4. Definitions

- **Epilimnion:** The warm, well-mixed, oxygen-rich surface layer of a thermally stratified lake; bounded below by the thermocline; the zone of primary production and gas exchange with the atmosphere.
- **Hypolimnion:** The cold, dense, isolated bottom layer of a thermally stratified lake; oxygen depleted in productive lakes due to decomposition of settling organic matter; often enriched in dissolved nutrients and reduced metals (Fe^{2+}, Mn^{2+}).
- **Metalimnion:** The layer of rapid temperature change between the epilimnion and hypolimnion; also called the thermocline layer; may harbor distinct biological communities (e.g., deep chlorophyll maxima) and chemical gradients.
- **Oxycline:** The depth zone of rapid decline in dissolved oxygen concentration; typically coincides with or lies below the thermocline in stratified lakes; marks the transition from aerobic to anaerobic conditions.
- **Secchi depth:** The depth at which a Secchi disk (a white or black-and-white patterned disk lowered on a calibrated line) becomes invisible; an index of water transparency and light penetration; negatively correlated with phytoplankton biomass and suspended sediment concentration.
- **Sonde:** A multi-sensor instrument lowered through the water column on a cable to measure temperature, dissolved oxygen, conductivity, pH, turbidity, and chlorophyll a at defined depth intervals; the primary instrument for lake profiling.
- **Stratification:** The separation of a water body into distinct horizontal layers of differing density, driven primarily by temperature differences; limits vertical mixing and controls the distribution of oxygen, nutrients, and organisms.
- **Thermocline:** The plane or narrow zone of maximum rate of temperature decrease with depth; defined operationally as a gradient exceeding 1 °C m^{-1}; the boundary between the epilimnion and metalimnion.

- **Trophic state:** A classification of a lake's biological productivity based on nutrient concentrations, phytoplankton biomass, and water clarity: oligotrophic (low nutrients, clear), mesotrophic (intermediate), eutrophic (high nutrients, low transparency), hypereutrophic (very high nutrients, algal bloom prone).

5. Safety and Hazards

⚠ Hazards

- **Drowning:** All lake work involves drowning risk. Personal flotation devices (PFDs) are mandatory for all personnel on boats, docks, and platforms.
- **Weather:** Lightning, high winds, and sudden storms pose serious hazards on open water. Abort profiling if thunderstorms are within 10 miles or winds exceed safe boating conditions for the vessel.
- **Sun exposure:** Extended time on open water increases UV exposure and heat stress.
- **Equipment:** Heavy sonde and cable assemblies can cause hand and back injuries when deployed over the side of a boat.

5.1. Required PPE

☐ Personal flotation device (PFD) – worn at all times on the water
☐ Closed-toed shoes with non-slip soles
☐ Nitrile gloves (when handling calibration solutions)
☐ Sun protection (hat, sunscreen, sunglasses)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. All boat occupants must know the location of PFDs, throwable flotation devices, and communication equipment (VHF radio or charged cell phone). If a person falls overboard, deploy the throwable device immediately and maneuver the boat to the person.

6. Equipment and Materials

Item	Notes
Eureka Manta2 multiprobe or equivalent sonde	Calibrated per SOP-F200
Sonde cable (length sufficient for maximum expected depth)	Graduated at 0.5 m or 1 m intervals
Secchi disk and graduated line	Per SOP-F207
Depth sounder or weighted line	For measuring total water depth at the profiling station
Boat, canoe, kayak, or dock access	Appropriate to site conditions

Item	Notes
Anchor(s) or tie-off lines	To hold the boat stationary during profiling
GPS unit	For recording station coordinates per SOP-F006
Field notebook and profiling data sheet	Waterproof (Rite in the Rain); see Appendix A
Camera	For photo-documenting site and weather conditions
Water sample bottles and cooler	If discrete-depth water samples are collected alongside the profile

7. Procedure

7.1. Pre-Field Preparation

1. Calibrate the sonde per SOP-F200 Eureka Manta2 before departing for the field
2. Verify that the sonde cable is marked at depth intervals (0.5 m or 1 m). If using a direct-read cable, confirm depth readings against a tape measure.
3. Check weather forecast; postpone if thunderstorms are likely or sustained winds exceed the vessel's safe operating threshold
4. Review the profiling station coordinates and planned depth intervals

7.2. Station Setup

1. Navigate to the profiling station (typically the deepest point of the lake or a fixed monitoring location)
2. Record GPS coordinates, date, time, weather conditions (sky cover, wind speed and direction, air temperature), and total water depth
3. Anchor or tie off the boat to minimize drift during the profile
4. Perform the Secchi disk measurement per SOP-F207

7.3. Sonde Deployment and Profile Collection

1. Lower the sonde to 0.5 m below the surface. Allow at least 60 seconds for the sensors to equilibrate (longer for optical DO in cold water).
2. Record all parameters (temperature, DO, pH, conductivity, turbidity, chlorophyll) at that depth. If the sonde is logging internally, note the time and depth; if reading from the handheld display, record values on the data sheet.
3. Lower the sonde to the next depth increment (typically 0.5 m in the epilimnion and through the thermocline; 1 m increments are acceptable in deep hypolimnetic water where parameters change slowly).
4. At each depth, allow 30–60 seconds for sensor equilibration before recording.
5. Continue to within 0.5 m of the bottom. Do not allow the sonde to contact the sediment (stirs up sediment and can foul sensors).

6. After the deepest reading, raise the sonde steadily to the surface. If time permits, record an ascending profile for comparison.

> **Tip**
>
> In productive lakes, the most rapid changes in temperature and DO occur at the thermocline and oxycline. When you detect these transitions, switch to 0.5 m or even 0.25 m intervals to resolve the gradient.

7.4. Discrete-Depth Water Sampling (Optional)

If the study design requires water samples at specific depths (e.g., epilimnion, metalimnion, hypolimnion):

1. Use a Van Dorn or Kemmerer bottle lowered to the target depth
2. Trip the sampler and raise to the surface
3. Dispense into pre-labeled sample bottles per SOP-F300 preservation requirements
4. Record the collection depth and time for each sample

7.5. Post-Profiling

1. Rinse the sonde sensors with deionized water
2. Replace the sonde in its calibration/storage cup with storage solution (especially the pH sensor)
3. Download logged data or scan the data sheet for completeness
4. Confirm that GPS coordinates, Secchi depth, total depth, and all profile readings are recorded

8. Data Interpretation

A typical stratified lake profile shows three zones:

Zone	Characteristics
Epilimnion	Warm, well-mixed surface layer; high DO, uniform temperature
Metalimnion (thermocline)	Rapid temperature decrease with depth (at least 1 C per meter)
Hypolimnion	Cold, dense bottom layer; may be anoxic in productive lakes

> **Note**
>
> Not all lakes stratify. Shallow, wind-exposed, or polymictic lakes may be fully mixed during some or all seasons. Record whether stratification is present and the depths of the thermocline boundaries.

9. Quality Control

- Calibrate the sonde on the day of profiling; record calibration results on the calibration log
- Allow adequate sensor equilibration time at each depth (minimum 30 seconds; 60 seconds at the first depth and near the thermocline)
- Record an ascending profile periodically to check for sensor drift during the profile
- Verify depth markings on the cable against a tape measure at least once per season
- If the sonde includes an internal depth (pressure) sensor, compare cable-marked depth to the sensor depth at several points; discrepancies greater than 0.3 m require investigation
- For long-term monitoring, profile at the same station, same time of day (within 2 hours), and same depth intervals for comparability

10. References

Kalff, J. (2002). *Limnology: Inland water ecosystems*. Prentice Hall.
Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems* (3rd ed.). Academic Press.

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F200	Eureka Manta2 Multiprobe	Sonde calibration and operation
F207	Secchi Disk	Transparency measurement
F300	Surface Water Sampling	Discrete-depth water samples
F305	Sediment Sampling	Lake sediment collection
L300	pH and Conductivity (Bench)	Lab verification of field readings
L310	Chlorophyll Extraction	Lab chlorophyll from depth samples

12. Training Record

Before performing lake profiling independently, personnel must:

1. Read this SOP, SOP-F001, and SOP-F200 in full
2. Complete boat safety orientation (PFD use, boat handling, man- overboard procedures) for the vessel type used at the site
3. Observe a trained operator perform a complete depth profile including sonde calibration, Secchi measurement, and data recording
4. Perform a supervised profile and demonstrate proper sensor equilibration timing and depth-interval decisions at the thermocline
5. Be signed off by the PI or field lead

13. Appendix A: Profiling Data Sheet

Print on waterproof paper. One sheet per station per date.

Station ID: _____ **Date:** _____ **Time start:** _____ **Time end:** _____

GPS: Lat _____ Lon _____ **Total depth (m):** _____ **Secchi depth (m):** _____

Weather: Sky _____ Wind _____ Air temp _____ C

Sonde ID: _____ **Calibration date:** _____

Depth (m)	Temp (C)	DO (mg/L)	DO (%)	pH	SpC (uS/cm)	Turb (NTU)	Chl (ug/L)	Notes
0.5								
1.0								
1.5								
2.0								
2.5								
3.0								
3.5								
4.0								
5.0								
6.0								
7.0								
8.0								
9.0								
10.0								

Observer: _____

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F307

Wetland Delineation

Standard Operating Procedure

SOP Number	SOP-F307
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Author(s)	Marc Los Huertos
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1. Purpose

This SOP describes the field and office procedures for conducting a wetland delineation using the three-parameter approach established by the US Army Corps of Engineers (USACE). A jurisdictional wetland must exhibit all three criteria simultaneously: hydrophytic vegetation, hydric soils, and wetland hydrology (US Army Corps of Engineers, 1987). Delineation establishes the boundary between jurisdictional wetlands (and other waters of the US) and upland areas, which is a prerequisite for regulatory compliance under Section 404 of the Clean Water Act.

Wetland delineation at EA Program sites supports baseline characterization, impact assessment, restoration planning, and student training in applied environmental science.

2. Scope

- **Applies to:** Freshwater palustrine wetlands (marshes, swamps, riparian wetlands, vernal pools, seeps) at EA Program field sites in the Arid West region of California. This SOP follows the USACE Arid West Regional Supplement (US Army Corps of Engineers, 2010), which supersedes the 1987 Manual for all delineations in this region.
- **Does not apply to:** Tidal or estuarine wetlands (require separate tidal methods); riverine waters regulated solely as ordinary high water mark (OHWM) features; non-USACE state-level wetland determinations under California law (e.g., Porter-Cologne Act), which may apply different criteria.
- **Location:** All EA Program wetland field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve delineation design; coordinate permits and landowner access
Trained Personnel	Conduct three-parameter assessment per this SOP
New Students	Complete training before independent plot assessment

4. Definitions

- **Dominant species:** A species contributing 50% or more of the relative frequency or relative cover within a stratum; used in the 50/20 rule for hydrophytic vegetation determination.
- **Facultative (FAC):** A wetland indicator status category applied to plant species that occur in both wetland and upland habitats with roughly equal frequency (34–66% occurrence in wetlands); neither confirms nor denies wetland conditions when dominant.
- **Facultative upland (FACU):** Plants that usually occur in uplands (67–99% upland occurrence) but are occasionally found in wetlands; their dominance is evidence against hydrophytic vegetation.
- **Facultative wetland (FACW):** Plants that usually occur in wetlands (67–99% wetland occurrence); their dominance is strong evidence for hydrophytic vegetation.
- **Hydric soil:** A soil that formed under conditions of saturation, flooding, or ponding long enough to develop anaerobic conditions in the upper part; identified by USACE hydric soil field indicators (e.g., depleted matrix, redox concentrations, sulfidic odor); a required criterion for wetland delineation (US Army Corps of Engineers, 1987).
- **Hydrophytic vegetation:** Plant community dominated (by the 50/20 rule) by species with a wetland indicator status of OBL, FACW, or FAC; a required criterion for wetland delineation.
- **Obligate wetland (OBL):** Plant species that occur almost always (> 99% of the time) in wetlands; their dominance is the strongest evidence for hydrophytic vegetation.
- **Ordinary high water mark (OHWM):** The line on the shore established by the fluctuations of water leaving physical characteristics such as a clear natural line impressed on the bank, shelving, destruction of terrestrial vegetation, or presence of litter and debris; used to define the lateral extent of jurisdictional waters.
- **Problem area:** A site where one or more of the three wetland criteria are not clearly met or where atypical conditions (drought, altered hydrology, recent disturbance) complicate standard application of the three-parameter approach; requires additional documentation and may require a USACE district determination.
- **Sample plot (data point):** A defined area (typically 5 m radius for vegetation; a single soil pit for soils; a 10 m radius for hydrology indicators) within which all three wetland criteria are assessed together.
- **Upland:** An area that does not meet one or more of the three required wetland criteria; not subject to Section 404 jurisdiction.
- **Wetland boundary:** The line on the landscape separating areas that meet all three wetland criteria from areas that do not; the primary product of a wetland delineation.
- **Wetland indicator status (WIS):** A USACE classification of plant species by their probability of occurrence in wetlands: OBL (obligate wetland), FACW (facultative wetland), FAC (facultative), FACU (facultative upland), UPL (upland). Current designations are maintained by the USACE National Wetland Plant List (NWPL).

5. Safety and Hazards

⚠ Hazards

- **Physical:** Unstable substrates (soft muck, quicksand-like conditions in saturated soils); risk of sinking, tripping on submerged debris, or falling. Standing water may conceal holes and channels.
- **Biological:** Mosquitoes (disease vectors including West Nile virus), ticks (Lyme disease, RMSF), venomous snakes, poison oak/ivy (*Toxicodendron* spp.), stinging nettles, and waterborne pathogens. Avoid direct skin contact with standing water.
- **Environmental:** Heat stress in summer; hypothermia in winter and spring. Wetlands offer little shade in open marsh environments.

5.1. Required PPE

☐ Rubber boots or chest waders (knee boots minimum)
☐ Nitrile gloves (for soil pit work)
☐ Long sleeves and long pants (tick and mosquito protection)
☐ Insect repellent (DEET or picaridin)
☐ Sun protection (hat, sunscreen SPF 30+)
☐ Personal flotation device (if water depth exceeds knee level or if working from a watercraft)

5.2. Emergency Procedures

Work in pairs at minimum. If a team member becomes stuck in soft substrate, distribute weight on a board, backpack, or rope and pull laterally rather than vertically. Refer to SOP-F001 Field Safety.

6. Equipment and Materials

Item	Notes
USACE 1987 Wetland Delineation Manual	Required reference; bring to field
USACE Arid West Regional Supplement (2010)	Required for all CA Arid West delineations
Munsell Soil Color Charts (moist)	See SOP-F405
Soil auger or push probe (60 cm minimum)	For reading soil profile to 50 cm depth
Tile spade or sharpshooter spade	For opening soil pit
30 cm metric ruler	Depth to WTHS and horizon thickness
Flagging tape (two colors: wetland, upland)	For marking boundary and sample plots
GPS unit or smartphone with mapping app	See SOP-F006
50 m fiberglass measuring tape	Transect layout
Plant identification keys (regional)	*Jepson Manual* for California
National Wetland Plant List (NWPL)	Current WIS designations (usace.army.mil)

Item	Notes
Hydric Soils Field Indicators guide	USDA NRCS Field Indicators of Hydric Soils
Wetland delineation field data sheets	See Appendix A
Clipboard, mechanical pencils, waterproof paper	Rite in the Rain recommended
Camera (waterproof or in dry bag)	Photo-document each sample plot
Compass	Transect bearings
10x hand lens	Plant identification

7. Estimated Time

Task	Time (2-person crew)
Office pre-work (maps, records, NWPL lookup)	2–4 hours
Site reconnaissance walk	30–60 minutes
Sample plot assessment (per plot)	15–30 minutes
Boundary flagging and GPS waypoints	1–2 hours per 100 m boundary
Field data compilation	30–60 minutes
Office post-work (report, map, data entry)	4–8 hours

8. Procedure

8.1. Step 1: Pre-Field Office Work

Before entering the field, complete the following:

1. Obtain and review available information:
   - USFWS National Wetlands Inventory (NWI) maps (fws.gov/wetlands)
   - USDA Web Soil Survey (websoilsurvey.nrcs.usda.gov) – note mapped hydric soils and map unit descriptions
   - USGS topographic maps (7.5-minute quadrangles)
   - Aerial/satellite imagery (Google Earth historical layers) – look for inundation signatures, dark organic soils, and characteristic vegetation patterns
   - NOAA precipitation records for the region to assess whether current year is a drought, normal, or wet year (relevant to atypical situation determinations)
2. Generate a base map at a scale appropriate for the site (1:2,400 for small sites; 1:4,800 for large sites). Include NWI polygons, soil map unit boundaries, topographic contours, and property boundaries.
3. Look up current wetland indicator status (WIS) for all plant species expected at the site on the USACE National Wetland Plant List (plants.usace.army.mil).
4. Identify the appropriate USACE district and confirm whether a preliminary jurisdictional determination (PJD) or approved jurisdictional determination (AJD) is ultimately required.

8.2. Step 2: Site Reconnaissance

On arrival at the field site, conduct a reconnaissance walk before placing any sample plots:

1. Walk the entire site perimeter and any internal drainages or basins to develop a mental model of landscape position, drainage patterns, and vegetation transitions.
2. Identify probable wetland areas (standing or ponded water, dark organic soils visible at surface, wet-adapted vegetation) and probable upland areas (well-drained soils, upland vegetation).
3. Identify any obvious disturbances (ditching, filling, altered hydrology, grazing, invasive species) that could create problem areas.
4. Note the approximate location of vegetation transitions, which are often close to the wetland boundary.
5. Sketch a rough site map with probable wetland/upland zones and planned transect routes.

8.3. Step 3: Establish Transects and Sample Plots

1. Establish transects perpendicular to the suspected wetland boundary, spaced to adequately characterize the site. For EA Program sites, transects are typically spaced 25–50 m apart; closer spacing is used in complex or small-scale boundaries.
2. On each transect, place sample plots at:
   - A clearly wetland location (well within the apparent wetland)
   - A clearly upland location (well outside the apparent wetland)
   - One or more locations straddling the suspected boundary
3. Flag each sample plot center with flagging tape and record GPS coordinates. Use a consistent color for wetland plots and a different color for upland plots.
4. Number all sample plots sequentially and record them on the base map.

8.4. Step 4: Assess Hydrophytic Vegetation

At each sample plot (approximately 5 m radius), assess vegetation in all strata present (tree, shrub/sapling, herb, woody vine):

1. List all plant species in each stratum. Identify to species level using regional keys (*Jepson Manual* for California).
2. Estimate percent areal cover for each species within each stratum to the nearest 5–10%.
3. For each species, record the current wetland indicator status (WIS) from the USACE NWPL for the Arid West region.
4. Determine dominant species in each stratum using the **50/20 rule**:
   a. Rank species within each stratum by decreasing percent cover.

b. Add species from highest to lowest cover until the cumulative cover total reaches 50% or more of the total stratum cover.
c. Include one additional species if it contributes at least 20% of the total stratum cover.
d. All species added in steps (b) and (c) are dominant species for that stratum.

5. Tally the number of dominant species that are OBL, FACW, or FAC (hydrophytic) and the number that are FACU or UPL (upland).

6. The **hydrophytic vegetation criterion is met** if more than 50% of the dominant species across all strata are OBL, FACW, or FAC.

> **FAC-Neutral Test**
>
> In borderline cases where FAC species are dominant, apply the FAC-neutral test: exclude all FAC species and recalculate dominance using only OBL, FACW, FACU, and UPL species. If the remaining dominants are predominantly hydrophytic, the criterion is still met. The FAC-neutral test is described in the Arid West Regional Supplement (US Army Corps of Engineers, 2010).

8.5. Step 5: Assess Hydric Soils

At each sample plot, excavate a soil pit to at least 50 cm depth (or to the water table or bedrock, whichever is shallower):

1. Excavate with a sharpshooter spade. If the pit fills with water, record the depth to water table and excavate no deeper.

2. Describe the soil profile using the form in Appendix A:

   - Horizon designation (A, B, C), depth (cm), and thickness
   - Munsell color (matrix and mottles/redoximorphic features) per SOP-F405 – record moist colors only
   - Texture (field ribbon test; see SOP-F401)
   - Structure, consistence, roots

3. Check for USDA NRCS Field Indicators of Hydric Soils (Version 8.2 or current). For the Western Mountains, Valleys, and Coast (WMVC) region, the most applicable indicators include:

Indicator Code	Name	Key Feature
A1	Histosol	Organic soil (Oi, Oe, or Oa horizon > 40 cm)
A2	Histic epipedon	Organic surface layer 20–40 cm
A3	Black histic	Black (10YR 2/1) organic layer >= 20 cm
A11	Depleted below dark surface	Dark A over low-chroma (chroma <= 2) matrix

Indicator Code	Name	Key Feature
F2	Loamy gleyed matrix	Gley hue matrix in loamy soils
F3	Depleted matrix	> 60% low-chroma (chroma <= 2) matrix
F6	Redox dark surface	Dark A over depleted or reduced matrix
F8	Redox depressions	Redox concentrations in depressions

4. The **hydric soil criterion is met** if one or more applicable field indicators are present at the appropriate depth. For most indicators in the Arid West Supplement, this means within the upper 30 cm of the soil profile (US Army Corps of Engineers, 2010).

> ⚠ Problematic Hydric Soil Situations
>
> Soils in the Arid West may have low-chroma colors from parent material (e.g., some shales, volcanic tuffs) rather than from saturation. If in doubt, check for redox concentrations (bright orange/red Fe masses) as corroborating evidence, and note the soil series and parent material from Web Soil Survey.

8.6. Step 6: Assess Wetland Hydrology

At each sample plot, document evidence of wetland hydrology. The USACE defines wetland hydrology as saturation within 30 cm of the surface for a continuous period of at least 5% of the growing season (US Army Corps of Engineers, 1987, 2010).

1. Record depth to surface water or saturated soil (standing water in the pit).
2. Check for **primary hydrology indicators** (in order of reliability):

Indicator	Description
Surface water	Standing water present at time of visit
High water table	Water within 30 cm of surface in soil pit
Saturation	Saturated soil within 30 cm (water oozes when squeezed)

3. Check for **secondary hydrology indicators** (two or more required when primary indicators are absent):

Indicator	Description
Water marks	Staining on tree trunks or structures
Sediment deposits	Fresh mineral sediment on leaves or stems
Drift lines	Lines of debris deposited by receding water
Waterlogged soils	Confirmed by soil pit; no free water but saturated
Water-stained leaves	Grayish-brown discoloration of litter

Indicator	Description
Oxidized rhizospheres	Orange halos around living roots in grey matrix
FAC-neutral plant communities	Vegetation dominance by OBL/FACW species
Crayfish burrows	Chimney mounds indicating seasonal saturation

4. For arid-region sites, pay particular attention to oxidized rhizospheres (a primary indicator in the Arid West Supplement) and drift/wrack lines along swales and basins.
5. The **wetland hydrology criterion is met** if at least one primary indicator is present, or at least two secondary indicators are present, at the time of visit; or if the site has documented evidence of seasonal or periodic inundation from well records or gauges (see SOP-F306).

> **Arid-Region Hydrology Timing**
>
> At arid and semi-arid sites, surface water and high water tables may only be present during a brief seasonal window. When conducting delineations outside the wet season, rely on secondary indicators and, where available, water level logger data from monitoring wells. Document the date of the visit and regional precipitation conditions in all field data sheets. See SOP-F306 for long-term hydrology monitoring methods.

8.7. Step 7: Apply the Three-Parameter Test

At each sample plot, compile results from Steps 4–6:

1. Record whether each of the three criteria is met (yes/no) on the field data sheet (Appendix A).
2. **A sample plot is classified as wetland if and only if all three criteria are met.** If any criterion is absent, the plot is classified as upland (or a transitional/problem area if two of three are met).
3. If two of three criteria are met, flag the plot as a potential **problem area** and document the specific conditions. Common problem area types include:
   - Recently disturbed wetlands (vegetation removed, soil disturbed)
   - Drought-year assessments (hydrology not apparent)
   - Altered hydrology (ditched, drained, or impounded areas)
   - Atypical vegetation (recent burn, mowing, herbicide)
4. For problem areas, apply the appropriate USACE atypical situation procedures as described in the Regional Supplement (US Army Corps of Engineers, 2010).

8.8. Step 8: Flag and Map the Wetland Boundary

1. Between each pair of adjacent wetland and upland sample plots on a transect, locate the wetland boundary by walking perpendicular to the transect and reassessing the three criteria at intermediate points until the line of change is identified.

2. Flag the wetland boundary with a distinct color (use a third color different from the wetland and upland plot flags). Place flags every 5–10 m or at every change in direction.
3. Record GPS waypoints at each boundary flag using SOP-F006. Waypoint naming convention: `WB_[transect]-[sequence]` (e.g., `WB_T1-01`).
4. Photograph the boundary flags from multiple angles; include a scale bar (person or measuring tape) in at least one photo per transect.
5. Sketch the boundary on the field base map, noting all sample plot locations and transect lines.

8.9. Step 9: Field Quality Review

Before leaving the site, complete the following checks:

☐ All sample plots have GPS coordinates recorded
☐ Every plot has complete data for all three criteria
☐ All boundary flags are GPS-logged
☐ At least two representative photographs per sample plot
☐ Boundary is continuous between all transects (no gaps)
☐ Problem areas are flagged and documented
☐ Equipment has been decontaminated per SOP-F007

8.10. Step 10: Post-Field Office Work

1. Enter all field data into the project database within 48 hours per SOP-L803.
2. Import GPS waypoints and generate a georeferenced boundary map in GIS software (see SOP-L804).
3. Overlay the delineated boundary on aerial imagery, NWI maps, and soil survey maps to check for consistency; note and document any discrepancies.
4. Calculate wetland area (hectares) from the GIS boundary polygon.
5. Compile the delineation report with:
   - Site description and location map
   - Methods summary (reference to this SOP)
   - Summary table of all sample plots and three-parameter results
   - Wetland boundary map
   - Photographs
   - Conclusions regarding jurisdictional status
   - Any problem areas and how they were addressed
6. Archive all raw field data sheets (scanned), GPS track files, GIS shapefiles, and the final report per project data management protocols.

9. Quality Control

- A minimum of two observers must conduct the vegetation assessment at each sample plot; independent species lists must be reconciled before leaving the plot.
- Munsell color readings must be made by two observers and must agree within one value and one chroma step; discrepancies require a consensus re-read per SOP-F405.
- GPS boundary waypoints must be collected with horizontal accuracy < 3 m (WAAS-enabled or equivalent). Record DOP and fix type.
- At 10% of sample plots, return the following day and re-assess the hydrophytic vegetation criterion independently; dominant species lists must agree within 10 percentage points cover.
- Final delineation maps must be reviewed by the PI before submission to any regulatory agency or inclusion in project reports.

10. Data Management

1. Raw field data sheets must be scanned and uploaded to the project folder within 48 hours of fieldwork.
2. Enter tabular sample plot data into the project database using the standardized template in Appendix B.
3. GPS tracks and waypoints must be exported and backed up immediately on return from the field.
4. All GIS boundary files must be saved in both shapefile and GeoJSON formats and versioned in the project Git repository.
5. Version-control all analysis scripts and report documents per SOP-L801.

11. References

- US Army Corps of Engineers (1987)
- US Army Corps of Engineers (2010)
- Mitsch & Gosselink (2015)

Mitsch, W. J., & Gosselink, J. G. (2015). *Wetlands* (5th ed.). John Wiley & Sons.

US Army Corps of Engineers. (1987). *Corps of engineers wetlands delineation manual* (Technical Report Y-87-1). Waterways Experiment Station, US Army Corps of Engineers. https://www.lrl.usace.army.mil/Portals/64/docs/regulatory/Wetland-Delineation-Manual.pdf

US Army Corps of Engineers. (2010). *Regional supplement to the corps of engineers wetland delineation manual: Arid west region (version 2.0)* (ERDC/EL TR-08-28). Engineer Research; Development Center, US Army Corps of Engineers.

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Boundary waypoint collection
F007	Field Decontamination	Between-site gear decontamination
F306	Wetland Hydrology Monitoring	Hydrology criterion support; long-term records
F405	Soil Color (Munsell)	Required for hydric soil assessment
F407	Soil Profile Description	Soil pit documentation
L803	Data Entry and Database Management	Post-field data entry
L804	GIS and Spatial Data	Boundary mapping and area calculation

13. Training Record

Before conducting wetland delineations independently, personnel must:

1. Read this SOP, SOP-F001, SOP-F306, and SOP-F405 in full
2. Review the USACE 1987 Manual and Arid West Regional Supplement (US Army Corps of Engineers, 1987, 2010)
3. Complete at least one supervised delineation transect, including independent application of the 50/20 rule and hydric soil field indicators, with results reviewed by the PI
4. Demonstrate Munsell color reading proficiency per SOP-F405 inter-observer calibration requirements
5. Successfully enter and map a practice delineation dataset in GIS
6. Be signed off by the PI or field lead

14. Appendix A: Field Data Sheet

Print double-sided on waterproof paper (Rite in the Rain). Complete one sheet per sample plot.

Site: _____ **Date:** _____ **Observers:** _____

Plot ID: _____ **Transect:** _____ **GPS (lat/lon):** _____

Landscape position: upland / footslope / toeslope / depression / flat / other: _____

14.1. A. Hydrophytic Vegetation

Species	Stratum	% Cover	WIS

50/20 Rule Tally: Dominants that are OBL/FACW/FAC: _____ / Total dominants: _____

Hydrophytic vegetation criterion met? YES / NO / Problem area

14.2. B. Hydric Soils

Depth to water table or bedrock: _____ cm

Horizon	Depth (cm)	Munsell (moist)	Texture	Notes

Hydric soil field indicator(s) present: _____

Hydric soil criterion met? YES / NO / Problem area

14.3. C. Wetland Hydrology

Primary indicators (check all present):

- ☐ Surface water (depth: _____ cm)
- ☐ High water table (depth: _____ cm)
- ☐ Saturation within 30 cm

Secondary indicators (check all present):

- ☐ Water marks [ ] Sediment deposits [ ] Drift lines
- ☐ Water-stained leaves [ ] Oxidized rhizospheres
- ☐ Waterlogged soils [ ] Crayfish burrows [ ] FAC-neutral community

Wetland hydrology criterion met? YES / NO / Problem area

14.4. D. Determination

All three criteria met? YES – WETLAND / NO – UPLAND / PROBLEM AREA

Notes / atypical situation documentation:

---

15. Appendix B: Sample Plot Summary Table Template

Enter into `data/raw/wetland_delineation_[site]_[date].csv`:

```
plot_id,transect,lat,lon,veg_criterion,soil_criterion,hydro_criterion,
determination,dominant_species,hydric_indicator,primary_hydro_indicator,
notes,observers,date
```

Column definitions are in the project data dictionary (`data/metadata/wetland_delineation_datadictionary.cs`

16. Appendix C: Wetland Indicator Status Quick Reference

Code	Category	Wetland Occurrence	Delineation Implication
OBL	Obligate wetland	> 99%	Strong evidence for hydrophytic veg
FACW	Facultative wetland	67–99%	Evidence for hydrophytic veg
FAC	Facultative	34–66%	Equivocal; apply FAC-neutral test
FACU	Facultative upland	1–33%	Evidence against hydrophytic veg
UPL	Upland	< 1%	Strong evidence against hydrophytic veg

Current WIS designations for the Arid West region are available at plants.usace.army.mil. Always use the regional list, not the national list, for California delineations.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F308

eDNA Water Sample Collection

Standard Operating Procedure

SOP Number	SOP-F308
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
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1. Purpose

This procedure describes site-specific water collection strategies for environmental DNA (eDNA) sampling across the range of aquatic habitats encountered in EA Program projects: wadeable streams, non-wadeable rivers, lakes and reservoirs, ponds and vernal pools, and constructed wetlands. It supplements SOP-F700 eDNA Field Sampling and Preservation, which covers the core filtration, preservation, and contamination- control protocols that apply to all eDNA water samples regardless of habitat type.

The separation recognizes that contamination prevention, filtration, and preservation (SOP-F700) are universal, while the physical act of collecting water varies with habitat. This SOP addresses

the collection step: where to sample within a water body, how many samples to collect, and how to adapt the approach to different hydrological settings (Deiner et al., 2017; Goldberg et al., 2016).

2. Scope

- **Applies to:** Water collection for eDNA analysis at all EA Program aquatic field sites. Once water is collected, filtration and preservation follow SOP-F700.
- **Does not apply to:** Soil eDNA or sediment eDNA sampling (see SOP-F701); airborne eDNA; tissue-based DNA sampling; lab-side filtration and extraction (see SOP-L700)
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design, confirm permits
Trained Personnel	Collect eDNA water samples per this SOP and SOP-F700
New Students	Complete SOP-F700 contamination training before participating

4. Definitions

- **Capture efficiency:** The proportion of target eDNA in the water column that is retained on a filter during collection; influenced by filter pore size, clogging, sample volume, and the physical state (dissolved vs. particulate) of the eDNA.
- **Closed-system filtration:** An eDNA collection method in which water passes through a filter enclosed in a sterile capsule (e.g., Sterivex) without contact with the external environment; minimizes contamination risk compared to open filtration.
- **Decontamination:** The removal or inactivation of exogenous DNA from sampling equipment and surfaces using bleach (10% sodium hypochlorite), UV irradiation, or other validated methods; essential between sites to prevent cross-contamination.
- **eDNA (environmental DNA):** Genetic material shed into the environment by organisms through mucus, feces, gametes, skin cells, and other biological material; collected from water without direct capture of the target organism.
- **Field blank:** A bottle of laboratory-grade water opened and handled at the field site under identical conditions to real samples; processed through the full filtration and extraction workflow to detect field contamination; must yield no target amplification.
- **Filter pore size:** The nominal diameter of pores in the filter membrane; 0.45 µm is the standard for eDNA capture from surface water, retaining bacteria, cells, and particulate-bound DNA while allowing water to pass.
- **Longmire's buffer:** A non-freezing preservation solution (containing EDTA, Tris, NaCl, SDS, and sucrose) used to preserve eDNA on filters in the field when immediate freezing is not possible; inactivates nucleases and inhibits microbial degradation.

- **Peristaltic pump:** A battery-operated pump used to draw large volumes of water through a filter in the field without the water contacting the pump mechanism; preferred for high-volume eDNA filtration because it reduces contamination risk.
- **Sterivex filter:** A commercially available enclosed 0.22 µm polysulfone cartridge filter; widely used in eDNA studies because the sealed design minimizes contamination, allows direct addition of preservation buffer, and is compatible with standard DNA extraction kits.
- **Target species detection:** The determination of presence or absence of a specific taxon in a water sample based on amplification of taxon-specific eDNA; sensitivity depends on eDNA shedding rate, water volume filtered, detection method (PCR, ddPCR, metabarcoding), and inhibitor load in the extract.

5. Safety and Hazards

All safety requirements from SOP-F700 and SOP-F001 apply. Additional hazards specific to collection settings:

⚠ Additional Hazards by Habitat

- **Non-wadeable rivers and lakes:** Boat access required; drowning risk. PFDs mandatory.
- **Vernal pools:** Often in remote locations with limited access; fragile habitat. Minimize trampling of pool margins.
- **Constructed wetlands:** May contain treated wastewater. Wear doubled gloves and avoid ingestion or skin contact.

6. Equipment and Materials

All sterile sampling supplies, preservatives, and field documentation are listed in SOP-F700 Section 5. Additional items for specific habitats:

Item	Habitat	Notes
Extension pole with sterile bottle clamp	Non-wadeable streams, bridges	For mid-channel collection without entering the water
Van Dorn or Kemmerer bottle (dedicated to eDNA, pre-sterilized)	Lakes, deep pools	For discrete-depth collection
Peristaltic pump with sterile tubing	Any depth-specific application	Single-use tubing per sample
Telescoping boat hook	Boat-based sampling	For retrieving floating bottles

7. Procedure

7.1. General Principles (All Habitats)

These principles supplement SOP-F700:

- **Sample where the target organism is most likely detected.** eDNA concentration varies spatially: it is higher near organism activity centers (spawning areas, resting pools, feeding zones) and downstream of occupied habitat in lotic systems (Jane et al., 2015).
- **Sample volume:** Collect at least 1 L per replicate (the volume actually filtered may be less if the filter clogs). Plan for 3 replicates per site unless the study design specifies otherwise.
- **Sampling order:** Within a day, sample sites from least likely to most likely occupied (downstream to upstream in a connected system, or reference sites before impacted sites) to minimize carry-over contamination (Goldberg et al., 2016).

7.2. Wadeable Streams

1. Approach the sampling point from downstream
2. Face upstream; submerge the sterile bottle to 10–20 cm below the surface, tilted slightly into the current
3. Fill to the 1 L mark and cap immediately
4. For a reach-level composite: collect 250 mL from each of 4 microhabitats (pool tail, riffle margin, run, and backwater) and combine into one bottle
5. Proceed to filtration per SOP-F700 Section 6.4

7.3. Non-Wadeable Rivers and Bridges

1. From a bridge or bank, lower a sterile bottle attached to an extension pole or a pre-sterilized Van Dorn sampler to mid-depth
2. If using an extension pole: open the bottle at depth, fill by current flow, and cap using a pull-string mechanism or retrieve and cap immediately
3. Record the approximate depth and distance from shore
4. Decontaminate the extension pole between sites per SOP-F700 Section 6.7

7.4. Lakes and Reservoirs

1. Collect samples from a boat positioned over the target area (e.g., littoral zone for nearshore species, open water for pelagic species)
2. For surface eDNA: submerge a sterile bottle to 15–30 cm below the surface from the upwind side of the boat to avoid contamination from the vessel
3. For depth-specific eDNA (e.g., below the thermocline): deploy a pre-sterilized Van Dorn bottle to the target depth, trip, and retrieve. Transfer to a sterile sample bottle.
4. If sampling multiple depths, work from deepest to shallowest to minimize disturbance
5. Record collection depth, water depth, and location (GPS)

7.5. Ponds and Vernal Pools

1. Approach slowly to minimize sediment disturbance (eDNA can be resuspended from sediment, creating false-positive risk for species no longer present) (Turner et al., 2015)
2. Collect water from 5–10 cm below the surface near the center or deepest point of the pool
3. For very shallow pools (less than 15 cm), tip the bottle on its side to fill without disturbing the bottom

4. If the pool is small (less than 10 m^2), a single 1 L grab from the center is acceptable; for larger pools, composite from 3–5 points around the perimeter

7.6. Constructed Wetlands and Treatment Systems

1. Wear doubled nitrile gloves; these systems may contain treated wastewater, elevated pathogen loads, or chemical residues
2. Collect from the outflow or from an open water area; avoid collecting from areas with dense emergent vegetation where filtration may be rapid
3. Collect a field blank at the site using the molecular-grade DI water from the field kit (this is especially important in constructed systems where background DNA loads are high)

7.7. Filtration and Preservation

After collection in any habitat, proceed immediately to filtration and preservation per SOP-F700 Section 6.4. If on-site filtration is not possible, keep samples on ice and filter within 6 hours.

8. Quality Control

All QC requirements from SOP-F700 Section 8 apply. Additional habitat-specific considerations:

- For lake depth samples, collect a field blank at the same depth using the same equipment to detect cross-contamination from the Van Dorn or pump
- For bridge sampling with an extension pole, collect an equipment blank (DI water poured through the pole apparatus) at least once per event
- When sampling connected sites in a watershed, record the order of site visits and the time elapsed between sites

9. References

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10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site decon
F008	Sample Labeling and COC	Sample ID and chain of custody
F300	Surface Water Sampling	General water collection
F700	eDNA Field Sampling and Preservation	Core eDNA protocol (filtration, preservation, contamination control)
L700	DNA Extraction	Lab-side extraction
L706	eDNA Metabarcoding	Full lab workflow

11. Training Record

Before collecting eDNA water samples independently, personnel must:

1. Complete all training requirements in SOP-F700 Section 11
2. Review the habitat-specific collection methods in this SOP for the site(s) to be sampled
3. If boat-based sampling is required, complete boat safety orientation (PFD use, boat handling)
4. Demonstrate proper sterile collection technique (bottle handling, depth sampling) under supervision
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F400

Soil Sampling and Processing

Standard Operating Procedure

SOP Number	SOP-F400
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the procedures for collecting, processing, and storing soil samples for laboratory analysis. It covers field sampling using a soil auger, sample homogenization, sieving, and subdivision for biological assays, carbon analysis, and other soil properties (Food and Agriculture Organization of the United Nations, 2024; Robertson et al., 1999).

2. Scope

- **Applies to:** All field-based soil sampling for EA Program research, including photovoltaic panel studies, agricultural plots, and restoration sites
- **Does not apply to:** Deep soil coring (> 30 cm) requiring mechanized equipment; contaminated site sampling requiring chain-of-custody protocols
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate sampling logistics, ensure protocol adherence
Trained Personnel	Collect and process samples according to this SOP
New Students	Complete training before independent sampling

4. Definitions

- **Bulk sample:** A large, mixed sample collected from multiple locations within a sampling unit and combined before analysis; represents the average composition of the unit rather than any single point.
- **Composite sample:** A sample formed by combining and mixing a defined number of subsamples from a plot or area; reduces analytical cost while improving spatial representativeness; the number and pattern of subsamples must be recorded (Robertson et al., 1999).
- **Core (soil core):** A cylindrical soil sample extracted with a push-probe or hammer corer; preserves the depth profile and allows depth-discrete sampling; also used for bulk density determination (see SOP-F402).
- **Fine earth fraction:** Soil material passing through a 2 mm sieve; sand, silt, and clay particles; the standard fraction for chemical analysis; gravel and coarser material are removed and their mass fraction recorded (Robertson et al., 1999).
- **Grab sample:** A single, point-in-time sample collected from one location; appropriate when spatial variability is low or when individual point values are required; contrasted with composite sampling.
- **Pedogenic horizon:** A layer of soil with distinct physical, chemical, or biological properties developed through soil-forming processes; designated by letter symbols (O, A, E, B, C, R); sampled discretely in profile descriptions (see SOP-F407).
- **Push-probe (soil probe):** A hollow stainless steel tube pushed into the soil by hand or with a slide hammer to collect an intact soil core; the standard tool for rapid depth-discrete sampling in fine-textured soils without large stones (Robertson et al., 1999).
- **Sampling depth increment:** A defined depth interval (e.g., 0–10 cm, 10–20 cm) from which soil is collected; depth increments must be consistent within a study for results to be comparable (Robertson et al., 1999).

- **Subsample:** One of the individual small samples taken from within a plot that are combined to form a composite; also refers to a representative aliquot drawn from a bulk sample for laboratory analysis.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Repetitive strain from augering, sharp edges on sieving equipment, trip hazards at field sites.
- **Biological:** Soil-borne pathogens, tick and insect exposure at field sites.
- **Chemical:** Potential exposure to contaminated soils at some sites.

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Nitrile or latex gloves
- ☐ Sun protection (hat, sunscreen)
- ☐ Safety glasses (when sieving)

Refer to SOP-F001 Field Safety for general field safety procedures.

6. Equipment and Materials

Item	Notes
Soil auger (Dutch or bucket)	Diameter 5–10 cm; clean between sites
Push-probe (soil core sampler)	For depth-discrete sampling
Rubber mallet or slide hammer	For driving probe into hard soils
Stainless steel bowl or plastic bucket	For compositing subsamples
Stainless steel sieve (2 mm)	For fine earth fraction
Ziploc bags or Whirl-Pak bags (labeled)	For each sample
Permanent markers and labels	Waterproof; include site, depth, date
Cooler with ice	For biological subsamples (enzyme assays, MBC, microbial DNA)
Field notebook	Per SOP-F003
GPS unit	Per SOP-F006
Isopropyl alcohol and paper towels	For decontaminating tools between sites

7. Sampling Design

Before sampling, confirm the sampling design with the PI and reference SOP-F005 Random Sampling Design for plot layout and randomization (Robertson et al., 1999). Record the following in

the field notebook:

- Site name and GPS coordinates
- Sampling date and personnel
- Target depth(s) or horizon(s)
- Number of subsamples per composite
- Auger/probe diameter
- Any deviations from the sampling plan

8. Procedure

8.1. Field Collection

1. Navigate to the sampling location per SOP-F006.
2. Record the GPS coordinates of each sample location.
3. Inspect the surface for hazards. Clear surface litter only if required by the protocol; record whether litter was cleared.
4. Insert the auger or push-probe vertically to the target depth. For depth-increment sampling:
    - Collect the 0–10 cm increment first. Remove and place in a labeled sample container.
    - Re-insert the auger and collect the 10–20 cm increment. Place in a separate container.
    - Repeat for additional depth increments as required by the sampling plan.
5. For composite samples, collect subsamples at each designated subplot location (typically 5–10 per plot) and combine in the bowl or bucket before homogenizing.
6. Record the following for each sample:
    - Sample ID per the project labeling scheme
    - GPS coordinates
    - Depth increment
    - Observed soil conditions (color, texture, moisture, visible roots, stoniness)
    - Any anomalies (disturbance, compaction, visible contamination)

8.2. Field Homogenization

7. Break up clods and remove surface litter, roots greater than 5 mm, visible rocks, and obvious debris by hand (Robertson et al., 1999).
8. Mix the soil thoroughly in the bowl or bucket until the color and texture appear uniform.
9. If the sample is to be composited across subsamples, combine all subsamples before mixing.

8.3. Sieving (Field or Lab)

Sieving can be performed in the field (when fresh soil is required for biological assays) or in the lab after air-drying (for chemical analysis) (Food and Agriculture Organization of the United Nations, 2024; Robertson et al., 1999):

1. Pass the soil through a 2 mm stainless steel sieve.

2. Weigh and record the mass of coarse fragments (> 2 mm) retained if the project protocol requires correction for coarse fragment content.
3. The fine earth fraction (< 2 mm) is the standard fraction for all chemical and biological analyses.

8.4. Sample Subdivision for Multiple Analyses

Divide the sieved fine earth fraction into the required subsamples before further drying or freezing (Robertson et al., 1999):

Subsample type	Treatment	Storage
Microbial biomass C/N	Field-moist; process within 24 h	Cooler 4 °C then lab
Enzyme assays	Field-moist; process within 24 h or freeze	Cooler/freezer (-20 °C)
Mineral N (KCl)	Field-moist or frozen; extract within 48 h	Cooler 4 °C
Soil moisture (gravimetric)	Field-moist	Sealed bag
Carbon/nitrogen (EA-IRMS)	Air-dry, then grind	Room temperature
pH, texture, other chemical	Air-dry	Room temperature

Biological Subsamples

Do not freeze biological subsamples unless the analysis protocol explicitly permits it.

8.5. Tool Decontamination Between Sites

Wipe the auger, probe, and bowl with isopropyl alcohol and let dry before moving to the next sampling location. This prevents cross-contamination of soils between sites and is particularly important for sites with invasive species or contamination concerns (Food and Agriculture Organization of the United Nations, 2024).

9. Quality Control

- Collect field duplicates at a rate of 1 per 10 samples to assess sampling variability (Robertson et al., 1999).
- Record all deviations from the sampling plan in the field notebook.
- Verify auger depth calibration at the start of each sampling day.
- Ensure sieve integrity (no torn or damaged mesh) before processing.

10. Waste Disposal

Waste Type	Disposal Method
Used gloves	Standard trash
Decontamination rinse water	Pour on ground away from sampling plots
Coarse fragments	Return to field site or dispose in trash

11. References

- Robertson et al. (1999)
- Food and Agriculture Organization of the United Nations (2024)

Food and Agriculture Organization of the United Nations. (2024). *Standard operating procedure for soil microbial biomass (Carbon): Chloroform fumigation-extraction method*. FAO. https://www.fao.org/soils-portal/soil-survey/soil-analysis/en/

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research*. Oxford University Press.

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F005	Random Sampling Design	Plot layout and randomization
F006	GPS and Site Navigation	Sample location coordinates
F007	Field Decontamination	Tool decontamination between sites
F402	Soil Bulk Density	Companion core-based method
F407	Soil Profile Description	Horizon-based sampling context
L400	Soil Moisture	Required for dry-weight correction

13. Training Record

Before sampling independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Participate in a supervised sampling session
4. Demonstrate proper auger technique, homogenization, and sieving
5. Be signed off by the field lead or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F401

Soil Texture Determination by Feel (Ribbon Method)

Standard Operating Procedure

SOP Number	SOP-F401
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the field determination of soil texture using the texture-by-feel (ribbon) method. This rapid, equipment-free technique classifies soil into one of 12 USDA textural classes based on how a moistened soil sample behaves when formed into a ball, extruded into a ribbon, and rubbed between the fingers (Soil Survey Division Staff, 2017; Thien, 1979). Field texturing provides an immediate estimate of sand, silt, and clay content without laboratory analysis.

2. Scope

- **Applies to:** Field characterization of mineral soils at all EA Program sites; preliminary texture assessment for soil sampling campaigns
- **Does not apply to:** Soils with very high organic matter content (>20%, e.g., peat); quantitative particle size analysis (use the hydrometer method, SOP-L401); soils with large gravel or rock fragment content (assess the fine fraction only)
- **Location:** All EA Program field sites; can also be performed on samples brought back to the lab

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Field Lead	Coordinate sampling, verify observer calibration
Trained Personnel	Perform texture assessments, record results
New Students	Complete training and calibration exercise before independent use

4. Definitions

- **Clay:** The finest soil particle size class, with equivalent spherical diameter less than 0.002 mm; imparts stickiness, plasticity, and cohesion to the moist soil; forms a long, flexible ribbon when extruded between thumb and forefinger.
- **Loam:** A textural class with roughly balanced proportions of sand, silt, and clay; exhibits moderate ribbon length (25–50 mm), moderate grittiness, and moderate stickiness; the most common agricultural soil texture.
- **Plasticity:** The ability of moist soil to be molded and deformed without cracking or crumbling; highest in clay-rich soils; assessed by rolling soil into a thread between the palms.

- **Ribbon:** The flat, continuous strip extruded when moist soil is pushed between the thumb and forefinger; ribbon length is the primary diagnostic criterion in the field texture-by-feel method; longer ribbons indicate higher clay content.
- **Sand:** Soil mineral particles with equivalent spherical diameter 0.05–2.0 mm; feels gritty between the fingers; does not form a ribbon; individual grains are visible to the naked eye.
- **Silt:** Soil mineral particles with equivalent spherical diameter 0.002–0.05 mm; feels smooth and floury when dry; slippery and slightly plastic when moist; imparts a silky texture to the soil.
- **Texture class:** A USDA classification category (e.g., sandy loam, silt clay loam, clay) based on the relative percentages of sand, silt, and clay; determined from the USDA textural triangle and confirmed by laboratory hydrometer analysis (see SOP-L401).
- **Water content (field):** The moisture status of the soil at the time of testing; texture-by-feel must be performed on soil adjusted to a defined moist or wet state to produce consistent results across operators.

5. Safety and Hazards

⚠ Hazards

- **Biological:** Soil-borne pathogens; wash hands thoroughly after handling soil
- **Chemical:** At contaminated sites, soil may contain heavy metals or other hazardous materials; wear gloves
- **Physical:** Standard field hazards (ticks, snakes, heat exposure)

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Nitrile gloves (recommended, especially at unknown or contaminated sites)
☐ Sun protection (hat, sunscreen)
☐ Hand sanitizer or soap and water for post-handling cleanup

5.2. Required Training

- SOP-F001 Field Safety

6. Background

6.1. Soil Particle Size Fractions

Soil texture is defined by the relative proportions of three mineral particle size fractions (Soil Survey Division Staff, 2017):

Fraction	Diameter	Feel
Sand	2.0–0.05 mm	Gritty; individual grains visible and felt between fingers
Silt	0.05–0.002 mm	Smooth, floury, or silky; like rubbing flour or talc
Clay	$<$ 0.002 mm	Sticky when wet; plastic and moldable; stains fingers

6.2. The USDA Textural Triangle

The 12 USDA textural classes are defined by the percentages of sand, silt, and clay. The texture-by-feel method estimates which class a soil belongs to without measuring exact percentages.

6.3. Why Field Texture Matters

- Determines water-holding capacity, drainage, and aeration
- Guides sampling strategy (e.g., different sample weights for carbon analysis; see SOP-L406)
- Informs the soil texture correction factor for nutrient extractions (see SOP-L403)
- Rapid enough to characterize many points across a landscape in a single field day

7. Required Materials

Item	Notes
Water bottle with squeeze top	For wetting soil incrementally
Small ruler (cm)	For measuring ribbon length
Field data sheet	Printed from Appendix A
Clipboard and pencil	Mechanical pencil preferred
GPS unit or smartphone	For recording sample locations
Trowel or soil knife	For collecting subsurface samples
Sealable plastic bags	If bringing samples back for confirmation
Paper towels or rag	For cleaning hands between samples

No specialized laboratory equipment is required.

8. Estimated Time

Task	Time
Per sample (experienced observer)	2–5 minutes
Per sample (new observer)	5–10 minutes
Calibration exercise (training)	30–45 minutes with known-texture standards

9. Procedure

9.1. Collect the Sample

1. Select a representative location and record GPS coordinates
2. Clear surface litter and organic debris
3. Collect approximately a golf-ball-sized amount of mineral soil from the target depth (typically 0–15 cm)
4. Remove visible roots, gravel, and organic fragments

9.2. Moisten the Sample

1. Place the soil in your palm
2. Add water drop by drop from the squeeze bottle while kneading the soil
3. Continue adding water and kneading until the soil reaches the consistency of modeling clay or putty
4. The soil should be uniformly moist but not dripping wet
5. Crush all aggregates between your fingers during kneading

! Getting the Moisture Right

This is the most critical step. If the soil is too dry, it will crumble and you'll underestimate clay content. If too wet, it will be slippery and you'll overestimate clay. The target is a smooth, pliable consistency similar to play dough. This may take 2–3 minutes of kneading.

9.3. Test 1: Form a Ball

1. Try to form the moistened soil into a ball (bolus) by squeezing and rolling in your palm
2. **If the soil will not form a ball**, it is **Sand**. Record and move to the next sample.
3. If a ball forms, proceed to Test 2

9.4. Test 2: Ribbon Test

1. Place the ball between your thumb and forefinger
2. Gently push the soil forward with your thumb, squeezing it into a flat ribbon about 2 mm thick and 1 cm wide
3. Let the ribbon extend over your forefinger, unsupported, until it breaks from its own weight
4. Measure the ribbon length with a ruler before it breaks

5. Repeat 3–5 times and use the longest consistent ribbon

Ribbon length determines clay content:

Ribbon Length	Clay Content	Category
No ribbon forms	$< 10\%$	Low clay
< 2.5 cm (< 1 inch)	10–20%	Low-moderate clay
2.5–5 cm (1–2 inches)	20–35%	Moderate clay
> 5 cm (> 2 inches)	$> 35\%$	High clay

9.5. Test 3: Feel Test (Grittiness and Smoothness)

1. Take a pea-sized piece of the moistened soil
2. Place it in your palm and add excess water to make a muddy slurry
3. Rub the slurry between your thumb and forefinger using a circular motion
4. Classify the dominant feel:

Feel	Sensation	Dominant Fraction
Gritty	Like rubbing sugar or fine sand between fingers; individual grains felt and possibly heard	Sand-dominated
Smooth	Like rubbing flour or talc; silky, no grains felt	Silt-dominated
Neither	Neither gritty nor silky dominates; sticky	Sand and silt balanced

9.6. Determine Textural Class

Combine the ribbon length and feel test results using the following decision matrix (Thien, 1979):

	Gritty Feel	Smooth Feel	Neither Gritty nor Smooth
No ribbon	Sand	Silt	Loam
< 2.5 cm	Sandy loam	Silt loam	Loam
2.5–5 cm	Sandy clay loam	Silty clay loam	Clay loam
> 5 cm	Sandy clay	Silty clay	Clay

Additional Clues

- **Loamy sand** falls between sand and sandy loam: barely forms a ball, very gritty, ribbon <1 cm
- **Silt** (pure): very smooth, almost soapy when wet, forms a weak ball but no ribbon

- When in doubt between two classes, record both (e.g., "sandy loam / loam") and note the uncertainty

9.7. Record Results

Record the textural class, ribbon length, feel, and any notes on the field data sheet (Appendix A). Photograph the ribbon if possible for documentation.

10. Supplementary Test: Jar Sedimentation

For a semi-quantitative confirmation of field texture, you can perform a jar test on the same soil:

1. Fill a clear, straight-sided jar (mason jar or pasta sauce jar) one-third full with soil
2. Fill the jar with water, leaving 2 cm of headspace
3. Add a pinch of non-foaming dish soap (dispersing agent)
4. Cap tightly and shake vigorously for 2 minutes
5. Set the jar on a level surface and do not disturb
6. After 1 minute, mark the settled layer on the jar (this is **sand**)
7. After 2 hours, mark the next layer (this is **silt**)
8. After 24–48 hours, the remaining suspended material has settled (this is **clay**); the water above may still be slightly turbid
9. Measure the thickness of each layer and calculate approximate percentages

> **Note**
>
> The jar test provides a rough visual estimate only. For precise particle size distribution, use the hydrometer method (SOP-L401).

11. Quality Control

- **Calibration:** Before independent fieldwork, each observer must practice on at least 5 soils of known texture (confirmed by hydrometer analysis). Results should match the confirmed texture class for at least 4 of 5 samples.
- **Inter-observer agreement:** When multiple observers are surveying, have each observer independently classify at least 10% of the same samples. Agreement should be within one textural class. Inter-observer variability is a well-documented limitation of texture-by-feel; training and calibration reduce but do not eliminate systematic differences among observers (Salley et al., 2018).
- **Documentation:** Record ribbon length to the nearest 0.5 cm, not just the textural class, so results can be reviewed later.
- **Confirmation:** For critical projects, bring duplicate samples back to the lab for hydrometer analysis on at least 10% of field-textured samples.

When texture-by-feel results will inform quantitative analyses (e.g., pedotransfer functions for water-holding capacity), confirm at least 10% of field-textured samples with hydrometer analysis (SOP-L401). The accuracy of the feel method varies by textural class: sandy and clayey soils are identified most reliably, while loams and silt loams are misclassified more often (Salley et al., 2018).

12. Data Management

Enter results from field sheets into the project spreadsheet within 48 hours. Required fields:

- Date, site, plot ID, observer
- GPS coordinates
- Sample depth (cm)
- Ribbon length (cm)
- Feel (gritty, smooth, or neither)
- Textural class
- Notes (e.g., high gravel content, high organic matter, very dry)

13. References

Salley, S. W., Herrick, J. E., Holmes, B. M., Karl, J. W., Levi, M. R., McCord, S. E., Waal, C. van der, & Edwards, B. R. P. (2018). A comparison of soil texture-by-feel estimates: Implications for the citizen scientist. *Soil Science Society of America Journal*, *82*(6), 1526–1537. https://doi.org/10.2136/sssaj2018.04.0137

Soil Survey Division Staff. (2017). *Soil survey manual*. Government Printing Office. https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-survey-manual

Thien, S. J. (1979). A flow diagram for teaching texture-by-feel analysis. *Journal of Agronomic Education*, *8*, 54–55. https://doi.org/10.2134/jae.1979.0054

14. Training Record

Before performing field texture assessments independently, personnel must:

1. Read this SOP in full
2. Complete a calibration exercise on at least 5 soils of known texture
3. Demonstrate the moistening, ball, ribbon, and feel tests under supervision
4. Achieve agreement within one textural class on 4 of 5 known samples
5. Be signed off by the field lead or PI

15. Appendix A: Soil Texture Field Data Sheet

Print this sheet and use it in the field.

Site: _____ **Date:** _____

Observer: _____ **Project:** _____

Sample #	GPS / Location	Depth (cm)	Ball? (Y/N)	Ribbon (cm)	Feel (G/S/N)	Texture Class	Notes
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

Feel key: G = Gritty, S = Smooth, N = Neither

Texture class options: Sand, Loamy sand, Sandy loam, Loam, Silt loam, Silt, Sandy clay loam, Clay loam, Silty clay loam, Sandy clay, Silty clay, Clay

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F402

Soil Bulk Density – Core Method

Standard Operating Procedure

SOP Number	SOP-F402
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil bulk density using the volumetric core method. Bulk density (ρ_b) is the mass of oven-dried soil per unit volume of field-moist soil, expressed in g/cm^3. It is used to convert gravimetric measurements (e.g., nutrient concentrations in mg/kg) to volumetric or areal bases (e.g., kg/ha), to assess soil compaction, to calculate total porosity, and to evaluate management effects on soil physical quality. The core method is the most widely used direct measurement of bulk density and is described in the SSSA Methods of Soil Analysis (Blake & Hartge, 1986; Grossman & Reinsch, 2002).

2. Scope

- **Applies to:** Mineral soils at all EA Program field sites, including agricultural plots, photovoltaic panel study sites, and restoration sites. Suitable for depths of 0–30 cm using hand-driven cores.
- **Does not apply to:** Soils with abundant coarse fragments (greater than approximately 20% by volume of gravel, cobbles, or stones), which require the excavation or clod method. Also not applicable to organic soils (histosols) or very loose sandy soils where core insertion causes compaction of the sample.
- **Location:** All EA Program field sites; laboratory drying and weighing in SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate sampling logistics, verify equipment
Trained Personnel	Collect cores and process samples per this SOP
New Students	Complete training before independent sampling

4. Definitions

- **Bulk density (ρ_b):** The mass of oven-dry soil per unit volume of field-moist soil, including pore space; expressed in g cm^{-3} or Mg m^{-3}; typically ranges from 1.0–1.8 g cm^{-3} for mineral soils and 0.1–0.5 g cm^{-3} for organic soils.
- **Coarse fragment correction:** An adjustment to bulk density calculations that removes the volume and mass of rock fragments (greater than 2 mm) from the core sample; required in stony or gravelly soils where coarse fragments occupy significant core volume.
- **Core sampler:** A thin-walled, beveled steel cylinder of known volume pushed into the soil to extract an intact sample for bulk density determination; internal dimensions must be measured and recorded for each sampler.

- **Dry bulk density:** The mass of oven-dry soil divided by the total volume of the sample including pore space; the standard reported value for bulk density; calculated after drying at 105 °C to constant mass.
- **Porosity (ϕ):** The fraction of total soil volume occupied by pores (air + water): $\phi = 1 - \rho_b / \rho_p$ where ρ_p is particle density (assumed 2.65 g cm^{-3} for mineral soils); porosity decreases as bulk density increases.
- **Soil carbon stock:** The mass of organic carbon per unit land area (Mg C ha^{-1}) calculated from bulk density, sampling depth, and organic carbon concentration; bulk density measurement is the primary source of uncertainty in carbon stock estimates.
- **Soil compaction:** An increase in bulk density caused by mechanical pressure (tillage, vehicle traffic, trampling); reduces porosity, impedes root growth, and decreases infiltration rates.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Driving the core sampler with a mallet or hammer poses a risk of hand and finger injury. Trowels and soil knives are sharp. Repetitive hammering can cause strain.
- **Biological:** Soil-borne pathogens; wash hands thoroughly after handling soil. Tick and insect exposure at field sites.
- **Environmental:** Standard field hazards (heat, uneven terrain, sun exposure).

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Nitrile or leather work gloves
☐ Safety glasses (when driving the sampler)
☐ Sun protection (hat, sunscreen)

5.2. Related SOPs

Refer to SOP-F001 Field Safety for general field safety procedures, including transportation, weather hazards, and emergency response.

6. Equipment and Materials

6.1. Field Equipment

Item	Notes
Bulk density core rings	Known internal volume; typical dimensions: 5 cm height x 5 cm diameter (approximately 98 cm^3) or 7.5 cm x 7.5 cm

Item	Notes
Core sampler (drive head)	Fits over the core ring; beveled cutting edge
Rubber mallet or dead-blow hammer	For driving the sampler
Driving cap or block	Wooden or rubber block to protect the sampler from mallet strikes
Flat-blade trowel or soil knife	For excavating around the core and trimming ends
Straight-edged blade or spatula	For trimming flush with ring ends
Plastic caps or aluminum foil	Two per core, to seal both ends after trimming
Pre-labeled ziplock bags	For transporting sealed cores
Cooler with ice packs	If gravimetric moisture will be determined separately
Ruler or depth gauge	For measuring insertion depth
Field notebook	For recording site, depth, and core ID
GPS unit or smartphone	For recording sampling coordinates

6.2. Laboratory Equipment

Item	Notes
Drying oven	Set to 105 C; see SOP-L105
Analytical balance	0.01 g resolution minimum; SOP-L102
Desiccator	For cooling dried samples before weighing
Pre-weighed aluminum drying tins	Large enough to hold the full core volume
Calipers or ruler	For verifying core ring dimensions

7. Procedure

7.1. Pre-Sampling Preparation

1. Measure and record the internal dimensions of each core ring (inside diameter and height) using calipers. Calculate the volume:

$$V = \pi \left(\frac{d}{2}\right)^2 \times h$$

where d = inside diameter (cm) and h = height (cm).

2. Pre-label core rings or bags with site name, plot ID, depth, and replicate number
3. Pre-weigh aluminum drying tins and record the tare mass
4. Clean core rings and the sampler with DI water before use
5. File a field work planning form per SOP-F001

7.2. Field Sampling

1. Navigate to the predetermined sampling location and record GPS coordinates
2. Clear surface litter, loose organic debris, and any vegetation from a small area (approximately 15 x 15 cm) at the sampling point without disturbing the mineral soil surface

> **!** Important
>
> Do not compress or disturb the soil surface before inserting the core. The goal is to capture the soil at its natural field density.

3. Assemble the core ring inside the drive head. Ensure the beveled cutting edge faces downward.
4. Place the assembled sampler vertically on the soil surface
5. Position the driving cap or wooden block on top of the sampler
6. Drive the sampler into the soil using the mallet, applying steady, even blows. Drive until the top of the core ring is flush with, or slightly below, the soil surface.
7. Do not over-drive the sampler. If the ring is driven more than 1 cm past flush, the sample may be compacted and should be discarded. Over-driving and under-driving the core introduce bias in opposite directions; the magnitude of the bias depends on soil type and moisture status (Grossman & Reinsch, 2002; Page-Dumroese et al., 1999).
8. Carefully excavate the soil around the sampler using the trowel, leaving a pedestal of soil beneath and around the core
9. Slide the trowel or soil knife beneath the bottom of the core to sever it from the underlying soil
10. Lift the sampler and core out of the ground

7.3. Trimming and Capping

1. Remove the core ring from the drive head
2. Using a straight-edged blade, trim the soil flush with the top and bottom of the ring. Remove any material that extends beyond the ring face. Be careful not to smear or compress the soil.
3. If large roots or rocks protrude from the core face, note this in the field notebook. If coarse fragments constitute a substantial fraction of the core, record this and consider whether the core method is appropriate for this site.
4. Cap both ends of the ring with plastic caps or wrap tightly with aluminum foil
5. Place the capped core in a labeled ziplock bag
6. Store in the cooler for transport to the laboratory

> Tip
>
> Collect a minimum of three replicate cores per plot or treatment to account for spatial variability. Five replicates are preferred for statistical robustness, especially in heterogeneous soils or when detecting small differences between treatments.

7.4. Backfilling

Backfill the sampling hole with surrounding soil to minimize site disturbance and prevent tripping hazards.

7.5. Laboratory Processing

1. Remove the plastic caps or foil from the core
2. Transfer the entire soil core from the ring into a pre-weighed aluminum drying tin. Use a spatula or trowel to ensure all soil is transferred; scrape the inside of the ring if necessary.
3. Record the mass of the tin plus field-moist soil (this can also be used to calculate gravimetric moisture if a separate subsample is not taken)
4. Place the tin in the drying oven at 105 C for a minimum of 24 hours (until constant mass is achieved). See SOP-L105.
5. Remove the tin from the oven and cool in a desiccator for 20–30 minutes
6. Weigh the tin plus oven-dried soil and record the mass

8. Quality Control and Acceptance Criteria

8.1. Calculations

Bulk density (g/cm^3):

$$\rho_b = \frac{M_{\text{dry}}}{V_{\text{core}}}$$

where M_{dry} = mass of oven-dried soil (g) and V_{core} = internal volume of the core ring (cm^3).

Gravimetric moisture content (if determined from the same core):

$$\theta_g = \frac{M_{\text{wet}} - M_{\text{dry}}}{M_{\text{dry}}}$$

where M_{wet} = mass of field-moist soil (g).

Total porosity (fraction):

$$\phi = 1 - \frac{\rho_b}{\rho_s}$$

where ρ_s is the particle density, typically assumed to be 2.65 g/cm^3 for mineral soils. The assumed particle density of 2.65 g/cm^3 is appropriate for most mineral soils but should be verified for soils with high organic matter (where it may be lower) or heavy mineral content (Blake & Hartge, 1986).

8.2. Expected Ranges

Typical bulk density values for mineral soils:

Soil Type	Expected ρ_b (g/cm^3)
Well-aggregated forest or prairie topsoil	0.8 – 1.2
Cultivated agricultural topsoil	1.1 – 1.5
Compacted or traffic-affected soil	1.5 – 1.8
Sandy subsoil	1.4 – 1.8
Clay-rich subsoil	1.1 – 1.6

Values outside the range of 0.5 – 2.0 g/cm^3 for mineral soils should be investigated for sampling or measurement error.

Variability is typically higher in surface soils than in subsoils because of biological activity and tillage effects (Al-Shammary et al., 2018). For carbon stock calculations, consistent sampling depth and core diameter are critical because small changes in bulk density propagate into large errors at the landscape scale (**walter2016?**).

8.3. QC Requirements

- Collect a minimum of three replicate cores per plot or treatment
- Calculate the coefficient of variation (CV) among replicates. A CV greater than 10% suggests high spatial variability or possible sampling problems (compaction, lost soil during extraction). Consider collecting additional replicates.
- Verify core ring dimensions at the start of each sampling campaign. If the ring is dented or deformed, replace it.
- Dry samples to constant mass. If the mass changes by more than 0.1 g between successive weighings (at least 4 hours apart), continue drying.

9. Waste Disposal

Waste Type	Disposal Method
Dried soil	Return to field site if uncontaminated, or standard trash
Used aluminum tins	Recycle or standard trash
Used gloves	Standard trash

10. References

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11. Training Record

Before collecting bulk density cores independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Complete SOP-F400 Soil Sampling training
4. Observe a trained operator perform core collection, trimming, and laboratory processing
5. Demonstrate proper core driving technique, trimming to flush, and capping without compacting the sample
6. Process at least three replicate cores through drying and weighing with a CV below 10%
7. Be signed off by the field lead or PI

12. Appendix A: Example Calculation

```
# Bulk density calculation from core method
# Core ring dimensions
diameter_cm <- 5.0
height_cm <- 5.0
volume_cm3 <- pi * (diameter_cm / 2)^2 * height_cm

# Masses
tin_g <- 15.32             # Tare mass of aluminum tin
tin_wet_g <- 112.45        # Tin + field-moist soil
tin_dry_g <- 95.78         # Tin + oven-dried soil (105 C, 24 h)

# Calculations
mass_dry_g <- tin_dry_g - tin_g
mass_wet_g <- tin_wet_g - tin_g

bulk_density <- mass_dry_g / volume_cm3
grav_moisture <- (mass_wet_g - mass_dry_g) / mass_dry_g
porosity <- 1 - (bulk_density / 2.65)
```

```
cat(sprintf("Core volume:           %.2f cm3\n", volume_cm3))
cat(sprintf("Oven-dry soil mass:    %.2f g\n", mass_dry_g))
cat(sprintf("Bulk density:          %.2f g/cm3\n", bulk_density))
cat(sprintf("Gravimetric moisture: %.3f g/g\n", grav_moisture))
cat(sprintf("Total porosity:        %.2f\n", porosity))
```

13. Appendix B: Coarse Fragment Correction

If the core contains gravel or rock fragments larger than 2 mm, the bulk density of the fine-earth fraction (less than 2 mm) can be calculated by removing the coarse fragments from the oven-dried sample, weighing them separately, and estimating their volume:

$$\rho_{b,\text{fine}} = \frac{M_{\text{dry}} - M_{\text{cf}}}{V_{\text{core}} - V_{\text{cf}}}$$

where M_{cf} = mass of coarse fragments (g) and V_{cf} = volume of coarse fragments (cm^3), estimated by water displacement or by dividing M_{cf} by an assumed rock density (typically 2.65 g/cm^3). The water-displacement method for estimating coarse-fragment volume is preferred when fragment shapes are irregular (Page-Dumroese et al., 1999).

Record the coarse fragment mass fraction and report whether the bulk density value refers to the whole soil or the fine-earth fraction.

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Core compacted during insertion	Soil too dry and hard; excessive force	Moisten the surface slightly (note in field notebook); do not hammer past flush
Soil falls out of the bottom	Loose sandy soil; insufficient cohesion	Try sampling when soil is slightly moist; use a core sampler with a retaining basket
Large roots fill the core	Heavily rooted location	Move to a nearby location; note the root density
CV among replicates exceeds 10%	High spatial variability; compaction artifacts	Collect additional replicates; inspect technique
Bulk density above 2.0 g/cm^3	Measurement error or rock fragment in core	Re-check masses and volume; inspect for gravel
Bulk density below 0.5 g/cm^3	Organic layer sampled; incomplete core	Verify that mineral soil was sampled; check for lost soil

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F403

Soil Infiltration Rate – Ring Infiltrometer Method

Standard Operating Procedure

SOP Number	SOP-F403
Revision	0.2
Author(s)	Marc Los Huertos
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Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the field measurement of soil infiltration rate using single-ring and double-ring infiltrometers. Infiltration rate is the velocity at which water enters the soil surface, typically expressed in cm/h or mm/h. It is a key parameter for evaluating stormwater management, irrigation efficiency, soil compaction, hydrologic modeling, and land use impacts on soil physical properties. The double-ring method follows ASTM D3385 (ASTM International, 2018); the single-ring method follows Bouwer (1986). Infiltration measurements are also essential for calibrating hydrologic models and for evaluating the effects of soil sealing in urban environments (Hillel, 1998; Reynolds et al., 2002).

2. Scope

- **Applies to:** Field measurement of infiltration rate on mineral soils at all EA Program sites, including agricultural plots, photovoltaic panel study sites, restoration areas, and urban soils
- **Does not apply to:** Soils with very low hydraulic conductivity (less than 1 x 10^{-5} cm/s; see ASTM D5093 for sealed-inner-ring method); rocky or gravelly soils that prevent ring insertion; laboratory column permeability tests
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve sampling plan, ensure training compliance
Field Lead	Coordinate logistics, select test locations
Trained Personnel	Conduct infiltration tests per this SOP
New Students	Complete training before independent testing

4. Definitions

- **Double-ring infiltrometer:** A measurement apparatus consisting of two concentric steel rings driven into the soil surface; the inner ring provides the measurement zone; the outer ring creates a buffer that minimizes lateral flow from the inner ring, enforcing predominantly vertical infiltration.
- **Final infiltration rate (steady-state rate):** The constant rate at which water enters the soil after the soil profile is saturated and the infiltration rate has stabilized; approximates the saturated hydraulic conductivity (K_{sat}) of the surface soil.

- **Green-Ampt model:** A physically based infiltration model that describes the rate of water entry into soil as a function of saturated hydraulic conductivity, matric suction at the wetting front, and soil moisture deficit; used to interpret falling-head infiltration data.
- **Infiltration rate:** The volumetric flux of water entering the soil surface per unit area per unit time, expressed in mm h^{-1} or cm h^{-1}; decreases from an initially high rate in dry soil to a lower, stable final rate as the soil wets.
- **Macropore flow:** Preferential movement of water through large continuous pores (earthworm burrows, root channels, structural cracks); produces non-uniform wetting and anomalously high initial infiltration rates that do not reflect bulk soil hydraulic conductivity.
- **Matric potential:** The energy status of soil water due to capillary and adsorptive forces; expressed in kPa or cm H_2O; drives water movement from wet to dry zones during infiltration.
- **Saturated hydraulic conductivity (K_{sat}):** The proportionality constant in Darcy's law relating water flux to hydraulic gradient under saturated conditions; the primary soil hydraulic property controlling drainage, runoff, and deep percolation; approximated by the steady-state infiltration rate.
- **Single-ring infiltrometer:** A simplified apparatus using one ring driven into the soil; easier to deploy than double-ring but subject to lateral flow error; appropriate for screening or comparative measurements when absolute K_{sat} is not required.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Driving metal rings into the soil with a sledge or mallet poses a risk of hand, finger, and foot injury. The rings have sharp edges.
- **Ergonomic:** Carrying water containers (20 L jugs) and rings to remote field sites can cause strain.
- **Environmental:** Standard field hazards (heat exposure, uneven terrain, insects).

5.1. Required PPE

☐ Closed-toed shoes or field boots (steel-toed preferred when driving rings)
☐ Leather work gloves (when handling rings and sledge)
☐ Safety glasses (when driving rings)
☐ Sun protection (hat, sunscreen)

5.2. Related SOPs

Refer to SOP-F001 Field Safety for general field safety procedures.

6. Equipment and Materials

6.1. Method A: Double-Ring Infiltrometer (ASTM D3385)

Item	Notes
Inner ring	Minimum 30 cm (12 in) diameter, 20 cm tall; 14-gauge or heavier steel
Outer ring	Minimum 60 cm (24 in) diameter, 20 cm tall; same gauge as inner ring
Sledge hammer (3–5 kg)	For driving rings
Driving cap or wooden plank	Placed on top of the ring to distribute hammer blows evenly and prevent ring deformation
Carpenter's level	For checking ring verticality
Ruler or graduated staff (mm)	For measuring water level in the inner ring
Stopwatch or timer	For recording time intervals
Water supply	40–80 L per test; carry in jerrycans or connect to a portable tank
Bucket or pitcher	For filling rings without disturbing the soil surface
Plastic sheeting or burlap	Placed inside the ring during initial filling to prevent soil disturbance
Mariotte bottle (optional)	For maintaining constant head automatically
Field data sheet	Printed from Appendix A
Clipboard and pencil	Mechanical pencil preferred
GPS unit or smartphone	For recording test locations
Tape measure	For measuring ring insertion depth

6.2. Method B: Single-Ring Infiltrometer

Item	Notes
Single ring	30 cm diameter minimum (larger is better; 90 cm preferred for accuracy); 20 cm tall
All other equipment	Same as Method A, minus the outer ring

7. Procedure

7.1. Site Selection and Preparation

1. Select test locations based on the project sampling plan. Avoid locations with visible animal burrows, large root channels, cracks, or other macropore features unless the objective is specifically to characterize preferential flow.
2. Record GPS coordinates and site description
3. Clear surface litter and loose organic debris from a 1 x 1 m area without disturbing the mineral soil surface
4. Note antecedent conditions in the field notebook: recent rainfall, soil moisture (qualitative: dry, moist, wet), and surface condition (bare, vegetated, crusted)

> **Tip**
>
> Collect a companion bulk density core (SOP-F402) and a texture-by-feel assessment (SOP-F401) at each infiltration test location. These measurements help interpret the infiltration results.

7.2. Ring Installation – Double-Ring Method

1. Center the inner ring at the test location
2. Place the driving cap or wooden plank on top of the ring
3. Drive the inner ring vertically into the soil to a depth of approximately 5–10 cm (2–4 in) using even blows from the sledge. Avoid tilting the ring; check periodically with the level.
4. Measure and record the insertion depth on at least two sides
5. Center the outer ring around the inner ring. Drive it to approximately 10–15 cm (4–6 in), deeper than the inner ring, to prevent test water from escaping beneath the outer ring.
6. Check that the inner ring is still centered and level

> **Important**
>
> Do not drive the rings deeper than necessary. Over-driving compresses the soil along the ring walls and creates an artificial seal that reduces measured infiltration rates. If the soil cracks or separates from the ring wall, abandon the test location and start at a new spot.

7.3. Ring Installation – Single-Ring Method

1. Place the single ring at the test location
2. Drive to approximately 5 cm depth using the same technique as above
3. For best accuracy, use the largest diameter ring available (90 cm or larger reduces lateral flow error) (Bouwer, 1986). Single-ring measurements tend to overestimate true vertical infiltration because lateral divergence below the ring cannot be eliminated; the magnitude of overestimation decreases as ring diameter increases (Gregory et al., 2006).

7.4. Initial Filling

1. Place a sheet of plastic or burlap on the soil surface inside the inner ring (and inside the annular space for the double-ring method) to cushion the water and prevent soil disturbance during filling
2. Gently pour water into the outer ring (annular space) first, then into the inner ring, to approximately 10–15 cm depth
3. Carefully remove the plastic or burlap
4. For the double-ring method, maintain the water level in the outer ring at approximately the same height as the inner ring throughout the test. This eliminates the lateral pressure gradient and forces flow beneath the inner ring to be vertical.

7.5. Measuring Infiltration Rate

Falling-head method (most common for EA Program):

1. Record the initial water level in the inner ring and the start time
2. Allow the water to infiltrate. Record the water level at regular time intervals. Suggested intervals:

Elapsed Time	Recording Interval
0–30 minutes	Every 5 minutes
30–60 minutes	Every 10 minutes
60–120 minutes	Every 15 minutes
120+ minutes	Every 30 minutes

3. When the water level drops to approximately 3 cm above the soil surface, refill to the original level and record the new starting level and time. Record each refill event.
4. Maintain the water level in the outer ring by refilling as needed.
5. Continue the test until the infiltration rate reaches a steady state (three or more consecutive readings with less than 10% change). This is the steady-state or "final" infiltration rate.
6. For most soils, steady state is reached within 1–3 hours. Clayey soils may require 4–6 hours or longer.

Constant-head method (Mariotte bottle):

1. Set up the Mariotte bottle to maintain a constant water depth (typically 5–10 cm) in the inner ring
2. Record the volume of water consumed from the bottle at regular time intervals
3. Calculate the infiltration rate from the volume consumed, the ring area, and the time interval
4. Continue until steady state is reached

7.6. Ending the Test

1. Record the final water level, time, and cumulative number of refills
2. Remove the rings by rocking gently and pulling upward
3. Backfill the holes and restore the site
4. Record any observations about the wetting front depth, root channels, or other features visible after ring removal

8. Quality Control and Acceptance Criteria

8.1. Calculations

Infiltration rate for each time interval (falling-head method):

$$f = \frac{\Delta h}{\Delta t}$$

where f = infiltration rate (cm/h), Δh = drop in water level (cm) during the interval, and Δt = duration of the interval (h). If the ring was refilled during the interval, add the refill depth to Δh.

Cumulative infiltration:

$$F = \sum_{i=1}^{n} f_i \times \Delta t_i$$

where F = cumulative infiltration (cm) and n = number of intervals.

Steady-state infiltration rate (f_c) is the final, approximately constant rate, typically reported as the mean of the last three or more consistent readings.

8.2. Expected Ranges

Steady-state infiltration rates vary widely with soil texture and structure:

Soil Type / Condition	Expected f_c (cm/h)
Coarse sand, well-structured	5 – 25+
Sandy loam	2.5 – 7.5
Loam, well-structured	1.0 – 5.0
Silt loam	0.5 – 2.5
Clay loam	0.1 – 1.0
Compacted or crusted soil	less than 0.5

Values outside the range of 0.01–50 cm/h should be investigated for measurement error or unusual soil conditions.

Rates measured at EA Program sites in the Pomona/Claremont area should be interpreted in the context of the local aridic moisture regime, where antecedent moisture is often very low and hydrophobicity may develop under prolonged dry conditions (Hillel, 1998). Urban and peri-urban soils may show infiltration rates well below their textural expectation due to compaction from foot traffic and construction (**lai2010?**).

8.3. QC Requirements

- Conduct a minimum of three replicate tests per plot or treatment to capture spatial variability
- Record antecedent soil moisture and recent precipitation for each test; these strongly affect initial rates
- Verify that the outer ring water level is maintained throughout the test (double-ring method)
- Inspect ring installation for gaps between the ring wall and the soil; if gaps are visible, the test is invalid

- Ring insertion depth and ponded water depth both influence measured infiltration rate; standardize these dimensions across replicates and sites to ensure comparability (Gregory et al., 2006).
- Plot the infiltration rate versus time curve for each test. A monotonically decreasing curve approaching a constant value is expected. Erratic increases may indicate macropore activation or ring leakage.

9. Waste Disposal

Waste Type	Disposal Method
Test water	Allowed to infiltrate on site; no disposal needed
Used plastic sheeting	Standard trash
Used gloves	Standard trash

10. References

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11. Training Record

Before conducting infiltration tests independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training
3. Observe a trained operator perform a complete double-ring infiltration test from ring installation through steady state
4. Demonstrate proper ring driving technique without ring deformation or soil cracking
5. Conduct at least one complete test under supervision and produce an infiltration curve that reaches steady state
6. Be signed off by the field lead or PI

12. Appendix A: Infiltration Test Field Data Sheet

Print this sheet and use it in the field.

Site: _____ **Plot ID:** _____ **Date:** _____

Observer: _____ **GPS:** _____

Method: Double-ring / Single-ring (circle one)

Inner ring diameter: _____ cm **Outer ring diameter:** _____ cm (if double-ring)

Ring insertion depth: Inner _____ cm, Outer _____ cm

Antecedent conditions: Last rain _____ days ago, _____ mm; Soil moisture: dry / moist / wet

Surface condition: bare / vegetated / crusted / mulched

Initial water depth in inner ring: _____ cm

Reading #	Clock Time	Elapsed (min)	Water Level (cm)	Refill? (Y/N)	Level After Refill (cm)	Interval Δh (cm)	Interval Rate (cm/h)	Notes
0		0		–	–	–	–	Start
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Steady-state rate (f_c): _____ cm/h (mean of last _____ readings)

Total test duration: _____ minutes

Additional notes: _____

13. Appendix B: Example Calculation and Plotting

```r
library(tidyverse)

# Example data from a falling-head double-ring test
# Ring inner diameter = 30 cm
ring_diam_cm <- 30
ring_area_cm2 <- pi * (ring_diam_cm / 2)^2

infiltration <- tibble(
  elapsed_min = c(0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180),
  water_level_cm = c(15.0, 13.8, 12.9, 12.1, 11.4, 10.2, 8.8, 7.6,
                     5.8, 4.2, 2.8, 1.5)
)

# Calculate interval infiltration rates
infiltration <- infiltration %>%
  mutate(
    delta_h_cm = -diff(c(NA, water_level_cm)),
    delta_t_h = c(NA, diff(elapsed_min)) / 60,
    rate_cm_h = delta_h_cm / delta_t_h,
    cumulative_cm = cumsum(replace_na(delta_h_cm, 0))
  )

# Steady-state rate (last 3 intervals)
f_c <- mean(tail(infiltration$rate_cm_h[!is.na(infiltration$rate_cm_h)], 3))
cat(sprintf("Steady-state infiltration rate: %.2f cm/h\n", f_c))

# Plot infiltration rate vs time
ggplot(infiltration %>% filter(!is.na(rate_cm_h)),
       aes(x = elapsed_min, y = rate_cm_h)) +
  geom_point(size = 3) +
  geom_line() +
  geom_hline(yintercept = f_c, linetype = "dashed", color = "blue") +
  annotate("text", x = max(infiltration$elapsed_min) * 0.7,
           y = f_c + 0.3,
           label = sprintf("f[c] == %.2f~cm/h", f_c),
           parse = TRUE, color = "blue") +
  labs(
    x = "Elapsed time (min)",
    y = "Infiltration rate (cm/h)",
    title = "Double-Ring Infiltration Test"
  ) +
  theme_minimal()
```

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Water leaks around the outside of the ring	Insufficient insertion depth; gap between ring wall and soil	Drive ring deeper; pack bentonite or moist soil around the outside base
Infiltration rate increases mid-test	Macropore activation; preferential flow path opened	Note in field notebook; this may be a real feature of the soil. If clearly an artifact (ring shifted), restart.
Very slow infiltration (less than 0.1 cm/h)	Dense clay; surface crust; compacted layer	Continue test for at least 4–6 hours to confirm steady state; consider ASTM D5093 sealed-ring method
Rate never stabilizes	Heterogeneous soil; macropores draining and refilling	Extend test duration; collect additional replicates; report the range rather than a single value
Ring deformed during installation	Rocks or roots; excessive force	Replace with a new ring at a nearby location
Soil disturbed inside the ring	Overly aggressive filling; ring bounced during driving	Restart at a new location; always use plastic sheeting during initial fill
Single-ring rate much higher than expected	Lateral flow beneath the ring	Use a larger-diameter ring (90 cm+) or switch to the double-ring method

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F404

Soil Temperature Profiles

Standard Operating Procedure

SOP Number	SOP-F404
Revision	0.3
Author(s)	Marc Los Huertos, Artemis Cruz
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for measuring soil temperature at multiple depths to construct soil temperature profiles. Soil temperature influences biological activity (microbial respiration, root growth, seed germination), chemical reaction rates (nutrient mineralization, decomposition), and physical processes (evaporation, frost heave) (Ham, 2002; Hillel, 1998). Temperature profiles are especially relevant for photovoltaic panel studies, where shading alters the soil thermal regime.

The amplitude and phase lag of diurnal and seasonal temperature waves attenuate with depth according to the thermal diffusivity of the soil profile, making multi-depth measurement essential for characterizing subsurface energy budgets (Hillel, 1998). Soil temperature also governs rates of microbial respiration and nitrogen mineralization, with Q_{10} values typically ranging from 2 to 3 for the biologically active zone (Onwuka & Mang, 2018). When continuous monitoring is not feasible, air-temperature-based models can be used to estimate soil temperature, but these require site-specific calibration (Zheng et al., 1993).

2. Scope

- **Applies to:** Spot measurements using handheld probe thermometers and continuous monitoring using datalogger-based thermocouple or thermistor systems at all EA Program field sites
- **Does not apply to:** Soil heat flux measurements (requires heat flux plates); fire-related soil heating studies
- **Location:** All EA Program field sites, including photovoltaic panel study areas, Bernard Field Station, and restoration sites

3. Responsibilities

Role	Responsibility
Lab PI	Approve monitoring plan, ensure training compliance
Field Lead	Coordinate installation, assign measurement schedules
Trained Personnel	Install sensors, collect data, maintain equipment
New Students	Complete training before independent measurement

4. Definitions

- **Diurnal temperature variation:** The cycle of soil temperature change over a 24-hour period driven by solar radiation and radiative cooling; amplitude decreases with depth and is nearly zero below about 50 cm in most soils.
- **Horizon temperature:** The temperature measured at a defined soil depth corresponding to a specific genetic horizon (e.g., A, B, C); used to characterize the thermal environment of the root zone and microbial communities.
- **Soil temperature:** The thermal energy state of the soil, expressed in °C; controls rates of microbial activity, organic matter decomposition, plant root growth, and soil gas fluxes.
- **Temperature gradient:** The rate of change in temperature with depth (°C cm^{-1}); drives heat flux between soil layers and between the soil surface and the atmosphere.
- **Temperature profile:** A set of soil temperature measurements taken at multiple depths simultaneously or sequentially at a single location; used to characterize heat storage, heat flux, and seasonal thermal dynamics.
- **Thermistor:** A temperature sensor whose electrical resistance changes predictably with temperature; commonly used in data loggers for continuous soil temperature monitoring; requires calibration against a traceable reference thermometer.
- **Thermocouple:** A temperature sensor composed of two dissimilar metals joined at a measuring junction; produces a small voltage proportional to the temperature difference between the junction and a reference; used in handheld soil thermometers.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Sharp probe tips, rebar stakes, hammering pilot holes in hard soil
- **Biological:** Ticks, snakes, and other field hazards (see SOP-F001)
- **Electrical:** Datalogger batteries and wiring (low voltage, minimal risk)
- **Environmental:** Heat exposure during summer measurements in southern California

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Gloves (when hammering pilot holes or handling rebar)
☐ Sun protection (hat, sunscreen)
☐ Safety glasses (when hammering)

5.2. Required Training

- SOP-F001 Field Safety

6. Summary of Methods

Two approaches are described in this SOP. Select based on your project requirements. Both methods are described in the SSSA Methods of Soil Analysis (Ham, 2002). The handheld method follows the GLOBE soil temperature protocol (**globe2014soil?**), which has been widely adopted for teaching and citizen-science applications.

Method	Best For	Temporal Resolution	Cost
Handheld probe	Spot measurements, surveys across many points	Single time point per visit	Low
Datalogger system	Continuous monitoring, diurnal and seasonal patterns	Minutes to hours (automated)	High

6.1. Standard Measurement Depths

Depth	Rationale
2 cm	Near-surface; responds rapidly to air temperature and solar radiation
5 cm	Root zone surface; standard GLOBE protocol depth
10 cm	Upper root zone; dampened diurnal signal
20 cm	Mid root zone; minimal diurnal variation
50 cm	Deep reference; seasonal variation only

Choosing Depths

For most EA Program projects, 5 cm and 10 cm are the minimum. Add 2 cm for surface energy balance studies and 20 cm or 50 cm for deep profile characterization.

7. Required Materials

7.1. Method A: Handheld Probe

Item	Notes
Thermocouple (Type T or Type K) or Resistance Temperature Detector (RTD) probes	Probe length must exceed deepest measurement depth + 2 cm. These probes carry a systematic measurement error; raw readings must be corrected using the probe-specific formula (see Data Correction under Data Management).
Electronic reader for probes	For the RTD probes, use either of the Omega HH804 readers. For the Type T probes, pair probe 1 with cable 1 and reader T819, and probe 3 with cable 2 and reader T299. The cable and reader both affect the reading, and the correction formulas are valid only for these specific equipment pairings.
Permanent marker, tape, or heat-shrink band	For marking measurement depths along the probe (see Prepare Depth Guide Marks)
Nail or awl	Same diameter as thermometer probe, for making pilot holes in hard soil
Hammer	For driving the nail into compacted soil
Field data sheet	Printed from Appendix A
Clipboard and pencil	Mechanical pencil preferred
GPS unit or smartphone	For recording measurement locations
Timer or watch	For tracking equilibration time

7.2. Method B: Datalogger System

Item	Notes
Datalogger	Campbell Scientific CR1000, CR300, or equivalent
Thermocouple probes	Type T or Type E; one per measurement depth
Thermistor probes (alternative)	Campbell Scientific 109SS or equivalent
Averaging thermocouple probe (optional)	Campbell Scientific TCAV-L for energy balance studies
Soil temperature profiler (optional)	Hukseflux STP01 or METER TEROS 06
Signal cable	Sufficient length from sensors to datalogger
Weatherproof enclosure	For datalogger and battery
12V battery and solar panel	For continuous power
PVC conduit	For protecting cable runs
Shovel or soil knife	For sensor installation
Laptop with datalogger software	For programming and data download

8. Estimated Time

Task	Time
Handheld: per measurement point (all depths)	10–15 minutes (including equilibration)
Datalogger: initial installation	2–4 hours per station
Datalogger: data download visit	15–30 minutes
Datalogger: sensor replacement	30–60 minutes

9. Procedure: Method A – Handheld Probe

9.1. Prepare Depth Guide Marks

Before going to the field, mark each measurement depth directly on the probe. The temperature sensor is at the tip of the probe, so each mark is placed at the target depth measured from the tip. In the field, push the probe in until the mark for the target depth is level with the soil surface.

Target Depth	Mark Distance from Tip
5 cm	5 cm
10 cm	10 cm
20 cm	20 cm

Use a permanent marker, a wrap of tape, or a heat-shrink band at each depth. Verify the marks against a ruler before each field season, since tape and marker can shift or wear off with use.

9.2. Select Measurement Locations

1. Navigate to the predetermined sampling location and record GPS coordinates
2. Select a representative spot with undisturbed soil surface
3. Gently brush away surface litter without disturbing the mineral soil

9.3. Take Measurements

1. If the soil is hard, use the nail and hammer to make a pilot hole to the target depth. Make the hole the same diameter as the probe to ensure good thermal contact.
2. Insert the probe and gently push until the mark for the target depth is level with the soil surface
3. Wait at least 2 minutes for the probe to equilibrate with the soil. Equilibration time depends on the thermal conductivity of the soil and the thermal mass of the probe; dry sandy soils may require 5 minutes or longer (Ham & Senock, 1992).
4. Read the temperature every 1 minute until two consecutive readings are within 0.5 C of each other
5. Record the stabilized temperature, depth, time, and location on the data sheet
6. Remove the probe and repeat at the next depth in the same hole (work from shallowest to deepest)

7. Move to the next measurement location

> **!** Equilibration Is Essential
>
> Do not rush readings. An un-equilibrated probe will read too warm in cool soil and too cool in warm soil. Two minutes is the minimum; 3–5 minutes may be needed in dry or very cold soil.

9.4. Timing

- For comparable data across sites, complete all measurements within 1 hour of local solar noon (approximately 12:00–13:00 PST)
- For diurnal profiles, measure at consistent intervals (e.g., every 2–3 hours over a 24-hour period)
- Record the exact time of each measurement

10. Procedure: Method B – Datalogger System

10.1. Sensor Installation

1. Select a representative location and record GPS coordinates
2. Dig a narrow trench or pit to the deepest sensor depth on one side of the measurement point (do not disturb the soil column directly above the sensors)
3. Insert sensors horizontally into the undisturbed soil wall at each target depth, pressing firmly to ensure good thermal contact
4. Backfill the trench carefully, maintaining the original soil layering as much as possible
5. Route cables through PVC conduit to the datalogger enclosure
6. Mark the sensor locations with discreet, flush-to-ground markers

> **i** Settling Period
>
> Soil disturbed during installation needs time to return to representative thermal conditions. Allow a minimum of 48 hours before using data for analysis. One to two weeks is ideal for deep sensors.

10.2. Datalogger Programming

1. Connect all sensors to the datalogger following the wiring diagram for your sensor type
2. Program the datalogger to scan sensors at the desired interval (typically every 1–5 minutes)
3. Configure storage to record averages at 15-minute or 30-minute intervals
4. Set the datalogger clock to local standard time (do not use daylight saving time)
5. Verify readings against a calibrated handheld thermometer at 5 cm depth

10.3. Data Download

1. Visit the station at regular intervals (weekly to monthly, depending on battery and storage capacity)
2. Connect a laptop and download data using the manufacturer's software
3. Check for gaps, sensor failures, or anomalous readings in the field
4. Transfer data to the project database within 48 hours

11. Calibration

11.1. Handheld Probes

1. Prepare an ice-water bath (crushed ice + deionized water, stirred); this should read 0.0 C
2. Insert the probe and allow to equilibrate for 3 minutes
3. Record the reading; it should be within 0.5 C of 0.0 C
4. If outside tolerance, adjust or replace the thermometer
5. Calibrate before each field season and after any suspected damage

The ice-point calibration is a single-point check; for research requiring higher accuracy, a two-point calibration (ice-water and warm-water bath at approximately 40 C) is recommended (Ham, 2002).

Type K Probes

Type K probes require a multi-point calibration to build their error-correction formula. Measure the reading at a minimum of three temperatures: an ice bath (approximately 0 C), room temperature, and a heating plate at approximately 50 C. Use a reference thermometer at each point and fit the correction from the paired readings (see Data Correction).

11.2. Datalogger Sensors

1. Before installation, place all sensors in a well-stirred water bath
2. Record readings from all sensors simultaneously
3. All sensors should agree within 0.3 C
4. Document any offsets and apply corrections in post-processing if needed

12. Quality Control

- Calibrate all thermometers and sensors before each field season
- For handheld measurements, take readings at a minimum of 3 replicate locations per plot
- Record the time of every measurement (soil temperature changes throughout the day)
- For datalogger systems, inspect data weekly for drift, gaps, and anomalous values
- Include a surface air temperature measurement alongside soil temperatures for context
- Photograph each measurement location at the start of each season

13. Data Management

13.1. Handheld Data

Enter data from field sheets into the project spreadsheet within 48 hours. Required fields:

- Date, time (local standard time), site, plot ID, observer
- Measurement depth (cm)
- Temperature (C)
- Surface condition (bare soil, litter, vegetated, shaded)
- Notes (e.g., soil was saturated, recent rain)

13.2. Data Correction

The handheld probes carry a systematic measurement error, so raw readings must be corrected before analysis. Apply the calibration formula matched to the specific probe, cable, and reader combination; the corrections below are valid only for the equipment pairings listed. Let T_{raw} be the reading in °C and T_{corr} the corrected temperature in °C.

RTD Probe 1

$$T_{\text{corr}} = 1.160\,T_{\text{raw}} - 13.23$$

RTD Probe 2

$$T_{\text{corr}} = 1.167\,T_{\text{raw}} - 14.609$$

Type T Probe 1 (Cable 1, reader T819)

$$T_{\text{corr}} = 1.028\,T_{\text{raw}} - 8.797$$

Type T Probe 3 (Cable 2, reader T299)

$$T_{\text{corr}} = 1.004\,T_{\text{raw}} - 8.121$$

Record which probe, cable, and reader were used for each measurement so the correct formula can be applied during processing. Type K probes must be calibrated and corrected using a formula derived from their own three-point calibration (see Calibration).

13.3. Datalogger Data

- Download raw files and archive unmodified copies
- Process data into a standardized format: timestamp, site, depth, temperature
- Flag and document any corrections (sensor offsets, gap-filling)
- Store processed data in the project database with metadata

14. Waste and Site Impact

- Minimize soil disturbance during installation; backfill trenches carefully
- Remove all temporary equipment, flagging, and markers after the study
- Leave permanent sensors in place for long-term monitoring unless the study is complete
- Pack out all trash and supplies

15. References

Ham, J. M. (2002). Soil temperature and heat flux. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis, part 4: Physical methods* (pp. 1181–1233). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.4.c50

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Zheng, D., Hunt Jr., E. R., & Running, S. W. (1993). A daily soil temperature model based on air temperature and precipitation for continental applications. *Climate Research*, *2*(3), 183–191. https://doi.org/10.3354/cr002183

16. Training Record

Before measuring soil temperature independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Calibrate a handheld probe using an ice-water bath
3. Demonstrate proper use of depth marks and equilibration timing
4. For datalogger work: demonstrate sensor installation, datalogger programming, and data download under supervision
5. Be signed off by the field lead or PI

17. Appendix A: Soil Temperature Field Data Sheet

Print this sheet and use it in the field.

Site: _____ **Plot ID:** _____ **Date:** _____

Observer: _____ **Instrument ID:** _____

Surface condition: _____ **Weather:** _____

Air temperature (C): _____ **Time of air temp reading:** _____

Location #	GPS / Description	Time	2 cm (C)	5 cm (C)	10 cm (C)	20 cm (C)	50 cm (C)	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Calibration check (ice bath reading): _____ C **Time:** _____

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F405

Soil Color Determination (Munsell)

Standard Operating Procedure

SOP Number	SOP-F405
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the field determination of soil color using the Munsell Soil Color Charts. Soil color is one of the most readily observable soil properties and provides information about organic matter content, drainage and aeration status, mineralogy, and degree of weathering (Munsell Color, 2009; Schoeneberger et al., 2012). Lower values (darker soils) generally reflect higher organic matter content, especially in A horizons, while high chroma indicates well-drained, oxidized conditions and low chroma suggests prolonged saturation or reducing environments (Lynn & Pearson, 1993; Shields et al., 1966). Red and yellow hues are controlled primarily by iron oxide mineralogy: hematite produces redder hues (5YR and redder), while goethite produces yellowish-brown hues (7.5YR–10YR) (Schwertmann, 1993; Torrent & Schwertmann, 1983). Consistent color determination is essential for soil profile descriptions (SOP-F407), wetland delineation, and site characterization.

2. Scope

- **Applies to:** Field determination of soil color for all EA Program soil sampling campaigns, profile descriptions, and site characterizations
- **Does not apply to:** Instrumental color measurement (colorimeters or spectrophotometers); rock or sediment color classification using the Rock Color Chart; Munsell readings on dried, ground samples in the lab (see SOP-L419)
- **Location:** All EA Program field sites; can also be performed on fresh samples in the lab

3. Responsibilities

Role	Responsibility
PI / Field Lead	Ensure Munsell charts are available and in good condition
Trained Personnel	Determine soil color per this SOP
New Students	Complete training and inter-observer calibration exercise

4. Definitions

- **Chroma:** The colorimetric purity or saturation of a color; the third component of Munsell notation (e.g., the 4 in 10YR 4/4); low chroma (2 or less) in subsurface horizons indicates gleying and periodic waterlogging.
- **Gleying:** A reduction process in waterlogged soils that depletes iron and produces low-chroma (gray, blue-gray, or green-gray) matrix colors; a diagnostic indicator of hydric soil conditions.
- **Hue:** The dominant spectral color in Munsell notation (e.g., 10YR, 7.5YR, 2.5Y, Gley 1); the first element of a Munsell designation; corresponds to the dominant wavelength of reflected light.
- **Matrix color:** The dominant color occupying more than 50% of the exposed soil face; recorded first in the Munsell notation before mottles or redoximorphic features.
- **Mottle:** A spot or blotch of color contrasting with the matrix color; described by abundance (few, common, many), size (fine, medium, coarse), and contrast (faint, distinct, prominent).
- **Munsell color system:** A standardized three-dimensional color notation (Hue Value/Chroma) used universally in soil science for objective color description; readings must be made in consistent natural or reference lighting conditions.
- **Redoximorphic features:** Color patterns caused by reduction and oxidation of iron and manganese under fluctuating saturation; include masses (concentrations of iron oxides) and depletions (zones of low chroma); diagnostic for hydric soil identification and wetland delineation.
- **Value:** The lightness or darkness of a color in Munsell notation; ranges from 0 (black) to 10 (white); the second component of Munsell notation (e.g., the 4 in 10YR 4/4).

5. Safety and Hazards

⚠ Hazards

- **Biological:** Soil-borne pathogens; wash hands after handling soil.
- **Physical:** Standard field hazards (ticks, snakes, heat exposure).
- **Chemical:** At contaminated sites, soil may contain heavy metals or other hazardous materials; wear gloves.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Nitrile gloves (recommended)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Equipment and Materials

Item	Notes
Munsell Soil Color Charts (current edition)	Handle with clean, dry hands; protect from rain and direct soil contact
Trowel or soil knife	For exposing fresh soil surfaces
Squeeze bottle with DI water	For moistening dry samples
Paper towels	For cleaning hands between samples
Field notebook	For recording colors

7. Background: The Munsell System

Munsell color notation has three components (Munsell Color, 2009; Soil Survey Staff, 2022):

- **Hue:** The dominant spectral color (e.g., 10YR, 2.5Y, 5YR). Pages in the chart book are arranged by hue.
- **Value:** Lightness, from 0 (absolute black) to 10 (absolute white). Vertical axis on each page.
- **Chroma:** Color intensity or saturation, from 0 (neutral gray) outward. Horizontal axis on each page.

A complete Munsell notation is written as: **Hue Value/Chroma** (e.g., 10YR 4/3). The corresponding color name (e.g., “brown” or “dark yellowish brown”) is listed on each chip.

8. Procedure

8.1. Sample Preparation

1. Expose a fresh soil surface by breaking a ped or cutting with a trowel. Do not use smeared or scraped surfaces, as smearing compresses the soil and alters the apparent color.
2. Determine color on **moist** soil. If the soil is dry, add a few drops of DI water to a small subsample and allow it to absorb for 30 seconds before reading.
3. If both moist and dry colors are needed (e.g., for profile descriptions), read the moist color first, then allow a portion to air-dry and read the dry color.

8.2. Reading the Color

1. Work in natural daylight, ideally under an overcast sky or in open shade. Avoid direct sunlight on the soil or the chart, as glare distorts color perception. Do not read colors under artificial light or at twilight.
2. Hold the soil sample behind the chart page apertures so that the soil is visible through the holes.
3. Compare the soil to the color chips (Schoeneberger et al., 2012). Start with the hue page that appears closest, then move to the value row that matches, then slide along the chroma axis.
4. Select the chip that most closely matches the soil. If the soil falls between two chips, record the notation of the nearest chip and note "between X and Y" in the field notebook.
5. Record the full Munsell notation and the color name:
    - Example: **10YR 3/2 (very dark grayish brown)**
6. If the soil contains mottles or redoximorphic features, record the matrix color and then the mottle color(s) separately, noting the abundance (few, common, many), size (fine, medium, coarse), and contrast (faint, distinct, prominent).

8.3. Special Cases

Organic soils (O horizons, peat): Use the Gley pages or the 10YR page with very low value and chroma. Very dark colors (value 2 or less) are common.

Gleyed soils: Use the Gley 1 and Gley 2 pages for soils with chroma of 2 or less and hues bluer than 5Y. Gleyed colors indicate prolonged saturation and reducing conditions.

Mottled soils: Record:

- Matrix color (dominant color occupying more than 50% of the surface)
- Mottle colors (each distinct color)
- For each mottle: abundance, size, and contrast

Abundance	Definition
Few	$< 2\%$ of exposed surface
Common	2–20% of exposed surface

Abundance	Definition
Many	> 20% of exposed surface

Size	Definition
Fine	< 5 mm diameter
Medium	5–15 mm diameter
Coarse	> 15 mm diameter

Contrast	Definition
Faint	Hue, value, and chroma differ by 1 unit or less from matrix
Distinct	Differ by 2–3 units in value or chroma
Prominent	Differ by more than 3 units

Redoximorphic features are diagnostic for hydric soil identification and wetland delineation; consistent recording of mottle properties is critical for regulatory applications (Soil Survey Division Staff, 2017).

9. Quality Control

- At the start of each field season, have two or more observers independently read the same soil samples to assess inter-observer agreement. Readings should agree within one chip (one unit of value or chroma) in at least 80% of comparisons.
- Replace Munsell charts when color chips are faded, soiled, or damaged. Faded chips produce systematic errors.
- Record lighting conditions (overcast, open shade, sunny) in the field notebook; lighting affects color perception.
- Always specify whether the reading was on moist or dry soil.

Digital colorimeters and smartphone-based color apps are increasingly used as supplements to visual Munsell matching; however, instrument readings and visual assessments can disagree, especially at low chroma and low value, so visual Munsell determination remains the standard for USDA soil survey and regulatory work (Viscarra Rossel et al., 2006).

10. References

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F400	Soil Sampling	Soil collection for color reading
F407	Soil Profile Description	Color is part of profile description
L419	Soil Color (Munsell – Lab)	Lab confirmation of field color

12. Training Record

Before determining soil color independently, personnel must:

1. Read this SOP in full
2. Complete an inter-observer calibration exercise with a trained operator, achieving agreement within one chip on at least 8 of 10 test samples
3. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F406

Soil Respiration Chambers (Field Static Chamber Method)

Standard Operating Procedure

SOP Number	SOP-F406
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment and operation of static (closed, non-flow-through) chambers for manual measurement of soil CO_2 efflux in the field. Static chambers are a simple, low-cost method for quantifying soil respiration across treatments, land uses, or seasonal gradients when the Picarro CRDS automated system (SOP-F504) is not available or when additional spatial replication is needed. The method captures headspace gas samples at timed intervals, which are analyzed for CO_2 concentration using a portable infrared gas analyzer (IRGA), syringe samples returned to the

lab, or soda-lime traps (Hutchinson & Mosier, 1981; Livingston & Hutchinson, 1995; Parkin & Venterea, 2012).

The static chamber method remains one of the most widely used approaches for point-scale soil CO_2 flux measurement because of its simplicity and low cost, though it is subject to known biases from headspace concentration buildup and pressure artifacts (Pumpanen et al., 2004; Rochette & Eriksen-Hamel, 2005). At a global scale, soil respiration represents one of the largest fluxes in the terrestrial carbon cycle, and small changes can have significant implications for atmospheric CO_2 concentrations (Davidson et al., 2000; Raich & Schlesinger, 1992).

2. Scope

- **Applies to:** Manual static chamber measurements of soil CO_2 efflux at EA Program field sites; applicable to grasslands, agricultural plots, restoration sites, and forest soils
- **Does not apply to:** Automated dynamic chambers with the Picarro CRDS analyzer (see SOP-F504); eddy covariance or gradient flux methods; aquatic CO_2 flux measurements; N_2O or CH_4 flux measurements requiring GC or CRDS analysis (though the same chambers can be used with different analytical endpoints)
- **Location:** All EA Program terrestrial field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve chamber design and deployment plan
Trained Personnel	Install collars, deploy chambers, collect gas samples
New Students	Complete training before independent use

4. Definitions

- **Basal soil respiration:** CO_2 efflux from undisturbed soil at ambient temperature and moisture without addition of labile substrate; reflects the combined metabolic activity of the soil microbial community and root respiration.
- **CO_2 efflux (soil respiration rate):** The flux of CO_2 from the soil surface to the atmosphere, expressed in µmol CO_2 m^{-2} s^{-1} or g C m^{-2} d^{-1}; the primary field measurement made with a closed or open dynamic chamber system.
- **Closed dynamic chamber:** A chamber placed over the soil surface in which headspace CO_2 concentration rises over time as CO_2 is released from the soil; flux is calculated from the rate of concentration increase, chamber volume, and collar area.
- **Collar:** A short ring of PVC or metal inserted several centimeters into the soil before measurement to create a gas-tight seal with the chamber; installed at least 24 hours before measurements to allow soil disturbance effects to dissipate.
- **Heterotrophic respiration:** CO_2 produced by soil microorganisms decomposing organic matter; one component of total soil respiration along with autotrophic (root and rhizosphere) respiration.

- **LI-COR analyzer (LI-8100A / LI-8150):** A portable infrared gas analyzer system used for automated soil CO_2 flux measurement; calculates flux from the exponential rise in headspace CO_2 concentration over a defined measurement period.
- **Q_{10} coefficient:** The factor by which soil respiration rate increases for a 10 °C rise in temperature; typically 1.5–3.5 for mineral soils; used to temperature-normalize measurements made under different field conditions.
- **Rhizosphere priming:** Enhanced decomposition of soil organic matter stimulated by root exudates; inflates field respiration measurements relative to bare-soil or root-exclusion treatments.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Driving collars into soil with a mallet poses impact injury risk. Carrying chambers and equipment over uneven terrain.
- **Heat:** Southern California field sites can exceed 40 C. Metal chambers become extremely hot in direct sunlight; handle with gloves.
- **Chemical:** Soda-lime ($NaOH/Ca(OH)_2$) is corrosive and causes burns on skin and eye contact. Concentrated NaOH solutions used for soda-lime traps are hazardous. Handle with gloves and safety glasses.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Nitrile or leather gloves
☐ Safety glasses (when handling soda-lime or driving collars)
☐ Sun protection (hat, sunscreen)

5.2. Related SOPs

Refer to SOP-F001 Field Safety for general field safety procedures.

6. Equipment and Materials

Item	Notes
PVC or metal collars	Installed in the soil at least 24 hours before measurement
Chamber lids	Opaque (dark) for total respiration; clear for net CO_2 exchange
Rubber mallet	For installing collars
Sealing material	Closed-cell foam gasket or water trough on collar rim

Item	Notes
Syringes (30–60 mL)	For headspace gas sampling (syringe method)
Evacuated vials (Exetainer)	For storing gas samples for lab analysis
Portable IRGA (optional)	For real-time CO_2 concentration readings
Soda-lime (optional)	For absorption-based CO_2 measurement
Timer or stopwatch	For tracking deployment intervals
Thermometer or soil temp probe	For concurrent soil temperature
Soil moisture probe (optional)	TDR or capacitance sensor
Ruler or tape measure	For measuring headspace height
Field data sheet	For recording deployment times and readings
GPS unit or smartphone	For recording chamber locations

7. Procedure

7.1. Collar Installation

1. Install PVC or metal collars into the soil to a depth of approximately 5 cm at least 24 hours (preferably 48 hours) before the first measurement to allow the soil to recover from disturbance.
2. Do not sever major roots during installation; if roots are encountered, reposition slightly.
3. Measure and record the collar inside diameter and the height of the collar above the soil surface on at least two sides. These dimensions are needed to calculate headspace volume.

7.2. Chamber Deployment

1. Record the start time, soil temperature (at 5 cm depth), and soil moisture at each collar location.
2. Place the chamber lid on the collar, ensuring a gas-tight seal (foam gasket compressed or water trough filled).
3. If using the syringe method, collect a headspace sample immediately after sealing (time zero), then at regular intervals (e.g., 10, 20, 30, and 40 minutes). The choice of deployment interval (typically 20–45 minutes) represents a trade-off: shorter deployments reduce the concentration-feedback bias that suppresses diffusion as headspace CO_2 rises, but longer deployments provide a more detectable concentration change in low-flux soils (Hutchinson & Mosier, 1981; Pumpanen et al., 2004).
4. If using a portable IRGA, connect the chamber to the analyzer and record CO_2 concentration continuously or at 1-minute intervals.
5. If using soda-lime traps, weigh the pre-dried soda-lime, place it inside the sealed chamber, and retrieve it after a fixed deployment period (typically 24 hours). Re-dry and reweigh to determine CO_2 absorbed.

7.3. Ending the Measurement

1. Remove the chamber lid at the end of the measurement period.

2. Record the end time.
3. Transfer syringe samples to evacuated vials (Exetainers) for transport to the lab if not using IRGA.

8. Quality Control and Acceptance Criteria

8.1. Flux Calculation

Linear method (syringe or IRGA):

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{P}{RT}$$

where F = CO_2 flux (micromol m^{-2} s^{-1}), $\Delta C/\Delta t$ = rate of change in headspace CO_2 concentration (ppm s^{-1}), V = headspace volume (m^3), A = collar area (m^2), P = atmospheric pressure (Pa), R = ideal gas constant (8.314 J mol^{-1} K^{-1}), and T = air temperature (K).

Soda-lime method:

$$F = \frac{(M_f - M_i) \times 1.69}{A \times t}$$

where M_f and M_i are the final and initial soda-lime masses (g), 1.69 is the stoichiometric correction factor, A = collar area (m^2), and t = deployment time (hours).

8.2. QC Requirements

- Deploy at least three replicate chambers per treatment or land use.
- Inspect collar seals before each deployment. Soil settling or animal disturbance can break the seal.
- Record soil temperature and moisture alongside every flux measurement; these are the primary drivers of soil respiration. Soil respiration typically increases exponentially with temperature, with Q_{10} values of 2–3 (Bond-Lamberty & Thomson, 2010; Raich & Schlesinger, 1992). Reporting concurrent soil temperature and moisture allows flux values to be normalized or modeled across campaigns and sites.
- Reject any measurement where the R^2 of the linear regression (concentration vs. time) is below 0.90, unless the flux is near zero.
- Avoid measuring during or immediately after rainfall, as water pooling on the collar disrupts gas exchange.

9. Waste Disposal

Waste Type	Disposal Method
Used soda-lime	Allow to dry; dispose as solid waste (non-hazardous once fully carbonated)
Syringe needles	Sharps container
Used gloves	Standard trash

10. References

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F005	Random Sampling Design	Chamber placement design
F400	Soil Sampling	Companion soil samples
F504	Picarro GHG Analyzer	Automated alternative

SOP	Title	Relationship
L400	Soil Moisture	Moisture alongside flux
L408	Solvita CO_2 Burst	Lab respiration complement

12. Training Record

Before deploying soil respiration chambers independently, personnel must:

1. Read this SOP and SOP-F504 in full
2. Demonstrate proper collar installation and headspace volume measurement
3. Perform a supervised syringe sampling or IRGA measurement at one site
4. Calculate flux from field data and demonstrate agreement with the trainer's calculation
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F407

Soil Profile Description

Standard Operating Procedure

SOP Number	SOP-F407
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the systematic description of soil profiles exposed in hand-dug pits or road cuts. A soil profile description documents the vertical sequence of horizons, their physical and morphological properties, and the relationships among them. Profile descriptions are fundamental to soil classification, land use assessment, understanding pedogenic processes, and interpreting laboratory analytical data in a landscape context (Schoeneberger et al., 2012; Soil Survey Staff, 2022). The systematic description of soil horizons is the foundation of pedology and provides the morphological evidence from which pedogenic processes (e.g., eluviation, illuviation, gleization) are inferred (Buol et al., 2011; Schaetzl & Anderson, 2015).

2. Scope

- **Applies to:** Field description of soil profiles at all EA Program sites, including research plots, restoration sites, and instructional field exercises
- **Does not apply to:** Auger-based sampling where no profile face is exposed (see SOP-F400); engineering soil classifications (Unified Soil Classification System); geotechnical borehole logging
- **Location:** All EA Program field sites where soil pits can be safely excavated

3. Responsibilities

Role	Responsibility
PI / Field Lead	Select pit locations; approve excavation; ensure safety
Trained Personnel	Excavate pits and describe profiles per this SOP
New Students	Complete training before independent profile description

4. Definitions

- **Consistence:** The resistance of soil to deformation or rupture; described at field moisture conditions (loose, very friable, friable, firm, very firm, extremely firm for moist soil) and separately for dry and wet conditions (sticky, plastic ratings).
- **Horizon:** A layer of soil approximately parallel to the land surface with distinct physical, chemical, or biological properties that differ from adjacent layers; the fundamental unit of soil profile description; designated by master symbols (O, A, E, B, C,

  R) and subordinate suffixes (e.g., Bt, Bhs).

- **Horizon boundary:** The contact between two soil horizons; described by distinctness (very abrupt, abrupt, clear, gradual, diffuse based on transition thickness) and topography (smooth, wavy, irregular, broken).
- **Master horizon:** A primary genetic horizon designation: O (organic surface), A (mineral surface with organic matter accumulation), E (eluviated), B (subsoil with illuvial accumulation or alteration), C (parent material), R (bedrock).
- **Ped:** A natural soil aggregate formed by soil-forming processes; described by type (granular, blocky, subangular blocky, prismatic, columnar, platy) and size class (very fine, fine, medium, coarse, very coarse).
- **Pedon:** The smallest three-dimensional body of soil that fully represents the range of properties of a given soil; typically 1–10 m^2 in surface area and extending to the C horizon or bedrock.
- **Soil profile:** A vertical cross-section of soil from the surface to the parent material or bedrock, exposing the sequence of genetic horizons; described in a pit or by augering.
- **Soil structure:** The arrangement and organization of soil particles into aggregates (peds); described by type (shape), class (size), and grade (distinctness of development: structureless, weak, moderate, strong).

- **Texture (field):** The relative proportions of sand, silt, and clay in the fine earth fraction (less than 2 mm); estimated in the field by the feel method (see SOP-F401) and confirmed by hydrometer analysis (see SOP-L401).

5. Safety and Hazards

⚠ Hazards

- **Physical:** Soil pits deeper than 50 cm can collapse, especially in sandy or dry soils. Pits deeper than 1.2 m (4 ft) require shoring or sloping per OSHA regulations (29 CFR 1926, Subpart P). Never enter an unshored pit deeper than 1.2 m.
- **Biological:** Soil-borne pathogens; ticks, snakes, and burrowing insects in riparian or wildland settings.
- **Environmental:** Heat exposure; dehydration during summer fieldwork.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Nitrile gloves
☐ Sun protection (hat, sunscreen)
☐ Safety glasses (when chopping through indurated horizons)
☐ Hard hat (if working in deep pits or near road cuts)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. If a pit wall collapses while someone is inside, call 911 immediately. Do not attempt excavation without proper rescue training and equipment.

6. Equipment and Materials

Item	Notes
Sharpshooter shovel	For excavating the pit
Flat-edged spade or soil knife	For cleaning the profile face
Hand pick or mattock	For indurated or cemented horizons
Munsell Soil Color Charts	See SOP-F405
Squeeze bottle with DI water	For moistening soil for color and texture
Metric tape measure (2 m)	For measuring horizon depths
Clinometer or hand level	For measuring slope at the pit site
Compass	For recording aspect
10% HCl solution in dropper bottle	For testing for carbonates (effervescence)
Field notebook or profile description form	NRCS-style form preferred
Camera	For photographing the profile face

Item	Notes
Ruler or scale bar	Placed against the profile for photo scale
GPS unit	For recording pit coordinates per SOP-F006
Flagging tape	For marking horizon boundaries
Sample bags (labeled)	If collecting horizon samples for lab analysis

7. Procedure

7.1. Pit Excavation

1. Select the pit location based on the project objectives and landscape position. Avoid disturbed areas, utility lines, and locations near structures.
2. Excavate a pit approximately 50 cm wide and 80–120 cm deep (or to the parent material or restrictive layer, whichever is shallower). One vertical face should be oriented to receive even light (face the sun or open shade).
3. Pile excavated soil upslope and on a tarp to facilitate backfilling.
4. Clean the profile face with the flat-edged spade or soil knife to expose a fresh, undisturbed surface. Avoid smearing.

7.2. Identifying Horizon Boundaries

1. Examine the profile face for changes in color, texture, structure, roots, pores, and cementation. Each distinct zone is a horizon.
2. Mark horizon boundaries with flagging tape pins or nails.
3. Measure the depth of each boundary from the soil surface using the metric tape. Record the upper and lower boundary depth for each horizon (e.g., A: 0–18 cm; Bt: 18–45 cm).
4. Describe the boundary distinctness and topography:

Distinctness	Width of Transition
Abrupt	< 2 cm
Clear	2–5 cm
Gradual	5–15 cm
Diffuse	> 15 cm

Topography	Description
Smooth	Nearly a plane
Wavy	Pockets wider than deep
Irregular	Pockets deeper than wide
Broken	Discontinuous

Boundary distinctness reflects the rate and nature of pedogenic change between horizons; abrupt boundaries often indicate a lithologic discontinuity or a strong process boundary (e.g., plowing), while diffuse boundaries suggest gradual, ongoing translocation (Schaetzl & Anderson, 2015).

7.3. Describing Each Horizon

For each horizon, record the following properties:

Horizon designation: Use standard USDA master horizon symbols (O, A, E, B, C, R) with appropriate suffixes (t, k, w, g, etc.) (Soil Survey Division Staff, 2017). When assigning subordinate suffixes (e.g., **t** for clay accumulation, **k** for carbonate, **g** for gleying), verify the morphological evidence for each suffix against the criteria in Keys to Soil Taxonomy (Soil Survey Staff, 2022) to ensure that the designation supports formal classification.

Color: Determine moist and dry color using Munsell charts per SOP-F405. Record matrix color and any mottles.

Texture: Determine texture by feel per SOP-F401. Record the USDA textural class (e.g., sandy loam, clay loam).

Structure: Describe the type, size, and grade:

Type	Shape Description
Granular	Roughly spherical, porous
Blocky (angular)	Sharp-edged blocks
Blocky (subangular)	Rounded-edge blocks
Platy	Flat, plate-like
Prismatic	Vertical columns, flat tops
Columnar	Vertical columns, rounded tops
Massive	No visible structure (coherent mass)
Single grain	No structure (loose, individual particles)

Size: very fine, fine, medium, coarse, very coarse (ranges depend on structure type; consult the *Field Book for Describing and Sampling Soils*).

Grade: weak, moderate, strong.

Soil structure type and grade influence water movement, root penetration, and aeration. Strongly developed angular blocky structure in B horizons often correlates with high clay content and shrink-swell behavior, while granular structure in A horizons is associated with biological activity and high organic matter (Schaetzl & Anderson, 2015; Soil Survey Division Staff, 2017).

Consistence: Describe the resistance to rupture at the appropriate moisture state:

- Dry: loose, soft, slightly hard, hard, very hard, extremely hard
- Moist: loose, very friable, friable, firm, very firm, extremely firm
- Wet: nonsticky/nonplastic to very sticky/very plastic

Roots: Record abundance (few, common, many) and size (very fine, fine, medium, coarse).

Pores: Record abundance and size.

Effervescence: Apply 1–2 drops of 10% HCl to the horizon face and record the reaction:

Effervescence	Description
None	No visible bubbles
Slight	Few bubbles, barely audible
Strong	Obvious bubbles, clearly audible
Violent	Vigorous bubbling, foam

Other features: Record any clay films, coatings, concretions, cementation, or biological features (krotovinas, worm casts).

7.4. Site Description

Record the following site-level information:

- GPS coordinates per SOP-F006
- Slope (percent) and aspect (degrees)
- Landscape position (summit, shoulder, backslope, footslope, toeslope)
- Parent material (if identifiable: alluvium, colluvium, residuum, loess, etc.). At EA Program sites in the Pomona/Claremont area, parent materials are commonly alluvium derived from the San Gabriel Mountains or residuum from granitic bedrock, and argillic horizons (Bt) are frequently developed in older geomorphic surfaces (Buol et al., 2011).
- Vegetation and land use
- Drainage class (excessively drained to very poorly drained)
- Depth to water table or bedrock (if encountered)
- Evidence of erosion or deposition

7.5. Photography

1. Place a ruler or scale bar vertically against the profile face.
2. Photograph the entire profile with horizon labels visible.
3. Take close-up photos of features of interest (mottles, clay films, cementation, root channels).
4. Record photo numbers in the field notebook.

7.6. Backfilling

Backfill the pit promptly after description and sampling. Replace soil in reverse order (subsoil first, topsoil last). Compact gently to prevent subsidence. Restore surface litter.

8. Quality Control

- Use the NRCS *Field Book for Describing and Sampling Soils* as the authoritative reference for all terminology and classification.
- Have a second observer verify at least the horizon designations and colors for profiles used in research publications.

- Photograph every profile with a scale bar as a permanent record.
- Cross-check field texture estimates with laboratory hydrometer results (SOP-L401) for at least one horizon per profile.

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10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F400	Soil Sampling	Horizon samples for lab analysis
F401	Texture by Feel	Field texture for each horizon
F402	Soil Bulk Density	Core samples from horizons
F405	Soil Color (Munsell)	Color determination per horizon
L401	Texture (Hydrometer)	Lab confirmation of field texture

11. Training Record

Before describing soil profiles independently, personnel must:

1. Read this SOP, SOP-F405, and SOP-F401 in full
2. Describe at least two supervised soil profiles with results reviewed by the PI or a trained pedologist
3. Demonstrate correct use of the Munsell charts, texture by feel, HCl effervescence test, and structure terminology
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F501

Air Temperature Monitoring with Portable Sensors

Standard Operating Procedure

SOP Number	SOP-F501
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the deployment, calibration, data retrieval, and post-processing of low-cost portable sensor networks for ambient air temperature and relative humidity monitoring. Three sensor platforms are covered: Maxim/Analog Devices Thermochron iButton loggers, Onset HOBO data loggers, and Raspberry Pi single-board computers with BME280 environmental sensor modules.

Dense networks of inexpensive air temperature sensors enable studies of urban heat island (UHI) effects, microclimatic gradients beneath photovoltaic arrays, canopy-vs.-open temperature contrasts, and diurnal and seasonal temperature variability at spatial resolutions not achievable with conventional weather stations (Chapman et al., 2015; Muller et al., 2013). The UHI is one of the most thoroughly documented examples of inadvertent human modification of local climate, driven by impervious surfaces, waste heat, reduced vegetation, and altered radiative and convective energy exchange (Oke et al., 2017; Voogt & Oke, 2003).

When designing a sensor network, characterizing the land cover at each site using the Local Climate Zone (LCZ) framework provides a standardized basis for comparing UHI intensities across cities and studies (Stewart & Oke, 2012).

2. Scope

- **Applies to:** Outdoor ambient air temperature and relative humidity monitoring at 1.5–2.0 m above ground level using portable sensor networks.
- **Does not apply to:** Soil temperature profiles (see SOP-F404), indoor temperature monitoring, or air quality (PM, gases; see SOP-F502).
- **Location:** EA Program field sites including the Pomona College campus, Bernard Field Station, photovoltaic study sites, and surrounding Inland Empire communities.

3. Responsibilities

Role	Responsibility
Lab PI	Approve monitoring plan, oversee data quality
Field Lead	Coordinate site access and logistics
Trained Personnel	Calibrate, deploy, retrieve data, process results

Role	Responsibility
New Students	Complete training before independent deployment

4. Safety and Hazards

⚠ Hazards

- **Working at height:** Mounting sensors at 1.5–2.0 m on poles or structures may require a stepladder. Follow ladder safety protocols.
- **Traffic:** Some monitoring locations are near roadways. Wear high-visibility vests.
- **Heat exposure:** Deployment and retrieval in summer in the Inland Empire involves significant heat risk. Carry water, work in pairs, and schedule field work for early morning when possible.
- **Electrical:** Raspberry Pi units use low-voltage USB power. Do not expose connections to rain or standing water without weatherproof enclosures.

4.1. Required PPE

☐ Closed-toe shoes
☐ High-visibility vest (if near roadways)
☐ Sun protection (hat, sunscreen)
☐ Safety glasses (during mounting/drilling)

5. Definitions

- **Urban Heat Island (UHI):** The phenomenon whereby urban areas experience higher air temperatures than surrounding rural areas due to impervious surfaces, waste heat, reduced vegetation, and altered energy balance. UHI intensity (ΔT_{UHI}) is quantified as the difference between an urban and a rural reference site at a given time (Oke et al., 2017).
- **Local Climate Zone (LCZ):** A classification system that describes the physical properties of the local environment (building density, height, surface cover, land use) at scales relevant to urban temperature variation. LCZ types range from compact high-rise (LCZ 1) to scattered trees (LCZ B), and provide a standardized framework for describing monitoring sites in UHI studies (Stewart & Oke, 2012).
- **Radiation shield:** A louvered or ventilated housing that protects a temperature sensor from direct and reflected solar radiation while allowing ambient air to flow past the sensor. Without shielding, sensors can read 5–15 C above true air temperature in direct sun (Hubbart et al., 2005; Terando et al., 2017).
- **BME280:** A Bosch environmental sensor measuring temperature, relative humidity, and barometric pressure. Used with Raspberry Pi platforms in the EA Program sensor network.
- **iButton:** A coin-sized temperature (and optionally RH) data logger manufactured by Maxim/Analog Devices (Thermochron series).
- **HOBO:** A family of portable data loggers manufactured by Onset Computer Corporation.

- **Collocation:** The practice of placing multiple sensors at the same location for a period to assess inter-sensor agreement and identify systematic offsets. Collocation is the primary calibration strategy for dense low-cost sensor networks (Meier et al., 2017).
- **Calibration offset:** A systematic difference between a sensor's reading and a known reference, determined during collocation and applied as a correction to field data.
- **Data completeness:** The percentage of expected readings actually recorded during a deployment period. A minimum of 75% completeness (18 of 24 hours per day) is required for daily summaries.

6. Prerequisites

- SOP-F001 Field Safety
- For Raspberry Pi deployments: basic familiarity with Raspberry Pi OS, SSH, and file transfer (see also SOP-F502)

7. Sensor Platforms

7.1. Comparison

Feature	iButton DS1921/DS1923	HOBO U23 Pro v2	Raspberry Pi + BME280
Approximate cost	$20–60	$130–200	$40–80
Temperature accuracy	$±$0.5–1.0 C	$±$0.21 C	$±$0.5 C (BME280)
RH measurement	DS1923 only	Yes	Yes
Logging interval	1 min – 255 min	1 s – 18 h	User-defined (script)
Memory	2048–8192 readings	~42,000 readings	SD card (unlimited)
Communication	1-Wire (download)	USB (download)	Wi-Fi / SD card
Battery life	~1 year	~1 year	Requires continuous power
Size	17 mm coin	6 x 5 x 2 cm	Variable (enclosure)

7.2. Choosing a Platform

- **iButton:** Best for dense spatial networks where low cost and small size are priorities. Limited memory requires periodic retrieval. Ideal for short-term (weeks to months) UHI transects.
- **HOBO:** Best general-purpose option with highest accuracy, long battery life, and large memory. Good for season-long deployments.
- **Raspberry Pi + BME280:** Best for real-time or near-real-time monitoring with Wi-Fi. Requires continuous power. Already available in the EA Program inventory (16 Pi Zero+ units).

8. Radiation Shielding

> **!** Radiation Shields Are Mandatory
>
> Unshielded sensors exposed to direct or reflected solar radiation can read 5–15 C higher than true air temperature. Every deployed sensor **must** be housed in a radiation shield (Hubbart et al., 2005; Terando et al., 2017).

8.1. Shield Options

Shield Type	Cost	Notes
6-plate gill shield (e.g., Davis 7714)	$20–30	Standard; good ventilation
10-plate aspirated shield	$30–50	Better in low-wind conditions
DIY stacked-plate shield (white plastic plates)	$5–10	Acceptable for student projects; see Appendix B for construction
Naturally aspirated PVC tube (white, louvered)	$10–15	Compact; fits iButtons

All shields must be white (high albedo) and mounted with the sensor opening facing downward to prevent rain intrusion.

9. Calibration

9.1. Ice-Bath Calibration (Single-Point Check)

1. Prepare an ice-water bath: fill an insulated container with crushed ice and deionized water, stir for 2 minutes
2. Submerge the sensor (in a waterproof sleeve for iButtons, or using a sealed bag for non-waterproof units)
3. Wait 5 minutes for equilibration
4. The sensor should read 0.0 $\pm$ 0.5 C. Record the reading.
5. If outside tolerance, note the offset; sensors with offsets greater than 1.0 C should be replaced.

9.2. Room-Temperature Inter-Comparison (Collocation)

1. Place all sensors to be deployed together in a shaded, well-mixed indoor environment for at least 24 hours
2. Calculate the mean temperature across all sensors for each timestep. Each sensor's offset is its deviation from the group mean.
3. Record offsets in a calibration log file: `data/calibration/offsets_YYYYMMDD.csv`
4. Apply these offsets as corrections during data processing (Section 11). Collocation under controlled conditions is the most practical calibration approach for networks of 10 or more sensors (Meier et al., 2017).

10. Deployment

10.1. Site Selection and Documentation

Select monitoring sites to represent the range of land cover types and microclimates relevant to the research question. For UHI studies, sample across multiple LCZ types (e.g., compact low-rise, open low-rise, large low-rise, scattered trees, dense trees) (Stewart & Oke, 2012).

At each site, record:

- GPS coordinates per SOP-F006
- Mounting height (standard: 1.5–2.0 m above ground level)
- Shield type and orientation
- Land cover within 50 m radius (impervious %, tree canopy %, grass %)
- LCZ type (see Stewart & Oke (2012) for classification guide)
- Nearest road (distance and type)
- Sky view factor (estimated or photographed)
- Sensor serial number and platform type

10.2. Mounting

1. Mount the radiation shield on a pole, post, fence, or structure at 1.5–2.0 m height. Ensure the shield is not directly above a paved surface, air conditioning exhaust, or heat-radiating wall.
2. Place the sensor inside the shield, ensuring the sensing element is centered within the shield stack.
3. For Raspberry Pi units, route the power cable through the base of the enclosure. Secure with cable ties.
4. Photograph the installation and record all metadata.
5. Record metadata as above.

11. Data Retrieval

Platform	Retrieval Method	Frequency
iButton	Remove, connect to 1-Wire reader, download in OneWireViewer	Every 2–4 weeks
HOBO U23	Connect USB shuttle or cable, download in HOBOware	Every 1–3 months
Raspberry Pi	SSH + `scp`, or remove SD card	Weekly or as needed

After retrieval, archive the raw file immediately. Never modify the original.

12. Data Processing

12.1. Import and Standardize

```
library(tidyverse)
library(lubridate)

# Example: import iButton CSV
raw <- read_csv("data/raw/site01_ibutton_ABC123.csv",
                skip = 14) %>%
  rename(timestamp = 1, temp_c = 2) %>%
  mutate(
    timestamp = mdy_hms(timestamp, tz = "America/Los_Angeles"),
    sensor_id = "ABC123",
    site_id   = "site01",
    platform  = "iButton"
  )

# Apply calibration offset (from Section 9)
offsets <- read_csv("data/calibration/offsets_YYYYMMDD.csv")

raw <- raw %>%
  left_join(offsets %>% select(sensor_id, offset), by = "sensor_id") %>%
  mutate(temp_corrected = temp_c - offset)
```

12.2. Quality Control

```
raw <- raw %>%
  mutate(
    qc_flag = case_when(
      temp_corrected < -10 | temp_corrected > 55 ~ "out_of_range",
      temp_corrected == lag(temp_corrected) &
        temp_corrected == lag(temp_corrected, 2) &
        temp_corrected == lag(temp_corrected, 3)   ~ "stuck_sensor",
      abs(temp_corrected - lag(temp_corrected)) > 10 ~ "spike",
      TRUE ~ "valid"
    )
  )

count(raw, qc_flag)
clean <- raw %>% filter(qc_flag == "valid")
```

12.3. Hourly and Daily Summaries

```
hourly <- clean %>%
  mutate(hour = floor_date(timestamp, "hour")) %>%
  group_by(site_id, sensor_id, hour) %>%
  summarize(
    temp_mean = mean(temp_corrected, na.rm = TRUE),
    temp_min  = min(temp_corrected, na.rm = TRUE),
    temp_max  = max(temp_corrected, na.rm = TRUE),
    n_obs     = n(),
    .groups   = "drop"
  ) %>%
  filter(n_obs >= 3)

daily <- hourly %>%
  mutate(date = as_date(hour)) %>%
  group_by(site_id, sensor_id, date) %>%
  summarize(
    t_mean = mean(temp_mean, na.rm = TRUE),
    t_min  = min(temp_min, na.rm = TRUE),
    t_max  = max(temp_max, na.rm = TRUE),
    n_hours = n(),
    .groups = "drop"
  ) %>%
  filter(n_hours >= 18)
```

12.4. Urban Heat Island Intensity

UHI intensity is typically calculated as the difference between an urban site and a rural (or least-developed) reference site (Oke et al., 2017):

$$\Delta T_{\text{UHI}} = T_{\text{urban}} - T_{\text{rural}}$$

```
ref_site <- "site_rural01"

uhi <- hourly %>%
  select(site_id, hour, temp_mean) %>%
  pivot_wider(names_from = site_id, values_from = temp_mean) %>%
  mutate(across(-c(hour, all_of(ref_site)),
                ~ . - .data[[ref_site]],
                .names = "uhi_{.col}"))
```

13. Quality Control Summary

Check	Criterion
Ice-bath calibration	Reading within $±$0.5 C of 0.0 C
Inter-sensor agreement (collocation)	Within $±$0.5 C at night
Hourly data completeness	$ $75% of expected readings
Daily data completeness	$ $18 of 24 hours
Stuck-sensor detection	$ $4 identical consecutive readings flagged
Spike detection	$ $10 C change between consecutive readings flagged

14. Data Management

- Raw files archived unmodified: `data/raw/siteID_platform_sensorID.csv`
- Calibration records: `data/calibration/`
- Processed files: `data/processed/`
- Site metadata (coordinates, height, shield type, land cover, LCZ type, sensor serial numbers, deployment/retrieval dates): `data/metadata/site_metadata.csv`
- All scripts version-controlled in the project Git repository

15. References

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16. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F101	Electrical Power in the Field	Power for Pi deployments
F404	Soil Temperature Profiles	Companion: soil vs. air temp
F502	Air Quality Monitoring	Companion: PM sensors on same Pi units
F510	Weather Monitoring	Companion: full meteorological context

17. Training Record

Before deploying air temperature sensors independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Perform a calibration (ice-water bath and room-temperature inter-comparison) under supervision
3. Assemble and mount a radiation shield
4. Deploy and retrieve a sensor at a test site
5. Import, clean, and summarize data using the R workflow
6. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F502

Air Quality Monitoring with Portable PM Sensors

Standard Operating Procedure

SOP Number	SOP-F502
Revision	0.2
Author(s)	Marc Los Huertos
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1. Purpose

This SOP describes the assembly, deployment, data retrieval, and post-processing of low-cost particulate matter (PM) sensor networks built around Raspberry Pi single-board computers and Plantower PMS5003/PMS5004 laser-scattering sensors. These sensors estimate PM_1, $PM_{2.5}$, and PM_{10} mass concentrations and report particle counts in six size bins (>0.3, >0.5, >1.0, >2.5, >5.0, >10.0 μm).

Low-cost PM sensors (LCS) provide spatial and temporal resolution that is not achievable with Federal Reference Method (FRM) or Federal Equivalent Method (FEM) monitors alone (Snyder et al., 2013). However, LCS data require quality screening, relative humidity correction, and calibration against reference instruments to produce scientifically defensible results (Ardon-Dryer et al., 2023; Barkjohn et al., 2021). When properly corrected and quality-controlled, LCS networks can complement regulatory monitoring by filling spatial gaps, supporting community exposure studies, and providing near-real-time information during wildfire smoke events (Morawska et al., 2018).

2. Scope

- **Applies to:** Outdoor ambient air quality monitoring using EA Program Raspberry Pi + PMS5003/PMS5004 sensor units for PM_1, $PM_{2.5}$, and PM_{10}.
- **Does not apply to:** Indoor air quality monitoring (different correction factors apply), gas-phase pollutant monitoring (O_3, NO_2, CO), or regulatory compliance monitoring.
- **Location:** Field deployment sites in the Pomona College area and surrounding Inland Empire communities.

3. Responsibilities

Role	Responsibility
Lab PI	Approve deployment plan, oversee data quality
Field Lead	Coordinate site access, logistics, and safety
Trained Personnel	Assemble, deploy, retrieve, and process data
New Students	Complete training before independent deployment

4. Definitions

- **$PM_{2.5}$:** Particulate matter with aerodynamic diameter less than 2.5 μm. $PM_{2.5}$ is a criteria pollutant regulated by the US EPA because of its association with cardiovascular and respiratory health effects.
- **PM_{10}:** Particulate matter with aerodynamic diameter less than 10 μm. Includes both fine ($PM_{2.5}$) and coarse (2.5–10 μm) fractions.
- **Low-cost sensor (LCS):** A PM sensor costing less than approximately $250 per unit, as distinct from regulatory-grade FRM or FEM instruments costing $10,000–$50,000 (Snyder et al., 2013).
- **Federal Reference Method (FRM):** The EPA-designated reference method for measuring criteria pollutants. For $PM_{2.5}$, the FRM is a gravimetric filter-based method (see SOP-F503).
- **Federal Equivalent Method (FEM):** An air quality measurement method approved by the US EPA as equivalent to the FRM (e.g., beta attenuation monitors).
- **CF=1:** "Standard particles" output from the Plantower sensor, factory-calibrated against Chinese ambient aerosol. This is the starting value for the EPA US-wide correction (Barkjohn et al., 2021).
- **AQI:** Air Quality Index. A standardized index for reporting daily air quality developed by the US EPA, with breakpoints tied to health-based $PM_{2.5}$ standards.
- **Collocation:** Placing LCS units alongside a reference instrument (or alongside each other) to assess accuracy, precision, and drift. Pre-deployment collocation is a minimum QC requirement.
- **Sensor drift:** Gradual degradation of sensor accuracy over months to years of continuous operation, caused by fan wear, laser aging, and contamination of the optical chamber (Malings et al., 2020).
- **Hygroscopic growth:** The increase in particle size (and thus light scattering) that occurs when ambient RH exceeds approximately 70%, causing LCS to overestimate PM mass concentration. The EPA correction includes an RH term to account for this (Barkjohn et al., 2021; Jayaratne et al., 2018).
- **PMS-O / PMS-U:** Original (pre-mid-2021) and updated (post-mid-2021) versions of the Plantower PMS5003, which differ in internal calibration. PMS-U sensors read lower at concentrations below approximately 16 $\mu g/m^3$ (Ardon-Dryer et al., 2023).

5. Safety and Hazards

⚠ Hazards

- **Electrical:** Raspberry Pi units and USB power supplies. Do not expose connections to rain or standing water. Use weatherproof enclosures for outdoor deployment.
- **Heat:** Enclosures can reach high temperatures in direct sunlight, especially in summer in the Inland Empire. Use ventilated or shaded enclosures.
- **Traffic and site access:** Many monitoring locations are near roadways. Follow all traffic safety procedures in SOP-F001.
- **Working at height:** If mounting sensors on poles or structures, follow ladder safety protocols.

5.1. Required PPE

☐ Safety glasses (during assembly)
☐ Closed-toe shoes
☐ High-visibility vest (if near roadways)
☐ Sun protection (hat, sunscreen)

6. Prerequisites

- SOP-F001 Field Safety
- Basic familiarity with Raspberry Pi OS (command line, SSH, file transfer)
- Basic familiarity with R for data processing

7. Background

7.1. How the Sensors Work

The Plantower PMS5003 and PMS5004 are optical particle counters that use a laser (680 ± 10 nm) and a photodiode to detect light scattered by individual particles drawn through the sensing chamber by a small fan. The sensor reports particle counts in six size bins and uses a proprietary algorithm to estimate PM_1, $PM_{2.5}$, and PM_{10} mass concentrations in $\mu g/m^3$ (Barkjohn et al., 2021).

The sensor outputs data in two formats: CF=1 ("standard particles," factory-calibrated against Chinese urban aerosol) and CF_atm ("atmospheric environment," with an additional adjustment at higher concentrations). For ambient monitoring in the US, the CF=1 output is used as the starting point for correction (Barkjohn et al., 2021).

7.2. Known Limitations

- **Relative humidity sensitivity:** At RH >70%, hygroscopic particle growth inflates readings. An RH correction term is essential (Barkjohn et al., 2021; Jayaratne et al., 2018).

- **Particle size dependence:** The sensors underestimate $PM_{2.5}$ when coarse particles (dust) dominate the size distribution. They perform best for fine-mode aerosol (PM_1) (Jaffe et al., 2023; Ouimette et al., 2026).
- **Batch variability:** Plantower changed the PMS5003 calibration in mid-2021, producing sensors that read lower at low concentrations. These can be identified by their particle count ratios and corrected (Ardon-Dryer et al., 2023).
- **Sensor aging:** Fan and laser degradation over months to years of continuous operation can reduce sensitivity. Long-term field evaluations show measurable drift after 6–12 months of continuous outdoor deployment (Malings et al., 2020). Periodic collocation with a reference instrument is needed.

8. Equipment and Materials

8.1. Sensor Unit Components

Item	Qty per Unit	Notes
Raspberry Pi Zero+ (or 3+/4+)	1	With microSD card (32 GB min)
Plantower PMS5004 (or PMS5003)	1	With ribbon cable
BME280 sensor module	1	Temperature, RH, pressure
USB power supply (5V, 2.5A)	1	Or solar panel + battery
Weatherproof enclosure	1	Ventilated; inlet tube for PM
microSD card (32 GB)	1	Pre-loaded with sensor software
Ethernet or Wi-Fi dongle	1	If Pi Zero (built-in on Pi 3+/4+)

8.2. Field Deployment Supplies

Item	Notes
Zip ties, mounting brackets	For securing enclosures
Extension cord or solar panel	Power supply for remote sites
GPS unit or smartphone	For recording coordinates
Field notebook	Site metadata
Laptop with SSH client	For on-site diagnostics

9. Procedure

9.1. Sensor Assembly and Software Setup

1. Flash the microSD card with Raspberry Pi OS Lite.
2. Install the sensor-reading Python script (maintained in the project repository). The script reads the PMS5003/5004 via serial (UART) and the BME280 via I2C, logging timestamped readings to a CSV file on the SD card.

3. Configure the Pi for headless operation (enable SSH, set Wi-Fi credentials if applicable, set timezone to America/Los_Angeles).
4. Connect the PMS sensor ribbon cable and the BME280 module.
5. Power on the unit and verify that the sensor LED illuminates and the fan spins. Check the log file for valid readings.
6. Allow at least 30 seconds of warm-up before trusting data (Plantower Technology, 2016).

9.2. Collocation (Pre-Deployment Calibration)

Before field deployment, collocate all sensor units at a single location for a minimum of 48 hours:

1. Place all units within 1 m of each other, at the same height, with inlets facing the same direction.
2. Compare readings across units to identify outliers or malfunctioning sensors.
3. If a reference instrument (FRM, FEM, or BAM) is available, collocate sensors with it to develop a site-specific correction factor (Barkjohn et al., 2021).
4. Calculate inter-sensor coefficients of variation (CV). Units with CV >15% relative to the group median should be inspected and recalibrated or replaced.

9.3. Site Selection

- Mount sensors 2–4 m above ground level, away from direct emission sources (exhaust vents, barbecues, construction).
- Orient the inlet away from prevailing wind to reduce rain intrusion.
- Ensure access to power (AC outlet, solar panel, or battery).
- Record GPS coordinates, mounting height, distance to nearest road, and land use context.

9.4. Deployment

1. Secure the enclosure at the selected location using zip ties, brackets, or straps.
2. Connect power.
3. Verify operation by SSH into the Pi and checking the live log.
4. Record the deployment date/time, site ID, sensor serial number, and any notes in the field notebook and project metadata file.

9.5. Data Retrieval

Retrieve data at regular intervals (weekly to monthly):

1. SSH into the Pi and copy the CSV log file to your laptop using `scp` or `rsync`.
2. Alternatively, remove the microSD card and copy files directly.
3. Archive the raw file immediately; never edit the original.
4. Check the log for gaps (power outages, sensor freezes).

9.6. Sensor Maintenance

- Inspect the inlet for debris, spider webs, or insect nests at each data retrieval visit.
- Replace sensors after approximately 2 years of continuous outdoor operation, or sooner if collocation checks show degraded performance (Malings et al., 2020).
- Clean the exterior of the enclosure. Do not open the PMS sensor housing.

9.7. Decommissioning

1. Power off the unit.
2. Retrieve the final data file.
3. Remove the enclosure and all mounting hardware.
4. Leave no trace at the site.

10. Data Processing and Correction

10.1. Overview

Raw $PM_{2.5}$ readings from PMS5003/5004 sensors overestimate FRM/FEM concentrations by approximately 40% under typical US ambient conditions (Barkjohn et al., 2021). The EPA US-wide correction reduces root mean square error from approximately 8 $\mu g/m^3$ to 3 $\mu g/m^3$ (Barkjohn et al., 2021).

10.2. Data Cleaning

```
library(tidyverse)

raw <- read_csv("data/raw/site01_20260101_20260201.csv")

raw <- raw %>%
  filter(
    pm25_cf1 >= 0,
    pm25_cf1 < 1000,
    rh_pct >= 0,
    rh_pct <= 100,
    temp_c > -40,
    temp_c < 60
  )

# Flag and remove sensor freeze periods
raw <- raw %>%
  mutate(frozen = pm25_cf1 == lag(pm25_cf1) &
                  pm25_cf1 == lag(pm25_cf1, 2) &
                  pm25_cf1 == lag(pm25_cf1, 3)) %>%
```

```
  filter(!frozen | is.na(frozen)) %>%
  select(-frozen)
```

10.3. EPA US-Wide Correction (Barkjohn et al., 2021)

$$\mathrm{PM}_{2.5,\mathrm{corrected}} = 0.524 \times \mathrm{PM}_{2.5,\mathrm{cf1}} - 0.0862 \times \mathrm{RH} + 5.75$$

where $\mathrm{PM}_{2.5,\mathrm{cf1}}$ is the CF=1 output (μg/m^3) and RH is relative humidity (%) (Barkjohn et al., 2021).

```
raw <- raw %>%
  mutate(
    pm25_corrected = 0.524 * pm25_cf1 - 0.0862 * rh_pct + 5.75,
    pm25_corrected = pmax(pm25_corrected, 0)
  )
```

> **!** Important
>
> This correction was developed for typical ambient and smoke-impacted conditions. It underestimates $\mathrm{PM}_{2.5}$ during dust-dominated events. For dust correction, see Jaffe et al. (2023) and Ouimette et al. (2026).

10.4. Identifying Updated (Post-2021) PMS5003 Sensors

If your sensors are PMS-U (post-mid-2021), apply the additional linear correction from Ardon-Dryer et al. (2023) before the EPA correction. PMS-U sensors can be identified by a $\mathrm{count}_{0.3}/\mathrm{count}_{0.5}$ ratio approximately 3 times lower than PMS-O sensors.

10.5. Hourly and Daily Averaging

```
hourly <- raw %>%
  mutate(hour = floor_date(timestamp, "hour")) %>%
  group_by(hour) %>%
  summarize(
    pm25_mean = mean(pm25_corrected, na.rm = TRUE),
    pm25_sd   = sd(pm25_corrected, na.rm = TRUE),
    rh_mean   = mean(rh_pct, na.rm = TRUE),
    temp_mean = mean(temp_c, na.rm = TRUE),
    n_obs     = n(),
    .groups   = "drop"
  ) %>%
  filter(n_obs >= 45)

daily <- hourly %>%
```

```
  mutate(date = as_date(hour)) %>%
  group_by(date) %>%
  summarize(
    pm25_daily = mean(pm25_mean, na.rm = TRUE),
    n_hours    = n(),
    .groups    = "drop"
  ) %>%
  filter(n_hours >= 18)
```

10.6. AQI Calculation

```
calc_aqi <- function(pm25) {
  breakpoints <- tibble(
    lo_pm  = c(0.0, 9.1, 35.5, 55.5, 125.5, 225.5),
    hi_pm  = c(9.0, 35.4, 55.4, 125.4, 225.4, 325.4),
    lo_aqi = c(0, 51, 101, 151, 201, 301),
    hi_aqi = c(50, 100, 150, 200, 300, 500)
  )
  bp <- breakpoints %>% filter(pm25 >= lo_pm, pm25 <= hi_pm)
  if (nrow(bp) == 0) return(NA_real_)
  with(bp, lo_aqi + (hi_aqi - lo_aqi) / (hi_pm - lo_pm) * (pm25 - lo_pm))
}

daily <- daily %>%
  mutate(aqi = map_dbl(pm25_daily, calc_aqi))
```

11. Quality Control

11.1. Pre-Deployment

- All sensors pass collocation check (inter-sensor CV <15%).
- Software logging verified (valid timestamps, all columns populated).

11.2. During Deployment

- Weekly data check (via SSH or at retrieval) for gaps and anomalies.
- Inspect enclosure and inlet monthly.

11.3. Post-Processing QC Flags

Flag	Condition	Action
F1	RH >95% for >4 consecutive hours	Flag; consider excluding
F2	$PM_{2.5}$ >500 $\mu g/m^3$ for >1 hour	Verify against regional monitors
F3	Sensor freeze (identical readings >10 min)	Exclude
F4	Hourly data completeness <75%	Exclude hour
F5	Daily data completeness <75% of hours	Exclude day

11.4. Periodic Collocation

Re-collocate sensors with a reference instrument (or with each other) at least once per year and after any sensor replacement. Document drift and update correction factors if needed (Malings et al., 2020).

12. Data Management

- Archive raw CSV files unmodified in `data/raw/` with the naming convention: `siteID_YYYYMMDD_YYYYMMDD_ra`
- Processed/corrected files in `data/processed/`
- Metadata in `data/metadata/`
- All processing scripts version-controlled per SOP-L801

13. Reporting

When reporting LCS data in publications or reports, include (Morawska et al., 2018):

- Sensor make and model (Plantower PMS5003 or PMS5004)
- Platform (Raspberry Pi model)
- Correction equation used (cite the reference)
- Whether sensors were collocated with a reference instrument
- Data completeness and QC criteria applied
- Known limitations (RH sensitivity, dust underestimation, sensor age)

14. References

Ardon-Dryer, K., Kelp, M. M., Stamber, B., & Kohl, S. (2023). Technical note: Identifying a performance change in the Plantower PMS 5003 particulate matter sensor. *Journal of Aerosol Science*, *173*, 106215. https://doi.org/10.1016/j.jaerosci.2023.106215

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Plantower Technology. (2016). *Digital universal particle concentration sensor: PMS5003 series data manual*. http://www.aqmd.gov/docs/default-source/aq-spec/resources-page/plantower-pms5003-manual_v2-3.pdf

Snyder, E. G., Watkins, T. H., Solomon, P. A., Thoma, E. D., Williams, R. W., Hagler, G. S. W., Shelow, D., Hindin, D. A., Kilaru, V. J., & Preuss, P. W. (2013). The changing paradigm of air pollution monitoring. *Environmental Science & Technology*, *47*(20), 11369–11377. https://doi.org/10.1021/es4022602

15. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F101	Electrical Power in the Field	Power supply guidance
F501	Air Temperature Monitoring	Companion: temp sensors on same Pi
F503	PM Filter Sampling	Reference-quality gravimetric companion
F504	Picarro GHG Analyzer	Companion field instrument SOP

16. Training Record

Before deploying air quality sensors independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Demonstrate Raspberry Pi setup (OS flash, SSH, sensor script configuration) under supervision
3. Participate in a supervised collocation and deployment

4. Perform a data retrieval and run the correction/QC pipeline
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F503

Particulate Matter Sampling – Gravimetric Filter Method

Standard Operating Procedure

SOP Number	SOP-F503
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the collection of ambient particulate matter (PM) on pre-weighed filters using active (pump-based) samplers for subsequent gravimetric and/or chemical analysis. Gravimetric filter sampling provides a reference-quality mass concentration that can be used to validate or

calibrate low-cost PM sensors (see SOP-F502) and to collect PM for compositional analysis (metals, ions, elemental/organic carbon) (Chow, 1995; US Environmental Protection Agency, 2017).

Ambient PM is a complex mixture of solid and liquid particles suspended in air, originating from both primary sources (combustion, dust, sea spray) and secondary formation (gas-to-particle conversion of sulfate, nitrate, and organic compounds) (Seinfeld & Pandis, 2016; **poschl2005?**). Gravimetric filter sampling remains the reference standard for PM mass measurement because it directly weighs the collected material, avoiding the indirect assumptions inherent in optical or beta-attenuation methods (US Environmental Protection Agency, 2017).

This SOP covers medium- and low-volume filter-based samplers operated by EA Program personnel. It does not cover Federal Reference Method (FRM) high-volume samplers operated by regulatory agencies.

2. Scope

- **Applies to:** Collection of $PM_{2.5}$ and PM_{10} on filters using portable or semi-permanent medium-volume or personal air samplers (e.g., MiniVol, BGI PQ200, SKC personal samplers) at EA Program field sites
- **Does not apply to:** Low-cost optical sensors without filters (see SOP-F502); FRM/FEM high-volume samplers operated by AQMD or EPA; indoor air quality sampling; gas-phase pollutant sampling
- **Location:** EA Program field deployment sites in the Pomona College area and surrounding Inland Empire communities

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling plan, coordinate site access
Trained Personnel	Condition filters, deploy sampler, retrieve and weigh filters
Lab Manager	Maintain balance, desiccator, and sampler calibration
New Students	Complete training before independent deployment

4. Definitions

- **Particulate matter (PM):** A mixture of solid particles and liquid droplets suspended in air, ranging in size from a few nanometers to tens of micrometers (Seinfeld & Pandis, 2016).
- **$PM_{2.5}$:** Particulate matter with aerodynamic diameter less than 2.5 μm ("fine particles"). Fine particles penetrate deep into the lungs and are associated with cardiovascular and respiratory health effects (**solomon2001?**).
- **PM_{10}:** Particulate matter with aerodynamic diameter less than 10 μm. Includes both fine ($PM_{2.5}$) and coarse (2.5–10 μm) fractions.

- **Gravimetric analysis:** Determination of PM mass by weighing a filter before and after exposure, under controlled temperature and humidity conditions (US Environmental Protection Agency, 2017).
- **Size-selective inlet:** An impactor or cyclone that removes particles above a defined aerodynamic diameter cutpoint (e.g., 2.5 or 10 μm) before they reach the filter.
- **PTFE filter:** Polytetrafluoroethylene (Teflon) membrane filter. Chemically inert, hydrophobic, and low in artifact mass; the standard substrate for gravimetric PM analysis.
- **Quartz fiber filter:** A filter made of quartz (SiO_2) fibers, used when thermal-optical analysis for elemental and organic carbon is planned. Must be pre-fired at 500 C for 4 hours to remove organic artifacts.
- **Filter conditioning:** Equilibrating filters at controlled temperature (20–25 C) and humidity (30–40% RH) in a desiccator for at least 24 hours before weighing. Conditioning removes adsorbed water that would bias the mass measurement.
- **Field blank:** A conditioned filter transported to the field site, briefly exposed to ambient conditions without running the pump, then resealed and returned for weighing. Field blank mass quantifies contamination introduced during handling and transport.
- **Flow rate:** The volumetric rate at which air is drawn through the sampler, typically expressed in L/min. The flow rate must match the design specification of the size-selective inlet (e.g., 5 L/min for a MiniVol $PM_{2.5}$ impactor).
- **Chemical speciation:** Analysis of the chemical composition of collected PM (e.g., metals by XRF or ICP-MS, ions by IC, carbon fractions by thermal-optical analysis) (**chow2015?**).

5. Safety and Hazards

⚠ Hazards

- **Electrical:** Sampling pumps require AC or battery power. Protect connections from rain and moisture.
- **Traffic:** Many monitoring locations are near roadways. Wear a high-visibility vest and follow SOP-F001 traffic safety procedures.
- **Lifting:** Medium-volume samplers (e.g., MiniVol TAS) and batteries can be heavy (10–15 kg). Use proper lifting technique.
- **Heat:** Enclosures and pumps can overheat in direct sunlight during Inland Empire summers.

5.1. Required PPE

☐ Nitrile gloves (when handling filters)
☐ Closed-toed shoes
☐ High-visibility vest (if near roadways)
☐ Sun protection

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Medium-volume sampler (e.g., MiniVol TAS, BGI PQ200) or personal sampler (SKC, Leland Legacy)	With size-selective inlet ($PM_{2.5}$ or PM_{10} impactor)
Calibrated flow meter (rotameter or primary standard, e.g., Bios DryCal)	For verifying and adjusting flow rate
Timer or elapsed-time indicator	Built into most samplers
Tripod or mounting pole	For positioning inlet at 1.5–2 m
Battery or AC power source	Sufficient for the planned sampling duration

6.2. Filter Supplies

Item	Notes
Pre-weighed PTFE filters, 47 mm	For gravimetric analysis
Pre-weighed quartz fiber filters, 47 mm	If EC/OC analysis is planned; pre-fire at 500 C for 4 h
Filter cassettes or holders	Matched to the sampler
Petri dishes or filter transport cases	For transporting conditioned filters
Forceps (flat-tipped, non-serrated)	For handling filters
Desiccator with active desiccant	For filter conditioning
Analytical balance (0.01 mg or better)	Per SOP-L200

7. Procedure

7.1. Filter Conditioning and Pre-Weighing

1. Place unused filters in a desiccator at controlled temperature (20–25 C) and humidity (30–40% RH) for at least 24 hours (US Environmental Protection Agency, 2017)
2. Weigh each filter on the microbalance to the nearest 0.01 mg. Record the filter ID and tare weight.
3. Weigh each filter a second time (at least 1 hour later) to confirm stability. The two weights should agree within 0.015 mg.
4. Place the conditioned filter in a labeled Petri dish or cassette. Seal and transport in a cooler if traveling to a remote site.

7.2. Sampler Setup and Flow Calibration

1. Install the appropriate size-selective inlet ($PM_{2.5}$ or PM_{10} impactor head) on the sampler

2. Load a blank filter cassette and connect the calibrated flow meter downstream of the filter holder
3. Start the pump and adjust the flow rate to the nominal design flow for the inlet (e.g., 5 L/min for a MiniVol $PM_{2.5}$ impactor)
4. Record the calibrated flow rate, temperature, and barometric pressure. If the site is at a different elevation than the calibration location, apply a volumetric correction.
5. Remove the blank cassette and load the pre-weighed sample filter using forceps. Handle the filter only by its edges.

7.3. Deployment

1. Position the sampler with the inlet at 1.5–2 m above ground level, unobstructed by walls or overhangs (at least 2 m from any vertical surface)
2. Record the start date, start time, site ID, filter ID, GPS coordinates, and weather conditions
3. Start the sampler and verify that the pump is running
4. For 24-hour integrated samples, allow the sampler to run for 23–25 hours

7.4. Retrieval

1. Record the stop time and elapsed run time
2. Verify the flow rate with the calibrated flow meter before stopping the pump (post-sampling flow check). Record the value.
3. Stop the pump
4. Remove the filter cassette using gloves. Transfer the exposed filter to its labeled Petri dish using forceps. Do not touch the deposit surface.
5. Seal the Petri dish and place in a cooler for transport
6. Calculate the average flow rate: $Q_{\text{avg}} = (Q_{\text{pre}} + Q_{\text{post}})/2$. If pre- and post-flow rates differ by more than 10%, flag the sample.

7.5. Post-Sampling Conditioning and Weighing

1. Condition the exposed filter in the same desiccator and at the same temperature/humidity as the pre-weighing step for at least 24 hours
2. Weigh the filter to the nearest 0.01 mg. Record the gross weight.
3. Repeat the weighing at least 1 hour later to confirm stability.
4. Calculate the net PM mass:

$$m_{\text{PM}} = W_{\text{post}} - W_{\text{pre}}$$

5. Calculate the PM concentration:

$$C_{\text{PM}}(\mu\text{g/m}^3) = \frac{m_{\text{PM}}(\mu\text{g})}{Q_{\text{avg}}(\text{L/min}) \times t(\text{min})} \times 10^3$$

7.6. Filter Archiving and Downstream Analysis

1. After gravimetric analysis, archive filters in sealed Petri dishes at room temperature (or at -20 C if chemical analysis is planned)
2. For elemental/organic carbon analysis, submit quartz filters to the analytical laboratory per their instructions
3. For metals analysis by XRF or ICP-MS, submit PTFE filters per SOP-L205 or SOP-L410
4. Chemical speciation of collected PM provides source apportionment data that complements the mass concentration measurement (**chow2015?**)

8. Quality Control

- Collect at least one field blank per sampling event: load a conditioned filter into a cassette, transport it to the site, open the cassette briefly (without running the pump), seal, and return to the lab for conditioning and weighing
- Collect duplicate samples (co-located samplers) for at least 10% of sampling events. Duplicate PM concentrations should agree within 15%.
- Verify the balance with NIST-traceable check weights at the start of each weighing session
- Document pre- and post-sampling flow rates; flag samples where the difference exceeds 10%
- Re-condition and re-weigh at least 10% of filters as laboratory duplicates; agreement should be within 0.015 mg
- Negative net masses (post-weight less than pre-weight) can occur due to filter handling artifacts or volatile loss; flag and investigate these, but do not discard without documentation

9. Waste Disposal

Waste Type	Disposal Method
Exposed PTFE or quartz filters (after analysis)	Standard trash (non-hazardous)
Impactor grease and oiled impaction substrates	Chemical waste; EH&S pickup
Used desiccant	Standard trash or regenerate per manufacturer

10. References

Chow, J. C. (1995). Measurement methods to determine compliance with ambient air quality standards for suspended particles. *Journal of the Air and Waste Management Association*, *45*(5), 320–382. https://doi.org/10.1080/10473289.1995.10467369

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US Environmental Protection Agency. (2017). *Ambient air monitoring reference and equivalent methods* (40 CFR Part 50, Appendix L). US EPA.

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F502	Portable PM Sensors	Low-cost sensor companion
F504	Picarro GHG Analyzer	Companion field instrument
F505	Passive Air Samplers	Companion gas-phase sampling
L200	Analytical Balance	Filter weighing
L205	XRF Analysis	Filter metals analysis

12. Training Record

Before deploying PM samplers independently, personnel must:

1. Read this SOP and SOP-F001 in full
2. Demonstrate proper filter handling (forceps, desiccator, conditioning, weighing) under supervision
3. Perform a flow calibration and sampler setup under supervision
4. Deploy and retrieve a sampler for one complete sampling period with acceptable QC results (field blank mass within 0.015 mg of pre-weight; pre/post flow agreement within 10%)
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F504

Greenhouse Gas Measurements – Picarro G2508 with Eosense Soil Flux Chambers

Standard Operating Procedure

SOP Number	SOP-F504
Revision	1.01
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes how to install Eosense eosAC soil gas flux chambers, connect them to the eosMX-P multiplexer, and operate the Picarro G2508 cavity ring-down spectroscopy (CRDS) analyzer for measuring greenhouse gas fluxes (N_2O, CH_4, CO_2, NH_3, and H_2O) from soil. The SOP also covers data acquisition using the eosAnalyze-AC software and data export for analysis (Eosense Inc., 2019; Picarro, Inc., 2021).

This SOP was originally developed for greenhouse gas emission measurements from strawberry fields in Northern California and has been modified as new projects have come on line.

2. Scope

- **Applies to:** Field deployment of the Picarro G2508 CRDS analyzer coupled with Eosense eosAC dynamic soil flux chambers and the eosMX-P multiplexer for automated soil greenhouse gas flux measurements
- **Does not apply to:** Laboratory (bench-top) Picarro configuration (see SOP-L504), atmospheric concentration monitoring without flux chambers, or eddy covariance measurements
- **Location:** All EA Program field sites within 30 m cable reach of a power source

3. Responsibilities

Role	Responsibility
Lab PI	Approve deployment plan, review data
Trained Personnel	Set up instruments, run cycles, retrieve data
New Students	Complete training before independent use

3.1. Acknowledgements

We thank Isaac Medina, Neha Vaingankar, and Bailey Lai, who worked with the instrument early on and developed early versions of this SOP. Improvements were made by Allison Joseph and Virginia Paschal.

4. Definitions

- **Cavity ring-down spectroscopy (CRDS):** The laser-based optical detection technique used by the Picarro G2508; a laser pulse is injected into a high-finesse optical cavity and the rate of light decay is measured; the decay rate is proportional to the concentration of absorbing gas molecules, enabling parts-per-billion sensitivity.
- **Chamber closure period:** The time interval during which the eosAC chamber lid is sealed over the soil surface; CO_2, N_2O, CH_4, and NH_3 accumulate in the chamber headspace during this period; flux is calculated from the rate of concentration change.
- **Dead volume:** The total volume of gas in the measurement system (chamber headspace, tubing, and instrument internal volume) that dilutes gas emitted from the soil; must be accurately characterized to calculate flux.
- **Drierite:** Anhydrous calcium sulfate desiccant used to remove water vapor from the gas stream before it enters the Picarro analyzer; must be regenerated or replaced when exhausted (color change from blue to pink for indicating Drierite).
- **eosMX-P multiplexer:** The Eosense controller that cycles sequentially through up to 16 chamber ports, directing flow from each chamber to the Picarro analyzer in turn; automates multi-chamber deployments without manual valve switching.
- **eosAC chamber:** An automated, transparent polycarbonate flux chamber manufactured by Eosense; opens and closes on a programmable schedule; connected to the eosMX-P multiplexer and Picarro analyzer via Teflon tubing.
- **Flux calculation:** Derivation of the gas flux (µmol m^{-2} s^{-1} or nmol m^{-2} s^{-1}) from the linear or non-linear rate of concentration increase in the chamber headspace, chamber volume, soil surface area enclosed, and air temperature and pressure.
- **N_2O (nitrous oxide):** A potent greenhouse gas (298x CO_2 warming potential over 100 years) produced in soil by nitrification and denitrification; the primary target of many EA Program field campaigns; measured simultaneously with CH_4, CO_2, NH_3, and H_2O by the Picarro G2508.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Compressed gas cylinders (calibration standards) pose crush and high-pressure hazards. The generator (if used) presents fuel spill, carbon monoxide, and noise hazards.
- **Chemical:** Drierite desiccant is a mild irritant. Calibration gas cylinders may contain pressurized gases.
- **Electrical:** Power strip, generator, and instrument connections in outdoor conditions; keep connections dry and off the ground.
- **Environmental:** High ambient temperatures (above 35 C) can cause instrument overheating.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Safety glasses (when handling gas cylinders)
☐ Work gloves (when handling cylinders and generator)
☐ Sun protection (hat, sunscreen)
☐ Hearing protection (if generator is running nearby)

5.2. Required Training

- SOP-F001 Field Safety
- SOP-F101 Electrical Power in the Field

5.3. Emergency Procedures

In case of gas leak from calibration cylinders, move upwind and ventilate the area. For generator fuel spills, contain with absorbent materials. Refer to SOP-F001 Field Safety for general field emergency response.

6. Equipment and Materials

6.1. Instruments

Item	Serial / Model	Notes
Picarro G2508 CRDS Analyzer	S/N 2143-JFAADS2048	Measures N_2O, CH_4, CO_2, NH_3, H_2O
Eosense eosMX-P Multiplexer	S/N listed inside device	Connects up to 12 eosAC chambers

Item	Serial / Model	Notes
Recirculation Pump (PN: A0702)	–	Provides vacuum for gas sequencing
Monitor, mouse, and keyboard	–	Connected to Picarro via USB

6.2. Chambers

Chamber S/N (old)	Firmware	Software ID	Field ID	Notes
AC20150501 (1001501)	2.2.4	50501	#1	
NA (1001502)	2.2.4	NA	#2	Unable to identify
AC20150503 (1001503)	2.2.4	50503	#3	
AC20150504 (1001504)	2.2.4	50504	#4	
AC20150505 (1001505)	2.2.4	50505	#5	
AC20190333 (1001506)	2.2.4	31333	#6	Repaired
AC20150507 (1001507)	2.2.4	50507	#7	
AC20150508 (1001508)	2.2.4	50508	#8	
AC20150509 (1001509)	2.2.4	NA	#9	Unable to identify
AC20150510 (1001510)	2.2.4	50510	#10	
AC20150511 (1001511)	2.2.4	50511	#11	Fails to close properly / not fully open
AC20150512 (1001512)	2.2.4	50512	#12	
AC20XX33714 (44714)	??	??	#14	Unsure of status
AC20190315	2.1.1	31315	#15	
AC20190316	2.2.4	31316	#16	
AC20190317	2.2.4	31317	#17	

6.3. Additional Supplies

Item	Notes
Teflon tubing and cables (30 m each)	Lettered; check for damage before use
Corrugated metal hose	Connects pump to analyzer VACUUM port
Swagelok washers	Use new washers each connection
Drierite desiccant	For moisture management
Generator (if no grid power)	Place 100+ feet downwind
Zip ties / Velcro straps	For cable management

6.4. Customer Support Contacts

Organization	Contact
Picarro	Melissa, 408-962-3978; Scott, 408-962-3987; Karrin Alstad (applications/calibration), 408-962-3991

Organization	Contact
Eosense	888-352-8313 (toll-free); support@eosense.com (Eastern Time Zone)

7. Procedure

7.1. Sampling Design

1. Locate the Picarro analyzer, multiplexer, and pump at the base station. Choose a level, shaded location if possible.
2. Use a random number generator to determine the chamber placement locations within the study area
3. All chambers must be within 30 m of the multiplexer (the length of the cables and tubing)
4. If a generator is used, place it at least 100 feet downwind of the chambers to avoid contaminating flux measurements with exhaust

7.2. Hardware Setup

1. Connect the recirculation pump to the analyzer VACUUM port using the corrugated metal hose. Use new Swagelok washers each time. Hand-tighten, then seal with a wrench.
2. Connect the multiplexer to the Picarro through USB ports on the Picarro (any port)
3. Connect the monitor, mouse, and keyboard to the Picarro via the remaining USB ports (two additional ports are available on the front of the Picarro)
4. Connect the blue monitor cable to the Picarro and the monitor power cable to the outlet
5. Ensure all machines are in the off position (circle symbol = off) before connecting power
6. Connect power cables for the CRDS analyzer, multiplexer, and pump
7. Connect the pump to the multiplexer:
    a. Connect the control and data ports on the back of the pump to the Picarro, ensuring the white triangles face upward
    b. Insert the other ends into any USB port on the Picarro
8. Connect clear tubing from the pump inlet (left side, clear tube) to the multiplexer inlet (right side, labeled)
9. Connect the outlet tubing: multiplexer outlet to the Picarro inlet (clear tube); pump outlet (right side, silver tube) to the Picarro outlet
10. Attach one end of the power/data cable to the COMM port on the pump and the other end to the matching COMM port on the multiplexer

> **!** Important
>
> Never turn off the pump while the Picarro is running. Never disconnect the vacuum while the analyzer is operating. Doing so can damage the instrument.

7.3. Installing Chamber Rings

Chamber ring installation varies based on experimental design. Install rings in advance of the measurement day to minimize soil disturbance during data collection. *TBD – add project-specific ring installation protocols as needed.*

7.4. Picarro Startup

1. Ensure all instruments are off and turn on the power strip
2. Turn on the recirculation pump first (no wait time required)
3. Switch on the Picarro. The boot-up sequence initializes the CRDS software and analyzer.
4. Switch on the multiplexer. The system requires approximately 30 minutes to warm up; warning alarms are normal until the cavity and other components reach operating temperature.
5. Keep ambient temperature below 35 C. If the DAS temperature approaches 45 C, shut down or use a fan to cool the air around the Picarro.

7.5. Placing Chambers

1. While the Picarro is warming up, connect all tubing and cables. Route tubing along the bed surface; do not let tubes fall into furrows.
2. Place chambers onto the ring bases, pressing down to achieve a good seal. Try to avoid disturbing the chamber after installation.
3. After all chambers have opened, perform a second check for foliage or debris that might obstruct the chamber seal.

7.6. Data Collection

Once the Picarro alarm indicator is green, the system is ready.

1. Verify typical atmospheric concentrations on the Picarro display:

Gas	Expected Ambient Concentration
N_2O	Approximately 0.3 ppm
CH_4	Approximately 2.4 ppm
CO_2	300–600 ppm
NH_3	Variable
H_2O	1–5%

2. Open the FP-Monitor multiplexer software
3. Uncheck “Default Cycle”
4. Load the appropriate cycle from the user folder. Available cycles include:

- *TBD – list current cycle names (e.g., "Straw 5 Chamber," "Full 12," "Full 12 minus #4")*

5. When the Picarro G2508 is warm, press "Start Cycle"
6. Check all chambers to ensure good seals
7. If a generator is in use, check fuel every two hours and top off. Release pressure before inserting the nozzle into the filler throat. Open the valve and press the green button for 3–5 second intervals, checking between fills to see if fuel is at or near the red line.
8. Aim for at least 3 complete measurement cycles. For irrigation studies, target 2 cycles before irrigation, 2 during, and 2 after.

7.7. Shutdown

1. After at least two post-irrigation cycles (or at the end of the measurement period), begin retrieving chambers after each one completes its measurement, starting with chamber #1
2. Cap all tube ends with a glove secured by tape
3. Carefully coil each set of tubes and cables to limit twisting and scratching of the Teflon. Zip-tie or Velcro together and stack in order on the ground.
4. Remove chambers from the sampling locations. Separate bases from chambers and repackage into boxes.
5. When the final chamber measurement is complete, end the sampling cycle in the software
6. Connect a spare chamber to a channel and select the "Desiccate" method. Run dry air through the Picarro for 10 minutes to prevent condensation damage (Picarro, Inc., 2021).
7. Initiate the Picarro shutdown sequence; select the option for moving the instrument
8. After the Picarro has fully shut down, manually switch off in this order:
    a. Multiplexer
    b. Picarro
    c. Vacuum pump
    d. Power strip

8. Calibration

The Picarro G2508 analyzer calibration is intended to be performed infrequently (Picarro, Inc., 2021). The procedure requires three calibration standards per gas species (two points define the calibration line and a third intermediate point is used for verification).

8.1. Calibration Procedure

1. Select calibration standards that span the representative range of concentrations the analyzer will typically measure. A concentration of zero is acceptable for the low-end standard.
2. Introduce the first calibration standard into the analyzer for long enough to yield a stable reading
3. Record the stated concentration of the standard and the analyzer's displayed value

4. Repeat for the remaining two standards
5. Plot the data with the analyzer's reported concentration on the horizontal axis and the standards' stated concentrations on the vertical axis
6. Calculate a linear best-fit equation. The slope and intercept are used to apply a correction to the analyzer's factory calibration.

> **Note**
>
> *TBD – develop a dedicated calibration SOP including parts to order (permeation tubes, calibration gas cylinders) and a step-by-step calibration workflow. Contact Karrin Alstad at Picarro (408-962-3991) for calibration supplies.*

9. Data Export and Analysis

9.1. Using eosAnalyze-AC

1. On the Picarro desktop, open the "eosAnalyze-AC" program. If it is not on the desktop, navigate to Start Menu and search "eosAnalyze-AC." Open the "eosAnalyze-AC-v3.4.2" folder and run the `.exe` application.
2. You do not need to wait for the Picarro system to warm up if you are only downloading data.
3. Click "Collect Data." Enter the start and end times for the period you want to download. Repeat for each cycle separately; combining multiple non-contiguous periods into one export will produce a single table with large time gaps.
4. Ensure the time zone is set correctly. Pacific Time Zone is UTC-8:00.
5. Verify the data under "Measurements." Check that the correct dates, times, and data graphs look reasonable.
6. Click "Export Data Table" and save the CSV to the desktop or an accessible location.
7. To export additional data tables, close and reopen the program before creating each new export.
8. Transfer the `.csv` files to a USB drive or external hard drive for analysis on another computer. The files are compatible with Excel or R (R Core Team, 2024).

9.2. Troubleshooting Data Export

If data does not load or you see a "No measurements detected" message:

1. Go to **Data > Analyzer Data Path** in eosAnalyze-AC. The data path should be `\UserData\DataLog_Forerunner\`. You should see subfolders organized by year.
2. Click OK, then retry collecting data.
3. If the problem persists, use Windows Explorer to search for `.DAT` files in `\UserData\DataLog_Forerunner\YE`. The file naming convention is: `JFAADS2048-YYYYMMDD-HHMMSS-DataLog_Forerunner.dat` where JFAADS2048 is the instrument serial number, followed by the date and time the file was created in 24-hour format.

9.3. Flux Calculation

Soil gas fluxes are calculated from the rate of change of gas concentration within the chamber headspace over the closure period. The eosAnalyze-AC software fits both linear and exponential models to the concentration time series for each closure event (Hutchinson & Mosier, 1981; Livingston & Hutchinson, 1995).

$$F = \frac{V}{A} \cdot \frac{dC}{dt} \cdot \frac{P}{RT}$$

where:

- F = flux (mass per area per time)
- V = chamber headspace volume (L)
- A = chamber area (m^2)
- dC/dt = rate of concentration change (ppm/s)
- P = atmospheric pressure (atm)
- R = ideal gas constant
- T = temperature (K)

TBD – clarify in eosAnalyze-AC output what (L) and (E) designations mean for flux values (likely Linear and Exponential fits). Confirm whether "dead band range" and "chamber offset" in the data processor options need to be set for each deployment.

10. Quality Control

- Verify atmospheric background concentrations before starting each measurement cycle (see table in Section 6.6)
- Inspect all tubing connections for leaks before each deployment
- Check chamber seals visually and by monitoring initial concentration traces
- Log any chambers that fail to open or close properly and remove them from the dataset
- Do not disconnect chambers from the multiplexer during an active measurement cycle, as this can cause the data processor to miss measurements
- Ensure the FRMonitor software recognizes all connected chambers at startup; use the "System Info" button to verify. Chambers not recognized will not record data even if they open and close.
- Use FRMonitor software version 1.6.2 or later to schedule measurements

10.1. Biases and Interferences

- Ambient temperature extremes can prevent the analyzer from stabilizing properly
- High-moisture gas samples can cause condensation damage. Always run dry air through the instrument for several minutes before shutdown (Picarro, Inc., 2021).
- *TBD – develop QC criteria for acceptable flux curve fits (r^2 thresholds, maximum allowable dead band)*

11. Waste Disposal

Waste Type	Disposal Method
Spent Drierite desiccant	Regenerate by heating at 210 C or dispose as non-hazardous solid waste
Generator fuel spills	Absorb with spill kit materials; dispose per EH&S
Damaged Teflon tubing	Standard trash (non-hazardous)

12. References

Eosense Inc. (2019). *eosAC automated soil CO textsubscript2 flux chamber and eosMX-P multiplexer: User manual*. https://eosense.com/products/eosac/

Hutchinson, G. L., & Mosier, A. R. (1981). Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*, *45*(2), 311–316. https://doi.org/10.2136/sssaj1981.03615995004500020017x

Livingston, G. P., & Hutchinson, G. L. (1995). Enclosure-based measurement of trace gas exchange: Applications and sources of error. *Biogenic Trace Gases: Measuring Emissions from Soil and Water*, 14–51.

Picarro, Inc. (2021). *G2508 gas concentration analyzer for N textsubscript2O, CH textsubscript4, CO textsubscript2, NH textsubscript3, and H textsubscript2O: User's guide*. https://www.picarro.com/environmental/products/g2508_gas_concentration_analyzer

R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

13. Training Record

Before deploying this instrument independently, personnel must:

1. Read this SOP, SOP-F001 Field Safety, and SOP-F101 Electrical Power in the Field
2. Observe a trained operator perform a full deployment (setup through shutdown)
3. Demonstrate competence with hardware connections (pump, multiplexer, tubing, chambers)
4. Perform a supervised deployment including startup, data collection, and shutdown
5. Demonstrate ability to export data using eosAnalyze-AC
6. Be signed off by the PI or field lead

14. Appendix A: Troubleshooting

14.1. Days with Unexpected Data

If data appears on unexpected Julian days, check whether the system clock or local time on the analyzer has been changed. Compare the processed measurements against field notes to identify obvious gaps that may correspond to mislabeled days.

14.2. Days That Should Have More Data

Errors in FRMonitor log files can cause the data processor to overlook measurements. If measurements appear to be missing, check the FRMonitor logs for errors. Do not disconnect chambers from the multiplexer while it is actively running a measurement cycle, as this can cause the data processor to miss measurements, especially if the chamber was closed when removed.

14.3. Days Where Some Chambers Are Missing

If certain chambers (e.g., #1 or #2) are not sending data, this indicates either a communication issue between the multiplexer and analyzer or that the chambers were not connected. Verify that the FRMonitor software recognizes all connected chambers at startup using the "System Info" button. Disconnecting and reconnecting chambers during a measurement cycle can also cause communication errors.

14.4. N_2O Noise

N_2O measurements from the Picarro G2508 can be noisy at ambient concentrations. *TBD – document strategies for reducing N_2O noise (e.g., longer closure times, post-processing smoothing, data filtering thresholds). Contact Picarro technical support for recommendations.*

15. Appendix B: Open Questions

The following questions from previous deployments remain unresolved. Update this appendix as answers are obtained.

1. What is the difference between "dry," "regular," and "timed" data columns in the Data Logger setup tool?
2. What is "etalon temp" (listed under sensors in the data source)?
3. Recommendations for car-mounting the instrument? Is it acceptable to stack the multiplexer on top of the Picarro during transport?
4. Is there a way to turn on the computer without powering on the gas analyzer and vacuum?
5. What files need to be sent to the PI for reprocessing?
6. Should chamber offset (distance between the bottom of the chamber and the soil surface due to the collar) be measured for each chamber at each deployment?

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F505

Passive Air Samplers – Diffusion Tubes

Standard Operating Procedure

SOP Number	SOP-F505
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the deployment, retrieval, and documentation of passive (diffusion-based) air samplers for time-integrated measurement of gaseous air pollutants. Passive samplers require no pump or power supply: the target gas diffuses along a concentration gradient through a defined path length and is captured by a sorbent or reactive medium at the closed end of the tube. After a

known exposure period, the sampler is sealed, returned to the laboratory, and analyzed to determine the time-weighted average ambient concentration (Cape, 2009; Palmes et al., 1976).

Common target analytes include nitrogen dioxide (NO_2), ozone (O_3), sulfur dioxide (SO_2), ammonia (NH_3), and volatile organic compounds (VOCs). Passive samplers are widely used in spatial surveys and community-based exposure studies because they are inexpensive, silent, and require no electricity, making dense network deployments feasible (Vardoulakis et al., 2005; **martin2014?**).

Passive diffusion tubes are subject to known biases from wind speed, temperature, and tube geometry that must be understood and managed through quality control (Plaisance et al., 2004; Tang et al., 2001). When operated according to harmonized protocols and validated against active reference methods, passive samplers can provide data suitable for regulatory screening and spatial mapping (**hafkenscheid2009?**).

2. Scope

- **Applies to:** Deployment and retrieval of passive diffusion tube samplers (e.g., Palmes tubes for NO_2, Ogawa badges for NO_2/O_3, Radiello cartridges for VOCs/NH_3) at EA Program field sites
- **Does not apply to:** Active (pump-based) gas sampling; passive PM samplers; low-cost electronic gas sensors; laboratory analysis of exposed samplers (handled by the analytical laboratory or per analyte-specific lab SOPs)
- **Location:** EA Program field deployment sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design, select sampler type and analytical lab
Trained Personnel	Deploy, retrieve, and document samplers
New Students	Complete training before independent deployment

4. Definitions

- **Passive (diffusion) sampler:** A device that collects a gaseous pollutant by molecular diffusion along a concentration gradient, without requiring a pump. The sampler relies on Fick's first law of diffusion to relate the mass of analyte captured to the ambient concentration over the exposure period (Palmes et al., 1976).
- **Palmes tube:** A cylindrical passive sampler (typically 7.1 cm long, 1.1 cm internal diameter) with a triethanolamine (TEA)-coated stainless steel grid at the closed end for NO_2 collection. Originally described by Palmes et al. (1976).
- **Ogawa badge:** A disk-shaped passive sampler with two collection pads separated by a diffusion barrier. Can be configured for NO_2, O_3, or SO_2.

- **Radiello cartridge:** A radial-symmetry passive sampler with a cylindrical diffusive body surrounding a sorbent cartridge. Used for VOCs (Carbograph sorbent), NH_3 (acid-coated), and other gases.
- **Uptake rate:** The rate at which the target analyte diffuses into the sampler, determined by the diffusion coefficient of the gas, the cross-sectional area, and the path length. Published uptake rates are specific to each sampler-analyte combination and are used to convert captured mass to ambient concentration.
- **Time-weighted average (TWA):** The mean ambient concentration over the exposure period, calculated from the total mass captured and the exposure duration.
- **Shortening ratio:** The effective reduction in diffusion path length caused by wind-induced turbulence at the open end of a Palmes tube, which increases the uptake rate and causes overestimation of ambient concentration in windy conditions (Tang et al., 2001).
- **Field blank:** A sampler transported to the site, briefly opened and re-sealed without exposure, then returned to the lab. Field blank analyte mass quantifies contamination from handling, storage, and transport.
- **Triethanolamine (TEA):** A viscous organic amine used as the absorbent medium for NO_2 in Palmes tubes. TEA reacts with NO_2 to form nitrite, which is subsequently analyzed by the Griess colorimetric reaction.
- **Fick's first law:** The principle that the flux of a gas through a still-air column is proportional to the concentration gradient and the diffusion coefficient:

$$J = D \times \frac{A}{L} \times C$$

where J = flux (mass/time), D = diffusion coefficient, A = cross-sectional area, L = path length, and C = ambient concentration (Palmes et al., 1976).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Some sorbent media (e.g., triethanolamine for NO_2) are mild irritants. Handle prepared tubes with gloves and avoid skin contact with the open end.
- **Traffic:** Many monitoring locations are near roadways. Wear a high-visibility vest and follow SOP-F001 traffic safety procedures.
- **Height:** Mounting samplers on poles or structures may require ladder work. Follow standard ladder safety.

5.1. Required PPE

☐ Nitrile gloves (when handling samplers)
☐ Closed-toed shoes
☐ High-visibility vest (if near roadways)
☐ Sun protection

6. Equipment and Materials

Item	Notes
Passive samplers (type matched to target analyte; see Section 7.1)	Pre-prepared by analytical lab or in-house
Protective rain shelters (inverted cup or tube shelter)	Shields the open end from rain and direct sunlight while allowing airflow
Mounting clips, zip ties, or cable ties	For attaching shelters and tubes to poles, posts, or structures
Airtight storage containers or bags	For transporting unexposed and exposed samplers
Cooler with ice packs (for heat-sensitive sorbents)	Maintain at 4 C during transport if required
GPS unit	Per SOP-F006
Field notebook and deployment log	For recording dates, times, and site conditions
Permanent marker (Sharpie)	For labeling tubes

7. Procedure

7.1. Sampler Selection

Select the sampler type based on the target analyte and exposure period:

Target Analyte	Sampler Type	Typical Exposure	Analytical Method
NO_2	Palmes diffusion tube (TEA-coated grids)	1–4 weeks	Spectrophotometry (Griess reaction)
NO_2 / O_3	Ogawa passive sampler	1–2 weeks	Spectrophotometry
NH_3	Radiello cartridge or acid-coated filter	1–4 weeks	Spectrophotometry or IC
VOCs (BTEX)	Radiello cartridge (Carbograph sorbent) or 3M OVM badge	1–4 weeks	GC-MS (thermal desorption)
SO_2	Impregnated filter or Radiello cartridge	1–4 weeks	Ion chromatography

> **Note**
>
> The analytical laboratory typically prepares and supplies the samplers, pre-loaded with the appropriate sorbent. Confirm sampler type, handling instructions, and shipping requirements with the lab before each deployment.

7.2. Pre-Deployment

1. Keep sealed samplers refrigerated (4 C) until deployment day unless the analytical lab specifies otherwise
2. Assign a unique ID to each sampler and record it in the deployment log
3. Designate at least 2 field blanks per batch: samplers that travel to the site, are briefly unsealed (less than 30 seconds), re-sealed, and returned to the lab with the exposed samplers

7.3. Deployment

1. At the monitoring location, mount the rain shelter at a height of 2–3 m above ground, away from walls (at least 1 m), exhaust vents, and vegetation canopy
2. Remove the sampler from its sealed container, note the date and time, and attach the open end downward inside the rain shelter
3. Secure the sampler with clips or zip ties
4. Record in the field notebook: site ID, sampler ID, deployment date/time, GPS coordinates, mounting height, distance to nearest road, weather conditions, and any potential sources nearby
5. Photograph the installed sampler for documentation

! Wind Exposure

Palmes tubes are sensitive to wind speed: turbulence at the open end of the tube effectively shortens the diffusion path, increasing the uptake rate and overestimating the ambient concentration by 10–30% at exposed sites (Plaisance et al., 2004; Tang et al., 2001). Rain shelters provide partial wind screening, but sheltered or recessed mounting locations should be avoided because they restrict airflow and cause underestimation. The harmonized EU protocol recommends mounting in locations with moderate, representative air exchange (**hafkenscheid2009?**).

7.4. Exposure Period

- The exposure period depends on the expected ambient concentration and the sampler's capacity. Follow the analytical lab's recommendation (typically 1–4 weeks for NO_2 Palmes tubes).
- Note any unusual events during the exposure period (wildfires, construction, power outages affecting nearby sources) in the field notebook.

7.5. Retrieval

1. Record the retrieval date and time in the field notebook
2. Remove the sampler from the shelter, immediately cap or seal it, and place in an airtight container or bag
3. Calculate the total exposure time in hours
4. Place sealed samplers in a cooler with ice packs for transport (if required by the analytical lab)

5. Retrieve and seal the field blanks in the same manner

7.6. Shipping to Analytical Laboratory

1. Pack sealed samplers securely with cushioning material in an insulated shipping container with ice packs (if heat-sensitive)
2. Include a chain of custody form listing all sampler IDs, site locations, deployment and retrieval dates/times, and total exposure hours
3. Ship via overnight courier or deliver in person to the analytical lab

7.7. Concentration Calculation

The analytical laboratory reports the mass of analyte captured on each sampler. The time-weighted average ambient concentration is calculated using Fick's first law (Palmes et al., 1976):

$$C = \frac{m}{D \times A/L \times t}$$

where:

- C = ambient concentration ($\mu g/m^3$ or ppb, after unit conversion)
- m = mass of analyte on the sampler (μg)
- D = molecular diffusion coefficient of the analyte in air (cm^2/s)
- A = cross-sectional area of the diffusion path (cm^2)
- L = length of the diffusion path (cm)
- t = exposure time (s)

> **Note**
>
> The analytical lab typically performs this calculation using published uptake rates for the specific sampler-analyte combination. Confirm which party is responsible for the calculation.

8. Quality Control

- Deploy at least 2 field blanks per batch (minimum 10% of total samplers). Field blank analyte mass should be below the method detection limit; if not, subtract the mean field blank mass from all samples.
- Deploy co-located duplicate samplers at 10% of sites. Duplicates should agree within 20% for NO_2 Palmes tubes (Cape, 2009). Duplicate agreement worse than 25% indicates handling problems or site-specific wind interference (Plaisance et al., 2004).
- If collocating with an active reference sampler (chemiluminescence NO_2 analyzer, UV O_3 analyzer), compare the passive sampler result with the reference average over the same period. Palmes tubes for NO_2 typically overestimate the reference by 10–30% at high-wind sites and underestimate at sheltered sites due to restricted airflow (Tang et al., 2001).

- Document any site disturbances (construction, shelter damage, vandalism) that could affect results.
- Archive field notebooks and chain of custody forms with the project data.

9. Waste Disposal

Waste Type	Disposal Method
Exposed Palmes tubes (TEA grids)	Return to analytical lab or dispose as chemical waste (TEA is a mild irritant)
Radiello cartridges (used)	Return to analytical lab
Rain shelters and mounting hardware	Clean and reuse

10. References

Cape, J. N. (2009). The use of passive diffusion tubes for measuring concentrations of nitrogen dioxide in air. *Critical Reviews in Analytical Chemistry*, *39*(4), 289–310. https://doi.org/10.1080/10408340903001613

Palmes, E. D., Gunnison, A. F., DiMattio, J., & Tomczyk, C. (1976). Personal sampler for nitrogen dioxide. *American Industrial Hygiene Association Journal*, *37*(10), 570–577. https://doi.org/10.1080/0002889768507522

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Tang, Y. S., Cape, J. N., & Sutton, M. A. (2001). Factors affecting the performance of palmes NO_2 diffusion tubes. *Atmospheric Environment*, *35*(30), 5199–5207. https://doi.org/10.1016/S1352-2310(01)00330-1

Vardoulakis, S., Gonzalez-Flesca, N., Fisher, B. E. A., & Pericleous, K. (2005). Spatial variability of air pollution in the vicinity of a permanent monitoring station in central England. *Atmospheric Environment*, *39*(15), 2725–2736. https://doi.org/10.1016/j.atmosenv.2004.05.067

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F502	Portable PM Sensors	Companion low-cost sensor SOP
F503	PM Filter Sampling	Active filter sampling companion
F504	Picarro GHG Analyzer	Companion field instrument
F510	Weather Monitoring	Wind data for interpreting bias

12. Training Record

Before deploying passive air samplers independently, personnel must:

1. Read this SOP and SOP-F001 in full
2. Review the handling instructions provided by the analytical laboratory for the specific sampler type
3. Observe a trained operator demonstrate deployment and retrieval at one site
4. Deploy and retrieve samplers at one site under supervision, including field blank handling
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F506

Noise Level Monitoring

Standard Operating Procedure

SOP Number	SOP-F506
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes field measurement and documentation of environmental noise levels using handheld sound level meters and logging dosimeters. Environmental noise is an increasingly recognized pollutant affecting human health (cardiovascular risk, sleep disturbance, cognitive impairment) and wildlife behavior (altered communication, habitat avoidance) (Barber et al., 2010; World Health Organization, 2018).

Noise monitoring in the EA Program supports community environmental assessments, transportation corridor studies, urban ecology research, and environmental justice analyses where noise disproportionately affects disadvantaged communities.

2. Scope

- **Applies to:** Outdoor environmental noise measurements (community noise, traffic noise, construction noise, natural soundscapes) using Type 1 or Type 2 integrating sound level meters and personal noise dosimeters
- **Does not apply to:** Occupational noise exposure assessment (governed by OSHA standards and conducted by campus EH&S); indoor building acoustics; underwater acoustics
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve monitoring design, coordinate site access
Trained Personnel	Calibrate instruments, collect and document measurements
New Students	Complete training before independent monitoring

4. Definitions

- **A-weighting (dBA):** A frequency-weighting filter applied to sound level measurements that approximates the frequency-dependent sensitivity of the human ear; attenuates low and very high frequencies; the standard weighting for environmental and community noise assessments.
- **Calibrator:** A portable acoustic device that produces a reference tone of known sound pressure level (typically 94 dB or 114 dB at 1,000 Hz) used to verify sound level meter accuracy before and after each measurement session.
- **Day-night average sound level (L_{dn}):** A 24-hour energy-averaged sound level with a 10 dB penalty added to nighttime (10 pm–7 am) levels to account for increased sensitivity to noise during sleep; the primary US EPA regulatory descriptor for community noise exposure.
- **Equivalent continuous sound level (L_{eq}):** The steady-state sound level that would contain the same acoustic energy as the actual time-varying sound over a defined measurement period; the primary descriptor for environmental noise measurements; expressed in dB or dBA.
- **L_{max} / L_{min}:** The maximum and minimum instantaneous A-weighted sound levels measured during a monitoring period; used to characterize the range of noise events and identify peak exposures.
- **Percentile level (L_N):** The sound level exceeded for N% of the measurement period (e.g., L_{90} is the level exceeded 90% of the time); L_{90} approximates background noise; L_{10} approximates peak intrusive noise events.

- **Sound level meter:** An instrument that measures sound pressure level in decibels; classified as Type 1 (precision, laboratory and field) or Type 2 (general purpose); Type 1 meters are required for regulatory compliance measurements; both types are acceptable for research.
- **Sound pressure level (SPL):** A logarithmic measure of the effective pressure of a sound wave relative to a reference pressure of 20 µPa (the threshold of human hearing): $SPL = 20 \log_{10}(p/p_0)$; expressed in decibels (dB).
- **Windscreen:** A porous foam or fur cover placed over the microphone to reduce turbulent airflow noise caused by wind; required for all outdoor measurements to prevent wind-induced artifacts from contaminating sound level data.

5. Safety and Hazards

Hazards

- **Traffic:** Noise monitoring often occurs near roadways. Wear a high-visibility vest. Never stand in a travel lane.
- **Hearing:** If monitoring near very loud sources (e.g., active construction, aircraft operations above 85 dBA), wear hearing protection.
- **Personal safety:** Monitoring in urban areas may involve working alone or in unfamiliar neighborhoods. Work in pairs when possible and inform someone of your schedule and locations.

5.1. Required PPE

- ☐ High-visibility vest (near roadways)
- ☐ Hearing protection (if sustained levels exceed 85 dBA)
- ☐ Closed-toed shoes
- ☐ Sun protection

6. Equipment and Materials

Item	Notes
Integrating sound level meter (Type 1 or Type 2, IEC 61672)	With A-weighting and slow/fast time weighting
Acoustic calibrator (94 dB or 114 dB reference)	Matched to the microphone; calibrate before and after each session
Windscreen (foam cover for microphone)	Required for all outdoor measurements
Tripod with microphone mount	Position microphone at 1.2–1.5 m above ground
Noise dosimeter (optional)	For unattended time-integrated measurements
GPS unit or smartphone	For recording coordinates
Field notebook and noise monitoring data sheet	Record metadata and observations

Item	Notes
Stopwatch or timer	For timed measurement intervals
Camera	For photo-documenting site and source conditions
Spare batteries	For the meter and calibrator

7. Procedure

7.1. Pre-Field Calibration

1. In a quiet indoor location, attach the acoustic calibrator to the microphone and turn on the calibrator
2. Adjust the sound level meter reading to match the calibrator reference level (e.g., 94.0 dB) per the meter's calibration procedure
3. Record the calibration date, time, calibrator serial number, and meter reading in the field notebook
4. If the meter reading deviates from the reference by more than 0.5 dB, recalibrate or service the instrument

7.2. Site Setup

1. Select a measurement location that represents the receptor of interest (e.g., residential facade, park boundary, school playground)
2. Position the microphone on a tripod at 1.2–1.5 m above the ground, at least 3.5 m from any large reflecting surface (building wall, barrier, parked vehicle) to minimize reflections (Acoustics – Description, Measurement and Assessment of Environmental Noise – Part 2, 2017)
3. Orient the microphone toward the dominant noise source (or upward if the sound field is diffuse)
4. Attach the windscreen
5. Record: site ID, GPS coordinates, date, time, weather conditions (wind speed and direction, temperature), distance and bearing to the dominant source(s), ground cover, and any shielding

> **!** Important
>
> Do not measure when wind speed exceeds 5 m/s (approximately 11 mph). Wind noise inflates readings even with a windscreen. Record the wind speed at the start and end of each measurement period.

7.3. Measurement Protocol

The following protocol collects standard community noise metrics. Adjust the duration and intervals to match the study design.

1. Set the meter to: A-weighting, fast time constant, and L_{eq} (equivalent continuous level) integration mode
2. Start the measurement and let it run for the designated period:
   - **Short-term survey:** 15 minutes per measurement location (the minimum recommended by most community noise standards)
   - **1-hour L_{eq}:** Run for 60 minutes if comparing to local noise ordinance limits
   - **Long-term (24-hour):** Use a dosimeter or logging meter; deploy and secure the instrument
3. During the measurement, note all significant noise events in the field notebook: vehicle passbys, aircraft overflights, dog barking, construction activity, etc. Record the time and estimated duration of each event.
4. At the end of the measurement period, record the following metrics from the meter display:
   - L_{eq} (equivalent continuous level)
   - L_{max} (maximum level)
   - L_{min} (minimum level)
   - L_{10}, L_{50}, L_{90} (statistical levels, if the meter computes them)
5. If the meter logs time-history data, download the file after the session

7.4. Post-Field Calibration Check

1. After the final measurement at the last site, re-check the calibration with the acoustic calibrator
2. Record the post-measurement calibration reading. If it differs from the pre-measurement value by more than 1.0 dB, flag all data from that session for review.

7.5. Data Recording

For each measurement, record on the data sheet:

- Site ID, GPS coordinates, date, start/end times
- Meter make, model, serial number
- Calibration readings (pre and post)
- Weather: wind speed/direction, temperature, sky conditions
- Source description and distance
- Measurement duration and metrics (L_{eq}, L_{max}, L_{min}, L_{10}, L_{50}, L_{90})
- Notable noise events and their times
- Observer name

8. Data Interpretation

Metric	Meaning
L_{eq}	Energy-averaged sound level over the measurement period; the single most important descriptor of community noise exposure
L_{max}	Highest instantaneous level recorded
L_{min}	Lowest instantaneous level recorded
L_{10}	Level exceeded 10% of the time (approximates "loud" events)
L_{50}	Median level
L_{90}	Level exceeded 90% of the time (approximates background or residual noise)
L_{dn}	Day-night average; L_{eq} with a 10 dB penalty applied to nighttime (10 PM–7 AM) hours

> **Note**
>
> Community noise standards in California (Title 24, local general plans) typically set limits in terms of L_{dn} (CNEL) or L_{eq} for specific land use categories. A residential exterior standard of 60–65 dB L_{dn} is common.

9. Quality Control

- Calibrate before and after every measurement session; flag data if drift exceeds 1.0 dB
- Use the windscreen for all outdoor measurements
- Do not measure when wind speed exceeds 5 m/s
- Record all extraneous noise events so they can be identified in the time-history data
- For long-term deployments, perform a calibration check at each data retrieval visit
- Maintain a calibration and maintenance log for each meter

10. Waste Disposal

No waste is generated by noise monitoring. Dispose of used batteries per campus battery recycling procedures.

11. References

Acoustics – Description, Measurement and Assessment of Environmental Noise – Part 2: Determination of Sound Pressure Levels, Pub. L. Nos. ISO 1996-2:2017 (2017).

Barber, J. R., Crooks, K. R., & Fristrup, K. M. (2010). The costs of chronic noise exposure for terrestrial organisms. *Trends in Ecology and Evolution*, *25*(3), 180–189. https://doi.org/10.1016/j.tree.2009.08.002

World Health Organization. (2018). *Environmental noise guidelines for the European Region*. WHO Regional Office for Europe. https://www.who.int/europe/publications/i/item/9789289053563

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F502	Portable PM Sensors	Companion air monitoring SOP
F505	Passive Air Samplers	Companion air monitoring SOP

13. Training Record

Before performing noise monitoring independently, personnel must:

1. Read this SOP and SOP-F001 in full
2. Demonstrate proper calibration of the sound level meter
3. Perform a supervised 15-minute measurement at one site, including correct microphone positioning, windscreen use, event logging, and post-calibration check
4. Demonstrate ability to read and record L_{eq}, L_{max}, L_{min}, and statistical levels from the meter
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F510

Weather Monitoring and Field Meteorology

Standard Operating Procedure

SOP Number	SOP-F510
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for recording weather and meteorological conditions during field activities. Documenting weather conditions at the time of sampling is essential for interpreting environmental data, identifying storm-driven events, establishing baseline climate conditions, and ensuring field safety. This SOP covers manual weather observations, use of portable weather instruments, and deployment of unattended data loggers for continuous monitoring (World Meteorological Organization, 2018).

2. Scope

- **Applies to:** All EA Program field activities where weather conditions may affect data interpretation, sample quality, or personnel safety; deployment and retrieval of portable weather stations and data loggers
- **Does not apply to:** Climate modeling or historical meteorological analysis; operation of fixed, permanently installed weather stations managed by other departments; air quality monitoring (see SOP-F502)
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Identify which weather variables are required for the project; approve deployment of data loggers
Trained Personnel	Record weather observations, deploy and retrieve loggers, download data
New Students	Complete training before independent use

4. Definitions

- **Beaufort scale:** A standardized scale (0–12) relating wind speed to observable effects on land or water; allows wind speed estimation without instruments; developed by Admiral Beaufort in 1805 and adopted as the international standard by the WMO (World Meteorological Organization, 2018).
- **Cloud cover:** The fraction of the sky obscured by clouds, expressed in tenths (0–10) or oktas (0–8); recorded with each manual weather observation (World Meteorological Organization, 2018).
- **Dew point:** The temperature to which air must be cooled at constant pressure for condensation to occur; a direct measure of absolute humidity; can be derived from wet-bulb temperature measurements (World Meteorological Organization, 2018).
- **HOBOconnect:** The Onset smartphone app used to program and download data from Bluetooth-enabled MX-series HOBO loggers (Onset Computer Corporation, 2021).
- **Kestrel meter:** A handheld weather meter (Kestrel 3000 or similar) that simultaneously measures air temperature, relative humidity, wind speed, barometric pressure, and derived quantities (dew point, wet-bulb temperature, heat index); the standard portable weather instrument for EA Program field use.
- **Radiation shield:** A ventilated white housing that surrounds a temperature or humidity sensor to prevent direct solar radiation from inflating measured air temperatures; mandatory for any sensor exposed to sunlight (World Meteorological Organization, 2018).
- **Relative humidity (RH):** The ratio of actual water vapor pressure to saturation vapor pressure at the same temperature, expressed as a percentage; affects evapotranspiration,

dew deposition, and physical and biological processes at field sites (World Meteorological Organization, 2018).

- **Screen height:** The standard height of 1.5 m above ground level for air temperature and humidity measurements; specified by the WMO to ensure comparability between stations and with published meteorological data (World Meteorological Organization, 2018).
- **Sling psychrometer:** A pair of wet-bulb and dry-bulb thermometers mounted on a frame that is swung to ventilate the sensors; used to determine relative humidity from the wet-bulb depression; a reliable low-cost alternative to electronic sensors.
- **WMO (World Meteorological Organization):** The United Nations agency responsible for international meteorological and hydrological standards; its Guide to Meteorological Instruments and Methods of Observation (WMO-No. 8) is the authoritative reference for field measurement practice (World Meteorological Organization, 2018).

5. Safety and Hazards

⚠ Hazards

- **Lightning:** Cease all field activities and seek shelter in a vehicle or enclosed structure when lightning is observed. Wait at least 30 minutes after the last lightning strike before resuming.
- **Heat:** Southern California field sites can exceed 40 °C. Carry adequate water, take shade breaks, and monitor for heat-related illness.
- **Wind:** High winds can topple tripod-mounted instruments and create hazardous conditions near exposed ridgelines or water bodies.
- **Flooding:** Rapidly changing weather can cause flash flooding in Southern California canyons and channels. Monitor upstream conditions and have an evacuation route planned.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Equipment and Materials

Item	Notes
Kestrel 3000 or equivalent meter	For air temperature, RH, wind speed
Sling psychrometer	Backup for RH measurement

Item	Notes
Rain gauge (manual graduated cylinder)	For precipitation measurement
HOBO MX-series data logger	For unattended monitoring per SOP-F204
Radiation shield (RS3 or similar)	Required for any temperature sensor exposed to sunlight (World Meteorological Organization, 2018)
Compass	For wind direction measurement
Field notebook	Per SOP-F003

7. Procedure

7.1. Manual Weather Observations

Record weather conditions at the start and end of each field session, and at any time conditions change notably. Enter all observations in the field notebook per SOP-F003 (World Meteorological Organization, 2018).

Variable	Method	Units
Air temperature	Kestrel, sling psychrometer, or digital thermometer at 1.5 m in shade	°C
Relative humidity	Kestrel or sling psychrometer	%
Wind speed	Kestrel or Beaufort scale estimate	m/s or Beaufort number
Wind direction	Compass bearing from which wind blows	degrees or cardinal direction
Cloud cover	Visual estimate	Tenths (0–10)
Cloud type	Identify dominant type	Name (cumulus, cirrus, etc.)
Precipitation	Observation and rain gauge	mm; note type (rain, drizzle, none)
Barometric pressure	Kestrel or smartphone app	hPa

> **Tip**
>
> If you do not have a weather instrument, still record qualitative observations. A note such as "clear sky, calm winds, warm" is far better than no record. Note the source of any online weather data (e.g., "NWS Ontario station, 08:00 reading").

7.2. Beaufort Wind Scale (Quick Reference)

Beaufort	Description	Speed (m/s)	Visual Indicator
0	Calm	< 0.3	Smoke rises vertically
1	Light air	0.3–1.5	Smoke drifts
2	Light breeze	1.6–3.3	Leaves rustle
3	Gentle breeze	3.4–5.4	Leaves and twigs in motion
4	Moderate breeze	5.5–7.9	Small branches move; dust rises
5	Fresh breeze	8.0–10.7	Small trees sway
6	Strong breeze	10.8–13.8	Large branches in motion

(World Meteorological Organization, 2018)

7.3. Deploying Portable Data Loggers

1. Select a deployment location representative of site conditions and unlikely to be disturbed by people, animals, or flooding.
2. Mount temperature and humidity loggers inside a radiation shield at 1.5 m above ground per WMO screen height standards (World Meteorological Organization, 2018). Secure to a tripod, post, or tree.
3. Mount rain gauges on a level surface with no overhead obstructions. The gauge orifice should be approximately 1 m above ground.
4. Program the logger using the HOBOconnect app or HOBOware software per SOP-F204 (Onset Computer Corporation, 2021):
   - Logging interval: typically 5 or 15 minutes
   - Start mode: delayed start
   - Time zone: Pacific Time
5. Record in the field notebook: logger serial number, GPS coordinates, deployment date and time, logging interval, sensor height.
6. Photograph the deployed logger with surrounding context.

7.4. Retrieving Data from Loggers

1. Connect to the logger via Bluetooth (HOBOconnect) or USB cable (Onset Computer Corporation, 2021).
2. Download the data and save using the naming convention: `YYYY-MM-DD_SiteID_LoggerSN_weather.csv`
3. Do not clear the logger memory until you have verified the file contains the expected date range.
4. Upload the raw data file to the project GitHub repository under `data/weather/` within 48 hours.

7.5. Using Online Weather Data

When on-site instruments are unavailable, supplement field observations with data from nearby weather stations. Preferred sources for Southern California:

Source	URL
NWS	https://www.weather.gov
MesoWest / SynopticData	https://synopticdata.com/mesowest
CIMIS	https://cimis.water.ca.gov
Weather Underground	https://www.wunderground.com

Record the station name, distance from field site, and retrieval date in the field notebook and data file metadata.

8. Quality Control

- Cross-check manually recorded air temperature against the nearest reliable weather station (NWS or CIMIS). Discrepancies greater than 3 °C at similar elevation and time should be investigated (World Meteorological Organization, 2018).
- Inspect data loggers for drift by deploying two loggers side by side for at least 24 hours once per year. Temperature readings should agree within 0.5 °C; humidity within 5%.
- Check rain gauge calibration at least once per year by pouring a known volume of water through the gauge and comparing the logged total to the expected value.
- Flag any suspicious data (e.g., sudden temperature jumps, constant humidity at 100% for days) during data review and note the issue in the project data log.

9. References

- World Meteorological Organization (2018)
- Onset Computer Corporation (2023)
- Onset Computer Corporation (2021)

Onset Computer Corporation. (2021). *HOBOware user's guide*. https://www.onsetcomp.com/files/manual_pdfs/10246-K-MAN-HOBOware.pdf

Onset Computer Corporation. (2023). *HOBO U23 Pro v2 external temperature data logger: User manual*. https://www.onsetcomp.com/products/data-loggers/u23-004

World Meteorological Organization. (2018). *Guide to meteorological instruments and methods of observation (WMO-no. 8)* (2018th ed.). World Meteorological Organization. https://library.wmo.int/idurl/4/41650

10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Lightning and heat safety
F003	Field Notebook Protocols	Weather recorded in notebook

SOP	Title	Relationship
F006	GPS and Site Navigation	Logger deployment coordinates
F204	HOBO Weather Station Deployment	Detailed logger procedures
F501	Air Temperature Monitoring	Dense sensor network SOP
L801	RStudio and GitHub	Data file version control

11. Training Record

Before recording weather observations or deploying loggers independently, personnel must:

1. Read this SOP in full
2. Demonstrate correct use of a sling psychrometer or Kestrel meter
3. Demonstrate logger programming, deployment, and data download during a supervised field visit
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F602

iNaturalist for Biodiversity Documentation

Standard Operating Procedure

SOP Number	SOP-F602
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
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1. Purpose

This SOP describes how to use iNaturalist (https://www.inaturalist.org) as a standardized tool for documenting biodiversity at EA Program field sites. iNaturalist is a community science platform that combines field observation, AI-assisted species identification, and expert community verification into a single workflow (**ueda2024?**). Observations that receive community agreement on identification reach "Research Grade" status and are automatically shared with the Global Biodiversity Information Facility (GBIF), making them part of the global scientific record (Global Biodiversity Information Facility, 2024).

Community science platforms like iNaturalist generate large volumes of occurrence data, but the scientific value of those data depends critically on the quality of individual observations, including photographic evidence, spatial accuracy, and taxonomic verification (Dickinson et al., 2010). When data quality is maintained, iNaturalist records can support large-scale biodiversity monitoring and contribute meaningfully to national and international biodiversity assessments (Callaghan et al., 2021; Chandler et al., 2017). This SOP covers making high-quality observations, creating and managing collaborative projects for EA Program research, and exporting data for analysis in R.

2. Scope

- **Applies to:** All EA Program biodiversity surveys, species inventories, and community science activities where iNaturalist is used to document plant, animal, fungal, or other organism occurrences
- **Does not apply to:** Quantitative vegetation transect surveys (SOP-F603); camera trap image processing (SOP-F620); formal specimen collection requiring voucher preparation; eDNA sampling
- **Location:** All EA Program field sites and campus

3. Responsibilities

Role	Responsibility
PI / Field Lead	Create and administer iNaturalist projects; review data quality
Project Admin	Manage project settings, curate observations, invite members
Trained Personnel	Make observations per this SOP; contribute identifications
New Students	Create an account and complete training before using iNaturalist for research data

4. Definitions

- **Observation:** A record of an encounter with an individual organism at a specific place and time, documented with at least one photo or sound recording.

- **Research Grade:** An observation that has a date, location, photo or sound, and community agreement on the identification (at least two-thirds of identifiers agree at the species level). Research Grade observations are shared with GBIF (Global Biodiversity Information Facility, 2024).
- **Needs ID:** An observation that has not yet received enough community identifications to reach Research Grade. These records are scientifically useful but carry higher taxonomic uncertainty.
- **Casual:** An observation that is missing date, location, or evidence (photo/sound), or that the community has voted as not meeting quality standards. Casual observations are excluded from most research exports.
- **Collection Project:** A project that automatically includes all observations matching defined criteria (taxa, place, users, dates, quality grade). No manual curation needed.
- **Traditional Project:** A project where observations must be added manually by members. Can require custom observation fields. Requires the creator to have at least 50 verifiable observations.
- **Umbrella Project:** A project that aggregates observations from multiple other projects. Useful for coordinating across sites or taxa.
- **Observation Field:** A custom data field that can be attached to observations (e.g., "habitat type," "number of individuals," "flowering status"). Only Traditional Projects can require observation fields.
- **Computer Vision (CV) Model:** The machine learning model trained on iNaturalist's community-verified image database that suggests identifications for new observations. The model is retrained periodically and performs best for taxa and regions with large training datasets; accuracy declines for rare, under-photographed, or morphologically cryptic taxa (**loarie2023?**).
- **Taxon Geoprivacy:** An automatic system that obscures the coordinates of observations for threatened, endangered, or sensitive species to protect them from collection or disturbance.
- **Coordinate Uncertainty:** The estimated spatial accuracy of the GPS location, expressed as a radius in meters. High uncertainty (greater than 1000 m) reduces the value of occurrence records for fine-scale spatial analyses.

5. Safety and Hazards

Standard field safety applies when making observations in the field. Refer to SOP-F001 Field Safety. Additional considerations specific to iNaturalist use:

- **Location privacy:** iNaturalist observations include GPS coordinates by default. Be aware that your location is visible to other users unless you obscure or hide it. Do not post observations from private residences without obscuring the location.
- **Sensitive species:** iNaturalist automatically obscures the locations of threatened and endangered species ("taxon geoprivacy"). Do not override this obscuring for sensitive taxa.
- **Disturbance:** Do not disturb, handle, or collect organisms solely for an iNaturalist photo. Photograph organisms in situ where possible.

6. Required Materials

Item	Notes
Smartphone with iNaturalist app	iOS or Android; free download
iNaturalist account	Free at https://www.inaturalist.org
Camera (optional)	For higher-quality photos; phone camera is sufficient for most uses
Field notebook	For recording additional data not captured in iNaturalist
GPS unit (optional)	Phone GPS is usually adequate

7. Procedure

7.1. Account Setup

1. Go to https://www.inaturalist.org or download the iNaturalist app
2. Create an account using your Claremont email address
3. In your profile settings:
    - Set your default photo license to **CC-BY** or **CC-BY-NC** (this allows your photos to be used in research with attribution)
    - Set your default observation license to **CC0** or **CC-BY** (this allows your observation data to be shared with GBIF)
    - Set your default location to "open" (you can obscure individual observations as needed)
4. Send your iNaturalist username to the project administrator so you can be added to the EA Program project

> **!** License Settings Matter for Research
>
> Observations with "All Rights Reserved" licenses are excluded from GBIF exports and many research datasets. For EA Program work, always use a Creative Commons license. CC-BY is recommended.

7.2. Making High-Quality Observations

A useful iNaturalist observation needs four things: a photo (or sound), a date, a location, and enough visual detail for identification. Follow these guidelines to maximize the chances of reaching Research Grade.

7.2.1. Photography Tips

1. Take multiple photos of each organism from different angles:
    - **Plants:** Whole plant habit, close-up of leaves (both sides), flowers or fruit, stem, bark (for trees)

- **Insects:** Dorsal view (top), lateral view (side), close-up of head and wing venation if possible
- **Birds:** Best angle available; include habitat context if possible
- **Fungi:** Top of cap, underside (gills/pores), stem, habitat context

2. Include something for scale (a finger, a coin, a ruler) when practical
3. Photograph the organism in its natural position; do not relocate it
4. For plants, photograph vegetative and reproductive structures together if both are present
5. Ensure the photo is in focus and adequately lit

7.2.2. Recording the Observation

6. Open the iNaturalist app and tap the camera icon to start a new observation
7. Take photos directly in the app, or select photos from your camera roll
8. The app will suggest an identification using its computer vision (CV) model (**loarie2023?**). Accept the suggestion if it looks correct, or refine the identification manually. When in doubt, identify to the broadest level you are confident in (e.g., "Plants" or "Oaks" rather than guessing a specific species). The CV model performs best for common, well-photographed taxa in North America and Europe; for under-represented groups (e.g., lichens, mosses, many invertebrate families), manual identification by the observer or community identifiers is essential.
9. Verify that the date and location are correct. The app captures these automatically from the photo metadata, but check that the GPS pin is accurate on the map. If coordinate uncertainty exceeds 500 m, manually reposition the pin.
10. Add any relevant notes in the "Notes" field (habitat, behavior, abundance, associated species)
11. If the observation is part of a Traditional Project, add it to the project before submitting (see Section 7.4)
12. Tap **Submit**

> Sound Observations
>
> For birds, frogs, and insects, a sound recording can be as useful as (or more useful than) a photo. The iNaturalist app can record audio directly. Hold the phone steady and minimize background noise.

7.3. Bulk Uploading Observations via CSV

For observations recorded outside the app (for example, transcribed from a field notebook, exported from another platform, or compiled in a spreadsheet during data entry), iNaturalist accepts a bulk CSV import. This is useful when you have many records to create at once and do not want to enter them one at a time. The importer creates the observation records only; it does not upload photos or sound files, so media must be attached afterward (see below).

7.3.1. Preparing the CSV

1. Build the spreadsheet with up to eight columns, in this order. Only the taxon column is mandatory.

Column	Format / example	Notes
Taxon name	text (e.g., `Quercus agrifolia`)	Mandatory. Use the scientific name for reliable matching.
Date observed	`YYYY-MM-DD HH:MM`	Use day-level precision or finer for research use.
Description	text	Free-text notes (habitat, behavior, abundance).
Place name	text	Optional named locality.
Latitude	decimal degrees (`34.1064`)	Use a decimal point, not a comma.
Longitude	decimal degrees (`-117.7120`)	Use a decimal point, not a comma.
Tags	`tag,tag`	Comma-separated tags.
Geoprivacy	`open`, `obscured`, or `private`	Set to `obscured` for sensitive taxa.

2. Keep the file under 10,000 rows. Split larger datasets into multiple files.
3. Wrap any field that contains a comma in double quotes so it is not misread as a column break.
4. Confirm that coordinates use decimal points. Spreadsheets configured for some locales export decimal commas, which the importer rejects.

7.3.2. Importing

1. Go to the import page at https://www.inaturalist.org/observations/import#csv_import (or from the upload page, click **More import options** and choose **CSV**).
2. Choose your file and upload it.
3. Open **Edit Observations** to review the created records. Use **Batch Edit** to select multiple observations, attach photos or sound files, and apply a shared location from the map.
4. Verify dates, coordinates, and identifications, then save.

CSV Imports Do Not Include Media

The CSV importer creates observation records without photos or sounds. An observation with no media cannot reach Research Grade and is treated as Casual, so it will be excluded from most research exports and from GBIF. Attach media through Batch Edit after import if the records are intended for analysis.

Large or Photo-Linked Datasets: Use the API

For large batches, or when you need to match many photos to the correct records automatically, the spreadsheet importer becomes impractical. In those cases, script the upload against the iNaturalist API (https://api.inaturalist.org). The `rinat` package is read-only (it retrieves data but does not create observations), so creating observations programmatically requires

direct authenticated calls, for example with `httr2` in R:

```
library(httr2)

# Requires an OAuth API token from your iNaturalist account.
# Create the observation, then POST the photo to /observation_photos
# using the returned observation id. See the API reference for the
# full request schema before running against your account.
```

Test any upload script on a handful of records first, and never use it to create observations for organisms you did not personally encounter (see Responsible Use).

7.4. Contributing Identifications

Identifying other people's observations is as valuable as making your own. It builds community expertise, helps observations reach Research Grade, and improves the CV model.

1. Go to the **Identify** tab on the website (or use the Explore page and filter by "Needs ID")
2. Filter by your area of expertise (taxa, location)
3. Add your identification. Agree with the existing suggestion if correct, or provide a different identification with a brief explanation.
4. Use the "Compare" tool to check similar species
5. Mark observations as "No, it's as good as it can be" only if the evidence is genuinely insufficient for identification

7.5. Creating and Managing Projects

iNaturalist offers three project types. Choose based on your research needs:

Feature	Collection Project	Traditional Project	Umbrella Project
Observations added	Automatically (by filter criteria)	Manually by members	From member projects
Custom observation fields	No	Yes (can be required)	No
Creator requirements	Any user	50+ verifiable observations	Any user
Best for	Site-based inventories, class bioblitzes	Projects needing custom data fields	Multi-site or multi-taxon coordination
Obscured coordinate access	Yes (with Trust enabled)	Yes (with member permission)	No (via member projects)

7.5.1. Creating a Collection Project

This is the recommended type for most EA Program site inventories and class projects. Observations matching your criteria are included automatically.

1. Go to https://www.inaturalist.org/projects/new
2. Click **Get Started** under Collection Projects
3. Fill in the project details:
   - **Title:** Use a descriptive name (e.g., "EA Program – Bernard Field Station Biodiversity 2025")
   - **Icon:** Upload the EA Program logo or a representative photo
   - **Banner:** Upload a site photo
   - **Description:** Explain the project's purpose, study site, and time frame
4. Set the project requirements (filters):
   - **Place:** Select the iNaturalist Place for your study area. If one does not exist, create a custom Place by drawing a boundary on the map (Community > Places > Add a New Place).
   - **Taxa:** Leave blank to include all organisms, or restrict to specific groups (e.g., Plantae, Aves)
   - **Users:** Leave blank to include anyone, or list specific usernames for a closed team
   - **Date range:** Set start and end dates if the project covers a specific period (e.g., a class semester or field season)
   - **Quality grade:** Select "Research Grade" and "Needs ID" for most research projects
5. Under **Trust** (optional but recommended for research): enable Trust to allow project admins to access the true coordinates of observations with obscured or private locations. Members must individually opt in to share their coordinates.
6. Click **Save**

Creating a Custom Place

If your study area (e.g., the Bernard Field Station, a specific watershed) does not exist as an iNaturalist Place, create one:

1. Go to https://www.inaturalist.org/places/new
2. Name the place (e.g., "Bernard Field Station, Claremont, CA")
3. Draw the boundary on the map using the polygon tool
4. Save

The place will be available as a filter in your Collection Project. Note that very small places (e.g., a single backyard) work better with Traditional Projects because GPS accuracy and obscured coordinates can cause observations to fall outside tiny boundaries.

7.5.2. Creating a Traditional Project

Use when you need custom observation fields or when the study area is too small for reliable GPS-based filtering.

1. You must have at least 50 verifiable observations on your account
2. Go to https://www.inaturalist.org/projects/new
3. Look for the small text link at the bottom of the page: "click here to create a traditional project"
4. Fill in the project details (title, icon, banner, description)
5. Set the membership model:
   - **Open:** Anyone can join
   - **Invite-only:** Only users you invite can join (recommended for research teams)
6. Set the submission model:
   - **Any member:** All members can add observations
   - **Project curators:** Only curators can add observations (recommended when the PI needs to control data quality)
7. Add observation fields if needed (e.g., "Habitat type," "Transect number," "Number of individuals").

8. Data Quality Considerations

iNaturalist data are heterogeneous in quality because they are generated by observers with widely varying skill levels, equipment, and motivations (Dickinson et al., 2010). For EA Program research, apply the following data quality filters when exporting records for analysis:

- **Quality grade:** Use Research Grade observations only for quantitative analyses. Needs ID observations can supplement species inventories but carry higher misidentification risk.
- **Coordinate uncertainty:** Exclude records with positional accuracy greater than 1000 m unless the analysis does not require fine spatial resolution.
- **Date precision:** Exclude records without day-level date precision for phenological analyses.
- **Taxonomic verification:** For critical analyses, manually verify identifications against the photographic evidence, especially for taxa where the CV model is known to struggle (e.g., grasses, juvenile insects, sterile plants).
- **Spatial bias:** iNaturalist data are spatially biased toward accessible, human-frequented areas (trails, roads, urban parks). This bias must be acknowledged and, where possible, corrected for in species distribution models (Callaghan et al., 2021).

These filters should be documented in the methods section of any publication or report using iNaturalist-derived data.

9. Data Export and Analysis

9.1. Exporting from iNaturalist

1. Go to the **Explore** page and filter to your project, place, or taxa of interest
2. Click **Filters** to narrow results
3. Click **Download** (bottom of the page) to download a CSV file

9.2. Exporting from GBIF

For Research Grade observations that have been shared with GBIF:

1. Go to https://www.gbif.org/occurrence/search
2. Filter by dataset (search for "iNaturalist"), location, and taxa
3. Click **Download** and select the desired format
4. GBIF assigns a DOI to every download; cite the DOI in your publications

9.3. Using `rinat` in R

The `rinat` package provides programmatic access to iNaturalist data (**barve2025?**):

```
library(rinat)

# Get observations from a specific project
obs <- get_inat_obs_project("ea-program-bfs-biodiversity-2025",
                            type = "observations")

# Get observations by taxon and place
birds <- get_inat_obs(taxon_name = "Aves",
                      place_id = 12345,
                      quality = "research",
                      maxresults = 500)
```

10. Citing iNaturalist Data

- **Platform citation:** iNaturalist. Available at: https://www.inaturalist.org. Accessed [date].
- **GBIF download citation:** Use the DOI provided with the GBIF download.
- **Individual observations:** Cite by observation URL (e.g., https://www.inaturalist.org/observations/123456) when referencing specific records.

When using iNaturalist data in publications, describe the data quality filters applied (e.g., Research Grade only, coordinate uncertainty threshold) and acknowledge the community identifiers whose expertise validated the records (Chandler et al., 2017; Dickinson et al., 2010).

11. Responsible Use

- Respect copyright: each user retains ownership of their observations and photos. Your rights to use them are governed by the license the user selected. Most iNaturalist photos use CC-BY-NC.
- Do not create observations for specimens you did not personally encounter (no uploading photos from field guides, the internet, or other people's cameras)
- Do not harass, collect, or relocate organisms for the purpose of getting a better photo

- For sensitive species (rare plants, nesting sites), manually obscure the location even if iNaturalist does not do so automatically
- Follow iNaturalist's Community Guidelines: https://www.inaturalist.org/pages/community+guidelines

> ⚠ Student Use
>
> Before assigning students to use iNaturalist, read the iNaturalist Teacher's Guide (https://www.inaturalist.org/pages/teacher's+guide). The majority of low-quality observations and inappropriate content on iNaturalist comes from poorly managed class assignments. Set clear expectations, demonstrate proper technique, and monitor student contributions. Structured class projects with specific data quality goals produce better scientific outcomes and learning (Aristeidou & Herodotou, 2020).

12. References

Aristeidou, M., & Herodotou, C. (2020). Online citizen science: A systematic review of effects on learning and scientific literacy. *Citizen Science: Theory and Practice*, *5*(1), 11. https://doi.org/10.5334/cstp.224

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Chandler, M., See, L., Copas, K., Bonde, A. M. Z., L'opez, B. C., Danielsen, F., Legind, J. orgen K. aebek, Masber, M., Miller-Rushing, A. J., Newman, G., et al. (2017). Contribution of citizen science towards international biodiversity monitoring. *Biological Conservation*, *213*, 280–294. https://doi.org/10.1016/j.biocon.2016.09.004

Dickinson, J. L., Zuckerberg, B., & Bonter, D. N. (2010). Citizen science as an ecological research tool: Challenges and benefits. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 149–172. https://doi.org/10.1146/annurev-ecolsys-102209-144636

Global Biodiversity Information Facility. (2024). *What is GBIF?* https://www.gbif.org/what-is-gbif

13. Training Record

Before using iNaturalist for EA Program research data, personnel must:

1. Read this SOP in full
2. Create an iNaturalist account with appropriate license settings (Section 7.1)
3. Make at least 10 observations that reach Research Grade (or "Needs ID" with correct identifications added by the community)
4. Contribute at least 10 identifications to other users' observations
5. Demonstrate familiarity with the project type relevant to their research (Collection or Traditional)
6. Be signed off by the PI or project lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F603

Vegetation Transect Surveys

Standard Operating Procedure

SOP Number	SOP-F603
Revision	0.3
Author(s)	Marc Los Huertos
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Organization	EA Program, Pomona College

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1. Purpose

This SOP describes field procedures for conducting vegetation transect surveys using three common methods: line-point intercept (LPI), line intercept (LI), and quadrat (cover class) sampling. These methods quantify plant species composition, percent cover, and community structure across field sites and form the basis for vegetation monitoring, ecological assessment, and restoration evaluation (Bonham, 2013; Kent, 2012; **mueller-dombois1974?**).

2. Scope

- **Applies to:** Terrestrial vegetation surveys at all EA Program field sites, including the Bernard Field Station, Pomona College Farm, the Pit, restoration sites, and photovoltaic panel study areas
- **Does not apply to:** Aquatic or submerged vegetation surveys; tree-only forest inventory (DBH-based methods; see SOP-F604); remote sensing vegetation analysis
- **Location:** All EA Program field sites

3. Definitions

- **Percent cover:** The proportion of the ground surface covered by the vertical projection of the aerial parts of a plant species, expressed as a percentage. Cover is the standard metric for characterizing non-woody vegetation communities because it integrates plant size and density into a single measure (Bonham, 2013).
- **Line-point intercept (LPI):** A method that estimates cover by recording what a vertically lowered pin contacts at fixed intervals along a transect. Originally described by Levy & Madden (1933), LPI produces objective, repeatable cover estimates with minimal observer bias (Herrick et al., 2005).
- **Line intercept (LI):** A method that measures the length of transect tape intercepted by the canopy of each species. Best suited for shrubs and other plants with clearly defined canopy boundaries.
- **Quadrat:** A fixed-area frame (typically 1 m x 1 m) within which percent cover is visually estimated for each species. Visual estimation introduces more observer subjectivity than point-based methods but permits rapid assessment and better detection of rare species (Daubenmire, 1959; God'inez-'Alvarez et al., 2009).
- **Daubenmire cover class:** A categorical scale for estimating cover within quadrats: 1 (0–5%), 2 (5–25%), 3 (25–50%), 4 (50–75%), 5 (75–95%), 6 (95–100%). The midpoint of each class is used in calculations (Daubenmire, 1959).

- **Top hit / lower hit:** In LPI, the "top hit" is the first species the pin contacts as it descends through the canopy; subsequent contacts with other species or canopy layers are "lower hits." Recording both allows multi-layer canopy analysis.
- **Species richness:** The total number of species recorded in a defined area. Richness is strongly influenced by plot size and sampling effort (**wilson2012?**).
- **Voucher specimen:** A pressed, dried, and identified plant specimen archived for verification of field identifications. See Section 9.
- **Basal cover:** The proportion of ground surface occupied by the base (stem or root crown) of a plant, as distinct from foliar cover. Basal cover is more stable than foliar cover across seasons and is sometimes used for perennial grass monitoring (Herrick et al., 2005).

4. Responsibilities

Role	Responsibility
Lab PI	Approve survey design, ensure training compliance
Field Lead	Coordinate logistics, assign transect locations
Trained Personnel	Conduct surveys, record data, collect voucher specimens
New Students	Complete training before independent surveying

5. Safety and Hazards

⚠ Hazards

- **Biological:** Poison oak, stinging nettle, ticks, snakes, bees (all common at local field sites)
- **Physical:** Uneven terrain, heat exposure, sharp stakes and rebar
- **Allergenic:** Pollen exposure during peak growing season

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Long pants (required in areas with poison oak or ticks)
☐ Sun protection (hat, sunscreen)
☐ Nitrile gloves (when handling unknown plants)
☐ Tick repellent (DEET or permethrin-treated clothing)

5.2. Required Training

- SOP-F001 Field Safety

6. Summary of Methods

Three survey methods are covered in this SOP. Select the method that best fits your vegetation type and research objectives (Elzinga et al., 1998; Herrick et al., 2005).

Method	Best For	Accuracy	Speed	Observer Bias
Line-point intercept	Grasslands, herbaceous communities	High	Moderate	Low
Line intercept	Shrublands, plants with dense canopies >1 m	High	Slow	Low
Quadrat (cover class)	Mixed communities, rapid assessment, rare species detection	Moderate	Fast	Moderate

Choosing a Method

For most EA Program projects in southern California grasslands and shrublands, **line-point intercept** is the default method because it minimizes observer bias in cover estimation (God'inez-'Alvarez et al., 2009; Herrick et al., 2005). Use **quadrats** when rare species detection is important or when rapid assessment is needed, recognizing that visual cover estimation varies significantly among observers even after training (Bonham, 2013). Use **line intercept** for shrub-dominated communities where canopy boundaries are distinct. When a study spans multiple years, method consistency across years is more important than selecting the "best" method in any given year (**symstad2008?**).

7. Required Materials

7.1. Field Equipment

Item	Notes
50 m measuring tape	Fiberglass preferred (doesn't stretch)
Chaining pins or rebar stakes	For anchoring transect endpoints
Pin flags	For marking transect start/end and quadrat locations
Sampling pin or rod	Thin metal rod (~3 mm diameter) for point intercept
1 m x 1 m quadrat frame	PVC or metal; for cover class method
Compass	For establishing transect bearings
GPS unit or smartphone	For recording transect coordinates
Field data sheets	Printed from Appendix A
Clipboard and pencils	Mechanical pencils work well in the field

Item	Notes
Plant identification guides	Regional flora; Jepson Manual for California (Baldwin et al., 2012)
Camera	For photo documentation at each transect
Collection bags and plant press	For voucher specimens of unknown species
Flagging tape	For temporary marking

8. Estimated Time

Task	Time
Site preparation and transect setup	15–30 minutes per transect
Line-point intercept survey	30–60 minutes per 50 m transect
Line intercept survey	45–90 minutes per 50 m transect
Quadrat survey	10–15 minutes per quadrat
Data entry and QC	30 minutes per transect (post-field)

9. Procedure

9.1. Survey Timing

Conduct vegetation surveys during the peak growing season for the target community. In southern California:

- **Grasslands and annual forbs:** March through May
- **Coastal sage scrub:** April through June
- **Riparian:** June through August

Consistency in timing across years is essential for detecting temporal changes (**symstad2008?**). Surveys conducted outside the peak window will underestimate cover for deciduous and annual species.

9.2. Transect Layout

1. Determine transect locations using the project sampling design (random, stratified random, or systematic placement within habitat zones). The choice of sampling design affects the inferences that can be drawn from the data; random or stratified random designs support unbiased estimation of population-level cover, while systematic designs maximize spatial coverage but can coincide with periodic landscape features (Kent, 2012).
2. Navigate to the starting point and record GPS coordinates
3. Establish the transect bearing using a compass (typically perpendicular to environmental gradients, e.g., slope, moisture, or distance from structures)
4. Anchor the 0 m end of the measuring tape with a chaining pin

5. Walk out the tape to the desired length (typically 50 m), keeping it taut and as straight as possible
6. Anchor the far end with a second chaining pin
7. Photograph the transect from both ends

> **Permanent Transects**
>
> For long-term monitoring, mark transect endpoints with permanent markers (capped rebar driven flush with the ground, GPS coordinates recorded to sub-meter accuracy). Take a compass bearing and distance to at least two nearby permanent features for relocation.

9.3. Method A: Line-Point Intercept

This method estimates percent cover by recording what a vertical sampling pin contacts at regular intervals along the transect. The method was originally developed by Levy & Madden (1933) for pasture analysis and has since become the standard for rangeland and grassland monitoring (Herrick et al., 2005).

1. Begin at the 0 m end of the transect; always work from the same side of the tape
2. At each sampling point (every 0.5 m or 1 m, depending on project design), lower the sampling pin vertically through the vegetation canopy directly over the tape
3. Record each plant species the pin contacts as it descends from the canopy to the ground (**top hit** is the first species contacted; record all species for multi-layer canopies)
4. If the pin does not contact any vegetation, record the ground surface type: bare soil, litter, rock, moss/lichen, or other
5. Record the height (cm) of the tallest species contacted at each point
6. Advance to the next sampling point and repeat

> **Pin Angle Matters**
>
> The pin must be lowered vertically (plumb). Tilting the pin even slightly increases the probability of contacting vegetation and inflates cover estimates. Some protocols use a pin guide or frame to enforce verticality.

Calculations:

$$\text{Percent cover for species } i = \frac{\text{Number of hits for species } i}{\text{Total number of sampling points}} \times 100$$

Total cover may exceed 100% when canopy layers overlap. The number of sampling points required depends on the diversity and patchiness of the vegetation; 100 points per 50 m transect (0.5 m interval) is standard for diverse grasslands, while 50 points (1 m interval) may suffice in low-diversity or homogeneous stands (Elzinga et al., 1998).

9.4. Method B: Line Intercept

This method measures the length of transect tape intercepted by each plant species. Best suited for shrubs and plants with distinct canopy boundaries (Bonham, 2013).

1. Begin at the 0 m end of the transect
2. Walk slowly along the tape, noting where each plant canopy begins and ends (intercepts the tape)
3. Record the species, the start point (cm), and the end point (cm) on the data sheet
4. If a gap occurs within a single plant's canopy that is longer than the canopy width on either side, treat it as two separate intercepts
5. Record gaps between plants as the appropriate ground cover type

Calculations:

$$\text{Percent cover for species } i = \frac{\sum \text{intercept lengths for species } i}{\text{Total transect length}} \times 100$$

9.5. Method C: Quadrat (Cover Class)

This method uses visual estimates of cover within fixed-area plots placed at intervals along the transect (Daubenmire, 1959).

1. Place quadrats at predetermined intervals along the transect (e.g., every 5 m or 10 m), or at random distances
2. Ensure enough distance between quadrats for them to be independent sampling units (typically at least 5 m apart)
3. For each quadrat, identify every plant species present
4. Estimate percent cover for each species using the modified Daubenmire cover classes:

Class	Cover Range	Midpoint
1	0–5%	2.5%
2	5–25%	15%
3	25–50%	37.5%
4	50–75%	62.5%
5	75–95%	85%
6	95–100%	97.5%

5. Also record percent cover of bare ground, litter, and rock
6. Note the maximum vegetation height (cm) in each quadrat
7. Photograph each quadrat from directly above

Visual estimation of cover is inherently subjective and sensitive to observer experience, viewing angle, and vegetation height; training with known-cover reference images or calibration frames improves consistency but does not eliminate inter-observer variability (Bonham, 2013; God'inez-'Alvarez et al., 2009).

10. Voucher Specimens

When a plant cannot be identified in the field:

1. Collect a representative sample including leaves, stems, and flowers or fruit if available
2. Place in a labeled collection bag (site, transect, date, collector)
3. Press specimens the same day
4. Identify in the lab using the Jepson Manual (Baldwin et al., 2012) or other regional flora
5. Update the data sheet with the confirmed identification

11. Quality Control

- Two observers should independently estimate cover for at least 10% of quadrats to assess observer agreement. Inter-observer variability in cover estimation is the largest source of error in quadrat-based surveys; training reduces but does not eliminate this bias (Bonham, 2013; Elzinga et al., 1998).
- For LPI surveys, duplicate readings at 10% of points should agree on species identity for at least 90% of hits. Disagreements on ground cover type (e.g., litter vs. bare soil) are most common and should be resolved by applying consistent definitions.
- Photograph each transect and quadrat for archival verification
- Review data sheets in the field before leaving the site (check for missing entries, illegible writing, and species code consistency)
- Enter data within 48 hours of collection
- Maintain a species list with standardized codes used across all surveys

12. Data Management

12.1. Field Data Sheets

Use the data sheets in Appendix A. Record in pencil (ink smears when wet). Use Rite in the Rain paper if available.

12.2. Data Entry

Enter data into the project spreadsheet or database within 48 hours. Each row should represent one observation (one point, one intercept, or one quadrat-species combination). Required fields:

- Date, site, transect ID, observer
- Method (LPI, LI, or Quadrat)
- Point/interval/quadrat number
- Species (use standardized 6-letter codes: first 3 letters of genus + first 3 of species, e.g., BRODIA for *Bromus diandrus*)
- Cover value or hit/no-hit
- Height (cm)
- Ground cover type (if applicable)

13. Waste and Site Impact

- Remove all flagging tape, pin flags, and temporary markers after the survey
- Do not trample vegetation outside the immediate transect corridor
- Leave permanent markers flush with the ground to avoid hazards to people and wildlife
- Pack out all trash and supplies

14. References

Baldwin, B. G., Goldman, D. H., Keil, D. J., Patterson, R., Rosatti, T. J., & Wilken, D. H. (Eds.). (2012). *The jepson manual: Vascular plants of california* (2nd ed.). University of California Press.

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Kent, M. (2012). *Vegetation description and data analysis: A practical approach* (2nd ed.). Wiley-Blackwell. https://doi.org/10.1002/9781118452592

Levy, E. B., & Madden, E. A. (1933). The point method of pasture analysis. *New Zealand Journal of Agriculture*, *46*, 267–279.

15. Training Record

Before conducting vegetation surveys independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Demonstrate familiarity with at least 20 common species at the study site
3. Participate in a supervised survey using each method to be employed
4. Demonstrate acceptable inter-observer agreement on cover estimates (within one cover class for quadrats, or within 5% for line-point intercept)
5. Be signed off by the field lead or PI

16. Appendix A: Field Data Sheets

16.1. Line-Point Intercept Data Sheet

Site: _____ **Transect ID:** _____ **Date:** _____

Observer: _____ **Bearing:** _____ **Length:** _____ m

Sampling interval: every _____ m **Start GPS:** _____

Point (m)	Top Hit Species	Lower Hit(s)	Ground Cover	Height (cm)
0.0				
0.5				
1.0				
1.5				
2.0				

(Continue on additional sheets as needed)

16.2. Quadrat Cover Class Data Sheet

Site: _____ **Transect ID:** _____ **Date:** _____

Observer: _____ **Quadrat size:** _____ m x _____ m

Cover classes: 1 = 0–5%, 2 = 5–25%, 3 = 25–50%, 4 = 50–75%, 5 = 75–95%, 6 = 95–100%

Quadrat #	Position (m)	Species	Cover Class	Max Height (cm)

Ground cover for each quadrat:

Quadrat #	Bare Soil (%)	Litter (%)	Rock (%)	Other (%)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F604

Tree DBH and Forest Inventory

Standard Operating Procedure

SOP Number	SOP-F604
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standardized procedures for measuring tree diameter at breast height (DBH), conducting fixed-area plot-based forest inventories, and estimating basal area using prism or angle-gauge sampling. These measurements are fundamental to characterizing forest structure, estimating stand density and biomass, monitoring tree growth, and providing inputs to allometric equations for carbon accounting (Avery & Burkhart, 2002; Westfall & Westfall, 2020). The measurement conventions follow the USDA Forest Service Forest Inventory and Analysis (FIA) national standards (USDA Forest Service, 2023).

Accurate DBH measurement is the single most important field variable in forest ecology because nearly all derived quantities (basal area, volume, biomass, and carbon) depend on it through nonlinear allometric relationships. Small systematic errors in DBH propagate disproportionately into biomass estimates, especially for large-diameter trees, which store a disproportionate fraction of stand-level carbon (Lutz et al., 2018; **west2009?**).

2. Scope

- **Applies to:** All EA Program field sites with tree cover, including riparian forests, oak woodlands, mixed conifer stands, and planted trees at restoration and campus sites
- **Does not apply to:** Shrubs with DBH below 2.5 cm (use SOP-F603 for shrub cover); timber cruising for commercial harvest; urban tree risk assessment
- **Location:** All EA Program terrestrial field sites with woody vegetation

3. Definitions

- **Diameter at breast height (DBH):** The diameter of a tree trunk measured at 1.37 m (4.5 ft) above ground level on the uphill side of the tree. DBH is the standard dimension for characterizing tree size (Avery & Burkhart, 2002; USDA Forest Service, 2023).
- **Basal area (BA):** The cross-sectional area of a tree trunk at breast height, calculated as $\text{BA} = \frac{\pi}{4} \times \text{DBH}^2$. Often expressed per hectare (m^2/ha) as a measure of stand density.
- **Basal area factor (BAF):** The conversion factor for a wedge prism or angle gauge that translates the number of "in" trees to stand basal area (m^2/ha).
- **Variable-radius plot (point sampling):** A sampling method where the probability of a tree being included is proportional to its basal area, achieved using a BAF prism. Originally described by

  1.

- **Fixed-area plot:** A plot of known area (e.g., 0.04 ha circular plot, radius 11.28 m) within which all trees above a minimum DBH threshold are measured (Laar & Ak cca, 2007).
- **Allometric equation:** A mathematical model relating an easily measured tree dimension (usually DBH) to a harder-to-measure quantity (usually biomass or volume) through a power function (Chojnacky et al., 2014; Jenkins et al., 2003).
- **Crown class:** A classification of a tree's position in the canopy: dominant, co-dominant, intermediate, or suppressed. Crown class reflects competitive status and light availability.

- **Snag:** A standing dead tree. Snags provide habitat for cavity nesters and are recorded separately from live trees in inventories.
- **Stand density index (SDI):** A relative measure of stand density that accounts for tree size, allowing comparison across species and sites (**pretzsch2009?**).
- **Size distribution:** The frequency distribution of tree diameters in a stand. Reverse-J distributions characterize uneven-aged stands with continuous recruitment; normal or bimodal distributions suggest even-aged cohorts or past disturbance (**muller-landau2006?**).

4. Responsibilities

Role	Responsibility
PI / Field Lead	Approve plot design and minimum DBH threshold
Trained Personnel	Measure trees and record data per this SOP
New Students	Complete training before independent measurement

5. Safety and Hazards

⚠ Hazards

- **Physical:** Dead or leaning trees (snags, widow-makers) may shed branches. Look up before working under any tree. Do not measure trees during high winds.
- **Biological:** Poison oak, ticks, stinging insects, and snakes in wooded habitats. Wasp nests may be present in tree cavities.
- **Terrain:** Steep slopes in forested areas increase fall risk. Use caution when navigating uneven terrain with equipment.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Long pants
☐ Hard hat (in areas with overhead hazard trees)
☐ Sun protection (hat, sunscreen)
☐ Tick repellent

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. Work in pairs in remote forested areas.

6. Equipment and Materials

Item	Notes
Diameter tape (DBH tape, metric)	Graduated in diameter units (pi tape); reads directly in cm
Standard measuring tape (50 m)	For establishing plot boundaries
Clinometer (Suunto or equivalent)	For measuring tree height (optional)
Basal area factor (BAF) prism or angle gauge	For variable-radius plot sampling
GPS unit or smartphone	Per SOP-F006
Field notebook or data sheet	Pre-printed inventory form (Appendix A)
Compass	For plot layout and stem mapping
Flagging tape	For marking plot center and measured trees
Tree tags and nails (aluminum)	For permanent plot re-measurement
Paint marker or timber crayon	For marking the DBH measurement point
Laser rangefinder (optional)	For measuring tree height and distances
Calculator	For basal area and density calculations

7. Procedure

7.1. Measuring DBH

DBH is measured at 1.37 m (4.5 ft) above ground level on the uphill side of the tree (Avery & Burkhart, 2002; USDA Forest Service, 2023).

1. Identify the measurement height: 1.37 m above the ground surface on the uphill side of the tree. If the ground is level, measure from any side.
2. Wrap the diameter tape around the trunk at 1.37 m, perpendicular to the axis of the trunk (not perpendicular to the slope).
3. Ensure the tape is snug against the bark, not twisted, and level. Read the diameter directly from the DBH tape to the nearest 0.1 cm.
4. Record the measurement in the field notebook or data sheet.

Special cases:

Situation	Measurement Rule
Tree on a slope	Measure at 1.37 m from the uphill side of the base
Forked below 1.37 m	Measure each fork as a separate tree
Forked at or above 1.37 m	Measure as one tree below the fork
Swelling or abnormality at 1.37 m	Measure above and below the abnormality and average
Leaning tree	Measure 1.37 m along the underside of the trunk
Buttressed tree	Measure above the buttress where the trunk becomes cylindrical

> **Tip**
>
> For permanent monitoring plots, mark the DBH measurement point on the bark with a paint marker or timber crayon so that subsequent measurements are taken at the exact same height. For long-term studies, tag each tree with a numbered aluminum tag nailed above breast height.

7.2. Fixed-Area Plot Inventory

Fixed-area plots are the standard method for forest inventory (Laar & Ak cca, 2007; **west2009?**). Plot size depends on tree density.

Tree Density	Recommended Plot Size	Radius (circular)
Dense forest	0.01 ha (100 m^2)	5.64 m
Moderate forest	0.04 ha (400 m^2)	11.28 m
Open woodland	0.10 ha (1000 m^2)	17.84 m

Procedure:

1. Navigate to the plot center and record GPS coordinates per SOP-F006.
2. Mark the plot center with a stake or flagging tape.
3. For circular plots, use the measuring tape as a radius to determine which trees are inside the plot boundary. A tree is "in" if more than half of its trunk at DBH lies within the plot radius.
4. Measure and record the following for every tree with DBH at or above the minimum threshold (typically 2.5 cm or 10 cm, as specified by the project):
   - Species (common name and/or scientific name)
   - DBH (cm)
   - Condition class: live, standing dead (snag), leaning
   - Crown class (optional): dominant, co-dominant, intermediate, suppressed
   - Height (optional; measured with a clinometer or rangefinder)
5. Record the total number of stems and species per plot.

7.3. Variable-Radius Plot Sampling (Basal Area Prism)

The BAF prism provides a rapid estimate of stand basal area without establishing fixed plot boundaries (Avery & Burkhart, 2002; Grosenbaugh, 1952).

1. Stand at the sample point and hold the BAF prism at arm's length, looking through it at each surrounding tree at breast height.
2. For each tree, the prism refracts the image of the trunk. Compare the offset image to the original:
   - **Tree is "in":** The offset image overlaps the unrefracted trunk. Count the tree.
   - **Tree is "out":** The offset image is completely separated from the unrefracted trunk. Do not count.

- **Borderline:** The offset image just touches the trunk edge. Count every other borderline tree.

3. Rotate 360 degrees, counting all "in" trees.
4. Calculate basal area:

$$\text{Basal area } (\text{m}^2/\text{ha}) = n \times \text{BAF}$$

where n is the number of "in" trees and BAF is the basal area factor of the prism (typically 1, 2, 4, or 5 m^2/ha per tree counted; check the prism label).

7.4. Tree Height Measurement (Optional)

Use a clinometer or laser rangefinder to measure total tree height (Laar & Ak cca, 2007; **west2009?**):

1. Stand at a known horizontal distance from the tree base (e.g., 15–30 m; measure with a tape or rangefinder).
2. Sight the tree top through the clinometer and record the angle to the top (α).
3. Sight the tree base and record the angle to the base (β). If the base is below eye level, β is negative.
4. Calculate height:

$$H = D \times (\tan\alpha - \tan\beta)$$

where D is the horizontal distance (m) and H is the total height (m). This formula assumes the observer's line of sight to the top of the tree is unobstructed and that the tree is vertical; for leaning trees, measure height along the leaning axis and record the lean angle.

8. Derived Calculations

8.1. Basal Area per Tree

$$\text{BA } (\text{m}^2) = \frac{\pi}{4} \times \left(\frac{\text{DBH}}{100}\right)^2$$

where DBH is in cm.

8.2. Stand Density and Basal Area per Hectare

$$\text{Trees/ha} = \frac{n}{\text{plot area (ha)}}$$

$$\text{BA/ha } (\text{m}^2/\text{ha}) = \frac{\sum \text{BA}_i}{\text{plot area (ha)}}$$

8.3. Biomass Estimation

Above-ground biomass can be estimated using published allometric equations of the form:

$$\text{Biomass (kg)} = a \times \text{DBH}^b$$

where a and b are species-specific coefficients from published sources (Chojnacky et al., 2014; Jenkins et al., 2003). Document the allometric equation, source, and species used in the project README. Because the exponent b typically ranges from 2 to 3, measurement errors in DBH are amplified in biomass estimates: a 1 cm error at DBH = 50 cm produces a roughly 4–6% error in biomass (**west2009?**). Large-diameter trees store a disproportionately large fraction of aboveground carbon in most forest types; accurate measurement of the largest individuals in a stand is therefore critical for unbiased biomass and carbon stock estimates (Lutz et al., 2018).

8.4. Interpreting Size Distributions

Plotting the diameter distribution (number of trees per DBH class) provides insight into stand history and dynamics. A reverse-J (negative exponential) distribution suggests continuous recruitment across size classes, typical of uneven-aged or mixed-species stands. A bell-shaped or bimodal distribution suggests one or more distinct cohorts established after disturbance events (**muller-landau2006?**; **pretzsch2009?**).

9. Quality Control

- Measure DBH twice (wrap the tape, read, remove, re-wrap, re-read). The two readings should agree within 0.5 cm. Record the mean.
- On 10% of plots, have a second observer independently measure all trees. DBH measurements should agree within 1 cm; tree counts should match exactly.
- Verify the diameter tape calibration at the start of each field season by measuring a cylinder of known diameter.
- For permanent plots, verify that tags are intact and measurement points are correctly located at each re-measurement.
- When comparing DBH across re-measurement intervals, confirm that the measurement point has not shifted due to bark ingrowth over tags or paint marks (Westfall & Westfall, 2020).

10. References

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Plot center coordinates
F603	Vegetation Transects	Understory and shrub cover
F605	Leaf Area Index	Canopy measurement companion
F606	Canopy Cover	Densiometer and photo methods

12. Training Record

Before conducting tree measurements independently, personnel must:

1. Read this SOP in full
2. Demonstrate correct DBH measurement technique, including special cases (forks, slopes, buttresses)
3. Participate in a supervised plot inventory
4. Demonstrate BAF prism technique with results within 10% of the trainer's count
5. Be signed off by the PI or field lead

13. Appendix A: Forest Inventory Data Sheet

Site: _____ **Plot ID:** _____ **Date:** _____

Observer: _____ **Plot size:** _____ m^2 **Min DBH:** _____ cm

GPS: _____ **Elevation:** _____ m **Slope:** _____ %

Tree #	Species	DBH (cm)	Height (m)	Condition	Crown Class	Notes

Basal area prism count (BAF = _____): _____ trees in = _____ m^2/ha

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F605

Leaf Area Index Measurement

Standard Operating Procedure

SOP Number	SOP-F605
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes field methods for estimating Leaf Area Index (LAI), the total one-sided area of leaf tissue per unit ground surface area (m^2 leaf / m^2 ground). LAI is a key structural parameter governing light interception, transpiration, and primary productivity (Jonckheere et al., 2004). It is used in the EA Program for characterizing canopy structure at photovoltaic panel sites, restoration areas, and agricultural plots.

Both ceptometer and hemispherical photography methods estimate an "effective" LAI that assumes a random spatial distribution of foliage elements within the canopy. In most real canopies, foliage is clumped rather than random, and the effective LAI underestimates the true LAI by 20–50% depending on species and canopy architecture (Br'eda, 2003; Chen et al., 1997). When reporting LAI values, specify whether a clumping correction has been applied and which correction method was used. Ground-based LAI measurements also serve as critical reference data for validating satellite-derived LAI products (**garrigues2008?**; **zheng2009?**).

2. Scope

- **Applies to:** Indirect optical measurement of LAI using hemispherical photography and/or a ceptometer (AccuPAR or equivalent linear PAR sensor) at all EA Program field sites
- **Does not apply to:** Direct destructive leaf area measurement (harvest methods); satellite-derived LAI products (though ground measurements may be used to validate them); root area measurements
- **Location:** All EA Program field sites

3. Definitions

- **Leaf Area Index (LAI):** The total one-sided area of leaf tissue per unit ground area. Dimensionless (m^2 m^{-2}). Typical values range from 0 (bare ground) to 1–3 (grasslands and crops) to 5–10 (closed-canopy forests) (Jonckheere et al., 2004).
- **Effective LAI (L_e):** The LAI value obtained from optical methods that assume random foliage distribution. Effective LAI underestimates true LAI in clumped canopies because light passes through gaps between clumps more easily than a random model predicts (Chen et al., 1997).
- **True LAI:** The actual one-sided leaf area per unit ground area, obtained either by destructive harvest or by applying a clumping correction (Ω) to the effective LAI: $\text{LAI}_{\text{true}} = L_e/\Omega$, where $\Omega < 1$ for clumped canopies (Chen et al., 1997).
- **Clumping index (Ω):** A dimensionless parameter (0 to 1) describing the degree to which foliage deviates from a random distribution. Values near 1 indicate nearly random distribution; values of 0.5–0.7 are typical for conifer canopies.
- **PAR:** Photosynthetically Active Radiation (400–700 nm).
- **Ceptometer:** A linear array of PAR sensors used to measure light transmission through a canopy. LAI is estimated from the ratio of below-canopy to above-canopy PAR using the Beer-Lambert extinction model (Norman & Campbell, 1989).
- **Gap fraction:** The proportion of the sky hemisphere visible through the canopy at a given zenith angle. Gap fraction is the primary measurement from hemispherical photography and is inverted to estimate LAI.
- **Extinction coefficient (K):** A parameter in the Beer-Lambert model relating LAI to light transmission; depends on solar zenith angle and leaf angle distribution.
- **Hemispherical photography:** A technique using a fisheye lens pointed upward to photograph the canopy. Gap fraction analysis of the image yields LAI estimates (Frazer et al., 2001).

4. Responsibilities

Role	Responsibility
PI / Field Lead	Approve measurement design, review data
Trained Personnel	Collect field measurements per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Uneven terrain, tripping hazards from dense vegetation
- **Biological:** Tick and insect exposure in dense canopy understories
- **Heat:** Measurements are often taken in open or partially shaded areas during peak growing season

5.1. Required PPE

☐ Closed-toe shoes or field boots
☐ Sun protection (hat, sunscreen)
☐ Long pants and long sleeves (tick protection in dense vegetation)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Required Materials

Item	Notes
Ceptometer (AccuPAR LP-80 or equivalent)	Linear PAR sensor with datalogger
External PAR sensor (if not built in)	For above-canopy reference
Camera with fisheye lens adapter	For hemispherical photography
Tripod with self-leveling head	For consistent camera positioning
GPS unit or smartphone	For recording measurement locations
Field data sheet	Pre-printed with site/date fields
Compass	For orienting measurements
Measuring tape (50 m)	For locating measurement points

7. Estimated Time

Task	Time
Site setup and orientation	10–15 minutes
Ceptometer measurements (10 points)	20–30 minutes
Hemispherical photographs (10 points)	30–45 minutes
Data download and QC	15 minutes

8. Procedure

8.1. Measurement Timing

LAI measurements should be taken under uniform overcast skies or within one hour of solar noon under clear skies (Weiss et al., 2004). Avoid measurement during patchy cloud conditions, as rapidly changing light causes inconsistent above-canopy reference readings. For hemispherical photography, early morning, late afternoon, or overcast conditions produce the best contrast between sky and foliage (Br'eda, 2003).

8.2. Sampling Design

1. Determine measurement locations using the project sampling design (random, stratified random, or collocated with vegetation transects from SOP-F603)
2. At each location, take a minimum of 5 below-canopy readings (ceptometer) or photographs (hemispherical) to capture spatial variability (Weiss et al., 2004)
3. Record GPS coordinates at each measurement location

8.3. Method A: Ceptometer (AccuPAR)

1. Power on the ceptometer and set the date, time, and site ID
2. Enter the leaf angle distribution parameter (χ). Use 1.0 for a spherical distribution (default for most broadleaf canopies) unless species-specific values are available (Norman & Campbell, 1989).
3. Take an above-canopy PAR reading by holding the sensor bar horizontally in full sunlight (or use the external above-canopy sensor if available)
4. Move to the first below-canopy measurement point. Hold the ceptometer horizontally at approximately 1 m height (or just above the understory layer), perpendicular to the dominant row or canopy orientation.
5. Record the below-canopy PAR reading
6. Rotate the ceptometer 90 degrees and take a second reading at the same point to account for canopy anisotropy
7. Average the two readings for that point
8. Repeat at each of the remaining measurement points (minimum 5 per location)
9. Take another above-canopy reading at the end of the measurement sequence to verify that light conditions have not changed substantially (within 10% of the initial reading)

10. The instrument calculates LAI internally using the Beer-Lambert model:

$$\text{LAI} = \frac{-\ln(\tau)}{K}$$

where τ is the ratio of below-canopy to above-canopy PAR and K is the extinction coefficient (a function of solar zenith angle and leaf angle distribution). This value is the effective LAI (L_e) and does not include a clumping correction.

8.4. Method B: Hemispherical Photography

1. Mount the camera with fisheye lens adapter on the tripod at 1 m height (or just above the understory)
2. Level the camera using the tripod's bubble level
3. Orient the top of the image toward magnetic north using a compass
4. Set the camera to manual exposure: use the sky brightness (not the canopy) to set exposure. Bracket exposures if uncertain (one stop above and below). Exposure settings strongly influence the sky/foliage classification threshold and can introduce systematic bias in LAI estimates (Br'eda, 2003).
5. Take the photograph pointing directly upward (zenith)
6. Record the image number, GPS coordinates, and any notes (e.g., understory density, canopy species) on the field data sheet
7. Repeat at each measurement point

8.4.1. Image Analysis

8. Transfer images to the lab computer
9. Analyze using Gap Light Analyzer (GLA) or the `hemiphot` R package (Steege, 2018):
    a. Threshold the image to classify pixels as sky (gap) or foliage
    b. Calculate gap fraction at multiple zenith angle rings
    c. Invert gap fractions to estimate LAI using a canopy model (e.g., Miller's formula or the ellipsoidal leaf angle model)
10. Report the mean LAI and standard error for each site. Specify whether the reported value is effective LAI or clumping-corrected true LAI.

i Ceptometer vs. Hemispherical Photography

The two methods measure slightly different quantities and may not agree at the same site. Ceptometers integrate PAR transmission along a linear transect and are sensitive to solar angle and sky conditions; hemispherical photographs sample the full sky hemisphere and are analyzed offline, allowing more flexible post-processing. Agreement between methods is typically within 0.5–1.0 LAI units, but systematic offsets can occur, especially in heterogeneous canopies (Jonckheere et al., 2004; **thimonier1996?**). Using both methods at a subset of sites allows cross-validation and helps quantify method-specific bias.

9. Quality Control

- Take at least 5 below-canopy readings per measurement location to quantify within-site variability
- Report the coefficient of variation (CV) across readings; if CV exceeds 30%, increase the number of readings
- For ceptometer measurements, verify that above-canopy PAR did not change by more than 10% between the start and end of the measurement sequence
- For hemispherical photography, visually inspect thresholded images to ensure correct sky/foliage classification; manually adjust the threshold if needed
- Cross-validate: where possible, compare ceptometer and hemispherical photography estimates at the same points (Br'eda, 2003)
- When LAI data will be used to validate remote sensing products, document the spatial footprint (measurement spacing and extent) and the timing relative to the satellite overpass (**garrigues2008?**)

10. Data Management

1. Download ceptometer data to the lab computer within 48 hours
2. Organize hemispherical images by site and date: `LAI_SiteID_YYYYMMDD/`
3. Archive raw data and images on the lab shared drive
4. Version-control analysis scripts per SOP-L801

11. References

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12. Training Record

Before measuring LAI independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety
2. Demonstrate correct ceptometer operation (power on, set parameters, above/below readings, data download)
3. Demonstrate correct hemispherical photography technique (leveling, orientation, exposure settings)
4. Process one set of images through gap fraction analysis under supervision
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F606

Canopy Cover (Densiometer and Hemispherical Photography)

Standard Operating Procedure

SOP Number	SOP-F606
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
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11 Training Record 7

1. Purpose

This SOP describes two methods for estimating canopy cover: the convex spherical densiometer and hemispherical (fisheye) photography. Canopy cover is a key variable in stream habitat assessments (SWAMP protocol), forest ecology, microclimate studies, and solar radiation modeling. Separating this SOP from SOP-F304 allows the methods to be applied in any habitat, not just stream reaches (Jennings et al., 1999; Lemmon, 1956).

The distinction between canopy cover and canopy closure is important for data interpretation. Canopy cover is the proportion of the forest floor covered by the vertical projection of tree crowns; canopy closure is the proportion of the sky hemisphere obscured by vegetation as seen from a single point below the canopy. The two metrics are related but not equivalent, and values can differ by 10–20% at the same location depending on canopy heterogeneity and the method used (Jennings et al., 1999; Paletto & Tosi, 2009).

2. Scope

- **Applies to:** Measurement of overhead canopy cover in forests, riparian corridors, oak woodlands, restoration sites, and any habitat where tree or tall shrub canopy is present; includes both the spherical densiometer method and hemispherical photography
- **Does not apply to:** Canopy closure measured by line-intercept or vertical projection methods (see SOP-F603); leaf area index (see SOP-F605); remote sensing canopy cover from aerial or satellite imagery
- **Location:** All EA Program field sites with tree or tall shrub cover

3. Definitions

- **Canopy cover:** The proportion of the forest floor covered by the vertical projection of tree crowns, expressed as a percentage. Canopy cover is an areal measurement that integrates across an entire stand or plot (Jennings et al., 1999).
- **Canopy closure:** The proportion of the sky hemisphere obscured by vegetation when viewed from a single point below the canopy. Canopy closure is a point measurement and is what both the densiometer and hemispherical photography directly estimate (Jennings et al., 1999).
- **Canopy openness:** The complement of canopy closure: openness = 100% - closure. Some software and instruments report openness rather than closure.
- **Convex spherical densiometer:** A hand-held instrument with a convex mirror etched with a grid. The observer counts sky-visible grid intersections to estimate canopy openness (Lemmon, 1956).
- **Gap fraction:** The fraction of the sky visible through the canopy at a given zenith angle, measured from hemispherical photographs. Gap fraction is the basis for estimating both canopy closure and effective LAI.
- **Binary threshold:** The image-processing step in hemispherical photography analysis where each pixel is classified as either sky (white) or canopy (black). The threshold value strongly influences the estimated canopy closure.
- **SWAMP:** Surface Water Ambient Monitoring Program (California). The SWAMP protocol uses densiometer readings to characterize stream canopy cover as part of physical habitat assessment.

4. Responsibilities

Role	Responsibility
PI / Field Lead	Specify method and number of measurement points
Trained Personnel	Perform measurements per this SOP
New Students	Complete training before independent measurement

5. Safety and Hazards

⚠ Hazards

- **Physical:** Looking upward while walking increases trip and fall risk. Stop walking before looking up.
- **Biological:** Ticks, poison oak, and snakes in forested and riparian habitats.
- **Environmental:** Bright sunlight reflecting off the densiometer can cause glare. Wear sunglasses when not actively reading the instrument.

5.1. Required PPE

- ☐ Closed-toed shoes or field boots
- ☐ Long pants
- ☐ Sun protection (hat, sunscreen)
- ☐ Tick repellent (riparian and woodland sites)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Equipment and Materials

6.1. Method A: Spherical Densiometer

Item	Notes
Convex spherical densiometer (Forestry Suppliers Model A or equivalent)	Concave models also exist; specify which model is used
Bubble level (built into the densiometer)	Essential for consistent readings
Field notebook or data sheet	For recording dot counts
Compass	For orienting the four cardinal readings

6.2. Method B: Hemispherical Photography

Item	Notes
Camera with fisheye lens	Full-frame fisheye (180 degrees circular) preferred; or smartphone with clip-on fisheye lens
Self-leveling tripod or monopod	Camera must be level and facing directly upward
Bubble level (hot-shoe mounted or tripod-integrated)	For leveling the camera
Gray card or exposure reference	For consistent exposure settings
Laptop with canopy analysis software	Gap Light Analyzer (GLA), hemIMAGE, or ImageJ with the Hemispherical plugin
GPS unit	Per SOP-F006

7. Procedure

7.1. Measurement Point Selection

Canopy cover measurements are typically taken at systematic intervals along a transect or at the center and edges of fixed-area plots.

- **Stream habitat surveys (SWAMP):** Measure at 11 equally spaced points along each cross-section transect (left bank, center, right bank at each of the main transect positions). See SOP-F304.
- **Forest inventory plots:** Measure at the plot center and at 4 points on the plot boundary (N, E, S, W).
- **Transect-based surveys:** Measure at regular intervals (e.g., every 10 m or 25 m along a 50 m transect).

Record the GPS coordinates of each measurement point or its position along the transect.

7.2. Method A: Convex Spherical Densiometer

The densiometer has a convex mirror with an etched grid. The observer counts the number of grid intersections that are open to the sky (not obstructed by vegetation) in each of four cardinal directions (Lemmon, 1956; Strickler, 1959).

1. Hold the densiometer at elbow height (approximately 30–40 cm in front of your body), level it using the bubble level.
2. Position your head so that its reflection is just outside the grid area. This ensures a consistent viewing angle.
3. Face **north**. Imagine the grid divided into 24 squares (4 rows x 6 columns). At each of the 24 grid intersections, determine whether the sky or vegetation is reflected. Count the number of open (sky) intersections. Record this count.
4. Rotate 90 degrees and face **east**. Repeat the count.
5. Repeat for **south** and **west**.
6. Calculate canopy cover:

$$\text{Canopy openness (\%)} = \frac{\text{sum of 4 counts}}{96} \times 100$$

$$\text{Canopy cover (\%)} = 100 - \text{canopy openness (\%)}$$

where 96 is the total number of possible open points across 4 directions (24 per direction x 4 directions).

> **!** Known Densiometer Bias
>
> The convex densiometer systematically overestimates canopy openness (and therefore underestimates canopy cover) compared to hemispherical photography because of the wide-angle optics and the observer's head position (Cook et al., 1995; Fiala et al., 2006). The magnitude of the bias varies with stand density, observer experience, and lighting conditions but is typically 5–15 percentage points (Korhonen et al., 2006; Paletto & Tosi, 2009). Report the method used alongside all canopy cover values, and do not mix methods within a dataset or monitoring program.

7.3. Method B: Hemispherical Photography

Hemispherical photographs capture a 180-degree view of the canopy and are analyzed digitally to classify sky vs. canopy pixels (Frazer et al., 2001).

In the field:

1. Mount the camera on the tripod at breast height (1.37 m) or at a height specified by the project protocol.
2. Level the camera using the bubble level. The lens must point directly upward (zenith).
3. Orient the top of the camera toward magnetic north. Record the declination if relevant.
4. Set the camera to manual exposure. Bracket the exposure (take 3 photos: one at the metered exposure, one stop under, one stop over) to ensure at least one well-exposed image.
5. Shoot in overcast or uniformly cloudy conditions whenever possible. Direct sunlight produces bright spots that confound automatic thresholding.
6. Include a gray card in one reference photo per site for exposure calibration.
7. Record the photo number, GPS coordinates, date, time, and sky conditions in the field notebook.

In the lab:

1. Import images into canopy analysis software (Gap Light Analyzer, hemIMAGE, or ImageJ with the Hemispherical plugin).
2. Register the image (set lens projection, define the circular boundary, orient to north).
3. Apply a binary threshold to classify each pixel as sky (white) or canopy (black). Adjust the threshold manually if automated thresholding produces obvious errors.
4. The software calculates canopy openness, canopy cover, leaf area index (effective), and gap fraction by zenith angle.
5. Export results as a `.csv` file. Save to the project repository under `data/canopy/`.

7.4. Reporting

Report canopy cover values with the following metadata:

- Method (densiometer or hemispherical photography)
- Instrument model (densiometer type; camera and lens model)
- Measurement height above ground
- Date, time, and sky conditions
- Software and thresholding method (for photography)
- Number of measurement points and their spatial arrangement
- Whether the reported metric is canopy cover (vertical projection) or canopy closure (hemispherical view); see Section 3 definitions

8. Quality Control

- **Densiometer:** Have two observers independently read the same point. Readings should agree within 5 percentage points. If they disagree by more than 10 points, re-read together to identify the source of discrepancy. Observer bias is the largest error source for densiometer measurements (Korhonen et al., 2006).
- **Hemispherical photography:** Process the same image twice using different threshold values (+/- 10 units). If the resulting canopy cover differs by more than 5 percentage points, the image may be poorly exposed; re-shoot if possible.
- At a subset of sites, measure canopy cover with both methods and report the comparison. This allows future users to relate datasets collected with different methods and to quantify the systematic offset between them (Fiala et al., 2006; Paletto & Tosi, 2009).
- Record the observer's name with every densiometer measurement.

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10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F304	Stream Physical Habitat	Canopy cover in stream reaches
F603	Vegetation Transects	Understory cover companion
F604	Tree DBH and Forest Inventory	Stand structure companion
F605	Leaf Area Index	Related canopy metric

11. Training Record

Before measuring canopy cover independently, personnel must:

1. Read this SOP in full
2. Demonstrate correct densiometer technique (leveling, head position, cardinal-direction counts) with results within 5 percentage points of the trainer's reading on at least 5 test points
3. If using hemispherical photography, demonstrate camera setup, exposure bracketing, and image analysis in the lab
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F607

Tree Tagging for Restoration Monitoring

Standard Operating Procedure

SOP Number	SOP-F607
Revision	0.1
Author(s)	Ellena Reposa
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standardized procedures for tagging trees during restoration field work at EA Program field sites. Tree tagging creates a permanent, individual record for each tree so that

management decisions are consistent, repeatable, and transparent across field crews and over time. A future student or technician should be able to revisit a tagged tree and understand exactly why it was flagged and how its condition has changed.

Tree tagging is used to:

- Identify which trees will be retained, removed, or monitored
- Track individual tree condition over time
- Create a permanent record for future groups and project documentation
- Support consistent decision-making across crew members

Recording a consistent set of fields for each individual tree allows data to be combined, compared, and reused across projects and seasons (Handley et al., 2021).

2. Scope

- **Applies to:** All EA Program field sites with tree cover, including riparian forest, oak woodland, mixed conifer stands, and planted trees at restoration and campus sites
- **Does not apply to:** Shrubs with DBH below 2.5 cm (use SOP-F603 for shrub cover); diameter, height, and plot-based inventory measurement (use SOP-F604); timber cruising for commercial harvest; formal urban tree risk assessment, which requires a qualified arborist (International Society of Arboriculture, 2017)
- **Location:** All EA Program terrestrial field sites with woody vegetation

3. Definitions

- **DBH (diameter at breast height):** The diameter of a tree trunk measured at 1.37 m (4.5 ft) above ground on the uphill side of the tree. The standard dimension for comparing tree sizes. Measurement procedure is defined in SOP-F604 (Avery & Burkhart, 2002; USDA Forest Service, 2023).
- **Tag:** A numbered aluminum disk fastened to the tree. Each tag number is unique within the project.
- **Flagging:** Colored plastic tape tied loosely around a tree at eye level. Color communicates the management decision for that tree.
- **Condition rating:** A five-level score (Excellent, Good, Fair, Poor, Dead) describing tree health and structure.
- **Crown:** The upper part of a tree, including all branches and foliage.
- **Crown spread:** The average horizontal width of the crown, taken as the mean of the widest spread and a second spread measured at right angles to it (American Forests, 2014).
- **Retain:** Leave the tree in place. Do not remove or damage.
- **Remove:** Tree is scheduled for cutting or restoration thinning.

4. Responsibilities

Role	Responsibility
PI / Field Lead	Approve flag color codes before the season starts; review completed field forms and photo logs weekly; ensure the project meets restoration goals
Trained Personnel	Tag trees per this SOP; complete all fields in the tree assessment record; take required photos; flag trees correctly the first time
New Students	Complete training before tagging independently

Minimum crew size is two persons at all times (safety requirement; see SOP-F001 Field Safety).

5. Safety and Hazards

⚠ Hazards

- **Overhead:** Dead branches and leaning or unstable trees (widow-makers) can fall without warning. Look up before approaching any tree. Do not work under or near hazard trees in high wind.
- **Sharp tools:** Hand saws, loppers, and pruners. Keep blades sheathed while walking, pass tools handle-first, and never carry open blades through brush.
- **Terrain, biota, and weather:** Uneven terrain, poison oak, ticks, snakes, stinging insects, and heat are common at EA field sites and are covered in SOP-F001 Field Safety.

5.1. Required PPE

☐ Hi-vis vest
☐ Closed-toed shoes or field boots
☐ Long sleeves and long pants (poison oak protection)
☐ Safety glasses or goggles
☐ Cut-resistant gloves (when using cutting tools)
☐ Hard hat (in areas with overhead hazard trees)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety for stop-work conditions, biotic and weather hazards, emergency contacts, and incident response. Work in pairs at all times in forested areas.

6. Equipment and Materials

Item	Notes
Numbered aluminum tags	Pre-numbered; one per tree
Aluminum or stainless nails	For attaching tags; see Section 8.2 on growth allowance
Flagging tape (color set)	Per the color codes in Section 7
Diameter (DBH) tape	Reads diameter directly in cm (pi tape)
GPS unit or smartphone	Record coordinates per SOP-F006
Camera or smartphone	Minimum three photos per tree
Field data sheets	Printed from Appendix A
Clipboard and pencils	Pencils work better than pens in wet conditions
Long measuring tape	For crown spread
Clinometer or laser rangefinder	For tree height (optional); see SOP-F604
Lumber crayon or paint marker	For marking the DBH measurement point
Hand saw or loppers (optional)	For small branch clearance to access the trunk

7. Flagging Color Codes

> **!** Important
>
> Confirm color codes with the crew and PI before working. Project-specific changes must be documented in the field log.

Flag Color	Designation
ORANGE	Confirmed removal: invasive species (primary target)
YELLOW	Confirmed removal: dead, dying, or hazard tree
PINK	Confirmed removal: restoration thinning
GREEN	Retain: do not remove
WHITE	Under review: secondary evaluation required (do not remove yet)
RED	Monitoring tree: reassess next cycle
BLUE	Approved native for transplant or seed collection

Rule: Tie flagging loosely at eye level. Do not wrap tightly; flagging can girdle the tree over time.

8. Procedure

8.1. Before Entering the Field (Daily Pre-Work)

Complete this checklist before leaving the vehicles each day:

- ☐ All PPE inspected and worn
- ☐ GPS units charged; project boundaries pre-loaded
- ☐ Tag number range assigned to each crew member (no duplicates)

- ☐ Communication check complete; phones functional
- ☐ Weather reviewed against the stop-work conditions in SOP-F001
- ☐ Crew briefed: target species, flag color codes, today's objectives
- ☐ Cutting tools sharpened, sheathed, and inspected

8.2. Tree Tagging Step-by-Step

Step	Action	Why it matters
1. Approach and evaluate	Assess tree form, lean, root zone, crown condition, and nearby hazards from more than one side before tagging	A tree that looks sound from one side may have rot, lean, or deadwood visible from another angle (International Society of Arboriculture, 2017)
2. Identify species	Confirm common and scientific name; cross-check against the project Invasive Priority List and Retain Species List. Use SOP-F602 iNaturalist for ID support where needed	Tagging the wrong species leads to the wrong management decision
3. Measure DBH	Measure at 1.37 m on the uphill side per SOP-F604	DBH drives removal priority, allometric calculations, and monitoring frequency
4. Assign flag color	Select the color per Section 7 and tie loosely at eye level	Color communicates the decision to every future crew member
5. Install tag	Fasten the numbered tag on the uphill side at DBH height with a single aluminum or stainless nail. Leave the nail head protruding about 2 to 3 cm so the tree can grow without engulfing the tag	A consistent, accessible tag is easy to relocate and read in later seasons; leaving the nail proud accommodates radial growth and bark ingrowth (USDA Forest Service, 2023)
6. Photograph	Take at least three photos: (a) full tree form, (b) tag number close-up, (c) notable defects or hazards	Photos resolve disputes and provide evidence if a tag is lost
7. Record data	Complete all fields in the tree assessment record (Appendix A) before moving to the next tree	If it is not written down, it did not happen

i Crown spread (optional field)

If crown spread is recorded, measure the widest spread across the crown, then measure a second spread at right angles to the first, and average the two: (A + B) / 2 (American Forests, 2014). Tree height, if recorded, is measured with a clinometer or laser rangefinder per SOP-F604.

8.3. Tree Condition Ratings

Use a consistent five-level scale. Note structural defects (lean, cracks, cavities, deadwood, included bark) that influence the rating and any hazard flag (International Society of Arboriculture, 2017).

Condition	Field indicators
EXCELLENT	No significant defects; vigorous, full crown; normal growth
GOOD	Minor defects; healthy overall; normal growth
FAIR	Moderate defects; reduced vigor; some dieback
POOR	Major defects; significant dieback; structural risk
DEAD	No live tissue; standing snag or fallen log

9. Quality Control

Perform these checks every tagging day:

1. **Tag uniqueness:** Confirm no tag number was used twice. Reconcile the day's tags against the assigned number ranges.
2. **Species cross-check:** Verify each species identification against the Invasive Priority and Retain Species lists. Flag uncertain IDs for review rather than guessing.
3. **Data completeness:** Confirm every tagged tree has a fully completed assessment record (Appendix A) with no blank required fields.
4. **Photo completeness (end of day):** Before leaving the site, scroll through the day's photos and confirm that every tag number has the three required photos (full form, tag close-up, defects). Re-photograph any tree that is missing photos before leaving.
5. **Second-observer check:** On a subset of trees (for example, 10%), have a second crew member independently confirm species, condition rating, and flag color. Resolve any disagreements with the field lead.

10. References

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11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite; hazards, stop-work, emergencies
F006	GPS and Site Navigation	Recording tree coordinates
F602	iNaturalist	Species identification support
F603	Vegetation Transects	Understory and shrub cover
F604	Tree DBH and Forest Inventory	DBH, height, and crown measurement
F606	Canopy Cover	Stand structure companion

12. Training Record

Before tagging trees independently, each student or new technician must:

1. Read this SOP in full
2. Read SOP-F001 Field Safety and pass a field safety check (PPE, stop-work conditions, emergency contacts)
3. Know the correct flag color codes
4. Demonstrate correct DBH measurement technique (per SOP-F604)
5. Correctly tag and flag three practice trees with the crew lead observing
6. Complete a tree assessment record without errors
7. Take an acceptable three-photo set (full tree, tag close-up, defects)
8. Be signed off by the PI or field lead

Trainee	Date read SOP	Date practice trees	Signed off by	Date

13. Appendix A: Individual Tree Assessment Record

Print one copy per tree (or one per plot) before going to the field. Circle the appropriate option where choices are given.

Tag number: _____ **GPS coordinates:** _____

Species (common): _____

Species (scientific): _____

DBH: _____ (circle: in / cm) **Est. height (ft):** _____ (clinometer or rangefinder; see SOP-F604)

Crown spread (ft): _____ (average of two perpendicular axes: (A + B) / 2)

Condition rating: EXCELLENT / GOOD / FAIR / POOR / DEAD (circle one)

Flag color: ORANGE / YELLOW / PINK / GREEN / WHITE / RED / BLUE (circle one)

Proximity hazards (e.g., lean toward trail, overhanging deadwood, near utility): _____

Adjacent utilities? YES / NO (if yes, note type and distance): _____

Removal rationale (e.g., invasive species, hazard, restoration thinning, dead): _____

Photo file references (e.g., TAG042_full, TAG042_tag, TAG042_defect): _____

Assessor initials: _____ **Date and time:** _____

Additional notes / sketch (trunk abnormalities, lean direction, nearby landmarks):

_____

_____

14. Appendix B: Daily Photo Log

Minimum three photos per tree. Store photos with the tag number in the filename (for example, `TAG042_full_form.jpg`).

Photo #	Tag #	Subject	Direction facing	Notes
1		Full tree form		
2		Tag number close-up		
3		Defects / hazards		
4				
5				

15. Appendix C: Field Note

This SOP is a field guide. It does not replace project permits or crew lead direction. Review this SOP with your crew lead at the start of each project season.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F608

Plant Tissue Sampling

Standard Operating Procedure

SOP Number	SOP-F608
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for collecting, handling, and preserving plant tissue samples in the field for subsequent laboratory analysis. Plant tissue analysis provides data on nutrient concentrations (C, N, P, K, metals), stable isotope ratios (δ^{13}C, δ^{15}N), and structural traits such as leaf mass per area (LMA) (Kalra, 1998; Mills & Jones, 2004). Proper sampling technique is essential to ensure that results represent the target tissue and are not contaminated or degraded during collection and transport.

Foliar nutrient concentrations and LMA form part of the "leaf economics spectrum" (LES), a global axis of plant strategy that links leaf structure to function across biomes: species with low LMA and high leaf nitrogen tend toward fast resource acquisition and short leaf lifespans, while species with high LMA and low nitrogen invest in durable, long-lived leaves (Reich, 2014; Wright et al., 2004). Extending this framework, the "fast-slow" plant economics spectrum integrates leaf, stem, and root traits into a whole-plant strategy axis (Reich, 2014; Westoby et al., 2002). Standardized sampling protocols allow EA Program data to be compared with global trait databases (e.g., TRY, BIEN) and regional reference values (Cornelissen et al., 2003; P'erez-Harguindeguy et al., 2013).

2. Scope

- **Applies to:** Collection of leaf, stem, root, and reproductive tissue from vascular plants for nutrient and isotope analysis at all EA Program field sites
- **Does not apply to:** Soil organic matter sampling (see SOP-F400); algal tissue sampling; plant tissue culture; DNA extraction from plant tissue (see SOP-L700)
- **Location:** All EA Program field sites; initial processing in SGM 133/134

3. Definitions

- **Tissue type:** The specific plant organ sampled (e.g., fully expanded mature leaves, current-year stem growth, fine roots less than 2 mm diameter, seeds).
- **Composite sample:** A sample formed by combining tissue from multiple individuals of the same species within a plot to represent the population average. Compositing reduces analytical cost but eliminates information about individual-level variation.
- **Voucher specimen:** A pressed and identified plant specimen collected alongside tissue samples to verify species identification.
- **Leaf mass per area (LMA):** The ratio of leaf dry mass to one-sided leaf area (g m^{-2}). LMA is a key functional trait that reflects the trade-off between resource capture (photosynthesis per unit area) and leaf durability. High-LMA leaves are thicker or denser, with lower nitrogen per unit mass but greater physical toughness (Poorter et al., 2009; Wright et al., 2004).
- **Specific leaf area (SLA):** The inverse of LMA: SLA = 1/LMA, expressed as m^2 kg^{-1}. SLA and LMA contain the same information but are used in different contexts; report whichever is standard for the analysis.
- **Leaf economics spectrum (LES):** A global pattern relating leaf traits (LMA, nitrogen, phosphorus, photosynthetic capacity, and leaf lifespan) along a single axis of variation. High-nitrogen, low-LMA species occupy the "fast return" end; low-nitrogen, high-LMA species occupy the "slow return" end (Wright et al., 2004).

- **Sun leaf / shade leaf:** Leaves developed in high-light (sun) or low-light (shade) conditions. Sun and shade leaves from the same tree can differ by 20–40% in nitrogen concentration and by a factor of two or more in LMA (Cornelissen et al., 2003; P'erez-Harguindeguy et al., 2013). Sampling must standardize canopy position to avoid confounding light environment with species-level traits.
- **Field blank:** An empty labeled sample container opened and exposed at the field site, then sealed and processed identically to real samples. Blanks detect contamination introduced during collection and transport.

4. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design and target species
Trained Personnel	Collect and process tissue per this SOP
New Students	Complete training before independent sampling

5. Safety and Hazards

⚠ Hazards

- **Biological:** Contact with allergenic plants (poison oak, stinging nettles). Some target species may have thorns or irritating sap.
- **Physical:** Use of pruning shears and scissors poses a laceration risk.
- **Chemical:** If using liquid nitrogen for snap-freezing, follow cryogenic safety procedures (gloves, face shield, ventilated area).

5.1. Required PPE

☐ Nitrile gloves
☐ Closed-toe shoes or field boots
☐ Sun protection
☐ Leather gloves (for thorny species)
☐ Cryogenic gloves and face shield (if using liquid nitrogen)

5.2. Emergency Procedures

For cuts from pruning shears, apply direct pressure and clean the wound. For poison oak exposure, wash with cold water and soap as soon as possible. Refer to SOP-F001 Field Safety.

6. Required Materials

Item	Notes
Stainless steel pruning shears or scissors	Clean between species
Paper coin envelopes or labeled paper bags	For drying samples; avoid plastic for fresh tissue intended for drying
Ziplock bags (gallon size)	For fresh tissue requiring freezing
Cooler with ice packs	For transport of fresh tissue
Permanent markers (Sharpie)	For labeling
Pre-printed labels	Site, species, tissue type, date, collector
DI water squeeze bottle	For rinsing contamination
Ethanol (70%) in a spray bottle	For decontaminating tools between species
Paper towels	For drying tools after decontamination
GPS unit	For recording sampling locations
Plant identification guides	For species verification
Plant press (if collecting vouchers)	See SOP-F603 Section 10
Field data sheet	Pre-printed
Liquid nitrogen dewar and cryo-vials	Only if snap-freezing is required
Analytical balance (field-portable, optional)	For recording fresh mass
Leaf area meter or scanner (optional)	For measuring fresh leaf area in the lab before drying (needed for LMA calculation)

7. Estimated Time

Task	Time
Site setup and species identification	15–30 minutes
Tissue collection (10 samples)	30–60 minutes
Labeling and packing for transport	15 minutes
Lab processing (cleaning, drying)	Variable (see Section 9)

8. Procedure

8.1. Sampling Design

1. Define the target species, tissue type, and number of replicates in the sampling plan before going to the field
2. Determine whether individual or composite samples are needed. For nutrient analysis where individual variation is important, collect tissue from individual plants. For site-level averages, composite tissue from 5–10 individuals per species per plot.
3. Select individuals randomly within the sampling area. Avoid plants at plot edges, obviously damaged or diseased individuals, and plants with heavy herbivory or epiphyte loads (unless these are the target condition).

8.2. Leaf Tissue Collection

1. Put on clean nitrile gloves. Change gloves between species to avoid cross-contamination.
2. Select fully expanded, mature leaves from the outer canopy (sun leaves) unless the project specifically targets shade leaves or another position. Standardizing canopy position is critical for comparability: sun and shade leaves from the same individual can differ by 20–40% in nitrogen concentration and by a factor of two in LMA (Cornelissen et al., 2003; P'erez-Harguindeguy et al., 2013). For trees, sample from the sunlit portion of the mid- to upper crown; for herbs and grasses, sample the most recently fully expanded leaf.
3. Using clean pruning shears, clip the petiole at its base. For grasses, clip the most recent fully expanded blade at the ligule.
4. Collect enough tissue mass for the planned analyses. As a guideline:

Analysis	Minimum dry mass
C, N, S by EA-IRMS	2–5 mg
δ^{13}C, δ^{15}N	2–5 mg
Total P, metals (acid digestion)	100–500 mg
LMA (requires leaf area scan)	3–5 leaves per individual
Multiple analyses from one sample	2–5 g fresh weight recommended

5. For composite samples, collect an approximately equal mass of tissue from each individual and combine in a single bag.
6. Place tissue immediately into a labeled paper envelope (if air-drying) or a labeled Ziplock bag (if freezing or transporting fresh). For frozen samples, place bags into the cooler with ice packs.

8.3. Measuring Leaf Area (for LMA/SLA)

If LMA or SLA is needed, leaf area must be measured on fresh tissue before drying (P'erez-Harguindeguy et al., 2013):

1. Transport fresh leaves to the lab within 6 hours of collection, keeping them in sealed bags in the cooler to minimize wilting
2. Scan leaves on a flatbed scanner (600 dpi) or measure with a leaf area meter. Record total one-sided area (cm^2) for each sample.
3. Dry and weigh as described in Section 9.1
4. Calculate LMA = dry mass (g) / area (m^2), or SLA = area (m^2) / dry mass (kg)

8.4. Stem, Root, and Reproductive Tissue

- **Stems:** Clip current-year growth. Record the diameter and length of the stem segment.
- **Fine roots (less than 2 mm diameter):** Excavate carefully around the base of the target plant using a trowel. Separate live roots from dead roots and soil. Rinse briefly with DI water in the field or at the first opportunity in the lab.
- **Seeds/fruits:** Collect from a minimum of 5 individuals. Record the phenological stage (e.g., immature, mature, dehisced).

8.5. Decontamination Between Species

1. Spray pruning shears with 70% ethanol
2. Wipe dry with a clean paper towel
3. Allow to air-dry for 30 seconds before cutting the next species
4. For metal analysis, use stainless steel tools only (no galvanized or painted tools) and rinse with DI water after ethanol to remove residual salts

8.6. Labeling

Every sample must be labeled with:

- Site name and plot/transect ID
- Species (use the 6-letter code from SOP-F603 Section 12.2)
- Tissue type (leaf, stem, root, seed)
- Individual or composite (if composite, record the number of individuals)
- Date (YYYY-MM-DD)
- Collector initials

Write on both the bag/envelope and a separate tag inside (in case the exterior label smudges).

8.7. Field Data Sheet

Record the following for each sample:

Field	Entry
Sample ID	Unique sequential number
Site / Plot	
Species	
Tissue type	
Number of individuals	(if composite)
Canopy position	Sun / shade / mixed
GPS coordinates	
Fresh mass (g)	(if field balance available)
Notes	Condition, herbivory, phenology

9. Sample Preservation and Lab Processing

9.1. Drying

The default preservation method for nutrient and isotope analysis (Jones, 2001):

1. Transport paper envelopes to the lab within 24 hours

2. Dry at 60 C in the drying oven (SOP-L105) for at least 48 hours or until a constant mass is reached
3. Record the dry mass
4. Store dried tissue in labeled coin envelopes in a desiccator or sealed container until grinding

> **!** Temperature Matters
>
> Do not exceed 60 C for tissue intended for nitrogen or carbon isotope analysis. Higher temperatures can volatilize nitrogen compounds and alter δ^{15}N values (Kalra, 1998). For samples intended only for total C and N by combustion (not isotope analysis), drying at 70 C is acceptable and reduces drying time.

9.2. Freezing

For analyses requiring fresh tissue (enzyme assays, gene expression, volatile compounds):

1. Transport in cooler with ice packs (or snap-freeze with liquid nitrogen in the field)
2. Transfer to the -80 C freezer (SOP-L106) within 6 hours of collection
3. Record in the freezer inventory

9.3. Grinding

1. Grind dried tissue to a fine powder using a ball mill or mortar and pestle
2. Clean the grinding equipment between samples (brush, then wipe with ethanol)
3. Store ground tissue in labeled microcentrifuge tubes or coin envelopes

10. Quality Control

- Collect at least one field blank per sampling event: open an empty labeled bag at the site, expose it to the same conditions as the samples, then seal and process identically
- For metal analysis, verify that tools are stainless steel and DI-rinsed
- Record the time between collection and drying/freezing on the data sheet; flag any samples exceeding the 24-hour (drying) or 6-hour (freezing) window
- For composite samples, verify that approximately equal mass was contributed by each individual
- When interpreting foliar nutrient results, compare against published reference ranges for the target species or functional group (Mills & Jones, 2004; Reuter & Robinson, 1997). Values outside the expected range may indicate contamination, analytical error, or genuine ecological signal (e.g., nutrient deficiency or toxicity).
- For LMA, verify that leaf area was measured on fresh, fully turgid tissue; wilted leaves underestimate area and inflate LMA (P'erez-Harguindeguy et al., 2013)

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12. Training Record

Before collecting plant tissue independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety
2. Demonstrate species identification for the target community
3. Demonstrate proper collection technique (glove changes, tool decontamination, labeling, canopy position standardization)
4. Process one set of samples through drying and grinding under supervision
5. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F610

Benthic Macroinvertebrate Sampling

Standard Operating Procedure

SOP Number	SOP-F610
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for collecting benthic macroinvertebrate (BMI) samples from wadeable streams. BMI communities are widely used indicators of water quality and biological integrity because different taxa have varying tolerances to pollution, habitat degradation, and hydrologic alteration. This SOP follows the California Surface Water Ambient Monitoring Program (SWAMP) protocols to enable comparison with statewide bioassessment datasets (Ode et al., 2016).

2. Scope

- **Applies to:** BMI sampling in wadeable streams (depth less than 1 m, velocity allowing safe wading) at all EA Program study sites
- **Does not apply to:** Non-wadeable rivers or lakes (require different sampling gear); estuarine or marine macroinvertebrates; terrestrial invertebrates; meiofauna (organisms passing through a 500 um sieve)
- **Location:** All EA Program stream sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling plan; ensure permit compliance
Trained Personnel	Collect and preserve samples per this SOP
New Students	Complete training before independent sampling

4. Safety and Hazards

⚠ Hazards

- **Drowning and swift water:** Do not wade in flows deeper than waist height or where footing is unstable. Always face upstream. Use a wading rod for support.
- **Slippery substrates:** Algae-covered rocks are extremely slippery. Wear felt-soled or studded wading boots.
- **Biological:** Waterborne pathogens, ticks in riparian vegetation, stinging insects
- **Chemical:** Ethanol (95%) preservative is flammable. Keep away from ignition sources.

4.1. Required PPE

☐ Chest or hip waders with felt or studded soles

- ☐ Nitrile gloves
- ☐ Safety glasses (when handling preservatives)
- ☐ Sun protection

4.2. Permits

BMI sampling may require a Scientific Collecting Permit (California Department of Fish and Wildlife). The PI must confirm permit status before fieldwork at any new site. Some sites may also fall under federal Section 7 or Section 10 requirements if listed species are present.

4.3. Invasive Species Prevention

Decontaminate all equipment (nets, waders, buckets) between sites to prevent transfer of invasive species and pathogens. Inspect, scrub, and dry all gear, or treat with dilute bleach or hot water (60 C). See SOP-F304 Section 4.3.

4.4. Emergency Procedures

A minimum of two people must be present for in-stream work. Refer to SOP-F001 Field Safety.

5. Definitions

- **Benthic macroinvertebrate (BMI):** An invertebrate organism living on or in the stream bottom substrate that is retained by a 500 um mesh sieve. Includes aquatic insects, crustaceans, mollusks, worms, and other taxa.
- **Riffle:** A shallow stream section with fast, turbulent flow over coarse substrate. The primary habitat sampled for BMI in wadeable streams.
- **Reach:** A 150 m to 250 m stream segment (typically 150 m in California SWAMP protocols) within which all sampling occurs.
- **Targeted riffle composite (TRC):** The SWAMP standard method in which BMI samples are collected from multiple riffles and composited into a single reach-level sample.
- **D-frame kick net:** A D-shaped net with 500 um mesh mounted on a frame, used for dislodging and capturing BMI from the streambed.

6. Required Materials

Item	Notes
D-frame kick net (500 um mesh)	Standard SWAMP gear
500 um sieve (brass or stainless steel)	For field processing
White sorting trays	For inspecting samples
Sample jars (1 L, wide-mouth, HDPE)	Pre-labeled

Item	Notes
95% ethanol	For preservation (final concentration approximately 70% after dilution by sample water)
Squirt bottles (DI water and ethanol)	For rinsing nets and transferring organisms
Forceps (soft-tipped)	For picking organisms from the net
Flagging tape	For marking transect locations
GPS unit	For recording reach coordinates
Measuring tape (50 m)	For delineating the reach
Field data sheets	SWAMP BMI collection forms
Camera	For photo-documenting reach conditions
Pencils and waterproof notebook	For field notes
Cooler	For transporting preserved samples

7. Estimated Time

Task	Time
Reach setup and reconnaissance	15–30 minutes
BMI collection (targeted riffle composite)	45–90 minutes
Field processing and preservation	15–30 minutes
Decontamination	10–15 minutes

8. Procedure

8.1. Reach Delineation

1. Identify the downstream boundary of the reach at the most downstream riffle or pool tail
2. Lay out the measuring tape upstream for 150 m (SWAMP standard reach length; adjust proportionally for streams narrower than 2 m or wider than 10 m per SWAMP guidance)
3. Record GPS coordinates at the downstream and upstream boundaries
4. Photograph the reach from the downstream end looking upstream

8.2. Habitat Assessment

Before disturbing the streambed for BMI collection, complete the physical habitat assessment per SOP-F304 if required by the project design. If habitat and BMI are collected on the same visit, always complete habitat measurements first (working upstream) before BMI sampling.

8.3. Targeted Riffle Composite Collection (SWAMP Method)

The SWAMP TRC method collects BMI from riffle habitats throughout the reach, compositing all kicks into a single sample.

1. Identify all riffles within the 150 m reach. A riffle is defined as a section with visibly turbulent flow over coarse substrate (gravel, cobble, boulder).
2. Allocate sampling effort across riffles proportional to their area. The target is 8 kicks distributed across all available riffles. If only one riffle is present, all 8 kicks are taken from that riffle.
3. Position yourself facing downstream at the downstream edge of the first riffle
4. Place the D-frame kick net on the substrate, opening facing upstream, with the bottom edge pressed firmly against the streambed
5. Disturb the substrate immediately upstream of the net opening by vigorously kicking and rubbing rocks with your boots for 30 seconds over an area of approximately 0.09 m^2 (one net width by one net width)
6. Lift the net and inspect the contents. Remove large rocks, sticks, and leaves after rinsing them in the net to dislodge clinging organisms.
7. Transfer the net contents into the composite sample bucket or directly into the sieve
8. Move upstream to the next kick location. Space kicks at least 2 m apart within each riffle.
9. Repeat until all 8 kicks are completed across the available riffles

> **!** Always Work Upstream
>
> Sampling must proceed from downstream to upstream to avoid disturbing habitats before they are sampled. Never walk through a riffle before sampling it.

8.4. Field Processing

1. Pour the composite sample through the 500 um sieve
2. Rinse the net and bucket thoroughly into the sieve to recover all organisms
3. Transfer the sieved material into a labeled 1 L sample jar
4. Add enough 95% ethanol to achieve a final preservative concentration of approximately 70% (the sample water dilutes the ethanol; adding ethanol equal to the sample volume is a reasonable approximation)
5. Seal the jar tightly. Invert gently to mix.
6. Label the jar with: site ID, date, "BMI-TRC," collector initials. Place a second label (pencil on waterproof paper) inside the jar.

8.5. Non-Riffle Habitats (Optional)

If the project requires sampling pools, runs, or other habitats in addition to riffles, collect these as separate samples (not composited with the TRC). Label accordingly (e.g., "BMI-POOL").

8.6. Field Data Sheet

Record the following:

Field	Entry
Site ID	
Date	YYYY-MM-DD
Collectors	
Reach length (m)	
Number of riffles sampled	
Number of kicks	(target: 8)
GPS (downstream boundary)	
GPS (upstream boundary)	
Weather conditions	
Flow conditions	Normal, low, high
Preservative used	95% EtOH
Notes	Unusual conditions, disturbances

9. Laboratory Processing

Laboratory identification and enumeration of BMI are typically performed by a contracted taxonomic laboratory or by trained students under PI supervision. The general workflow:

1. Transfer the preserved sample to a gridded sorting tray
2. Randomly select grid cells and pick all organisms from those cells until a minimum of 500 organisms are identified (SWAMP standard)
3. Identify organisms to the lowest practical taxonomic level (genus for most insects; family or higher for non-insect taxa) using standard keys (Merritt et al., 2019; Thorp & Rogers, 2015)
4. Record counts by taxon
5. Calculate summary metrics: total taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, percent tolerant taxa, and other indices as required
6. Archive voucher specimens of difficult identifications

SWAMP Data Submission

If data will be submitted to the California Environmental Data Exchange Network (CEDEN), use SWAMP-compatible data formats and QA/QC protocols. Consult the SWAMP Bioassessment SOP 004 (Ode et al., 2016) and the PI.

10. Quality Control

10.1. Field QC

- Record the number of kicks and their distribution across riffles on the data sheet
- If fewer than 8 kicks are possible (limited riffle habitat), record the actual number and the reason
- Take one field blank per sampling event: rinse the net into a clean jar with ethanol; this documents any organisms retained in the net from the decontamination process

10.2. Lab QC

- Re-sort 10% of processed samples to estimate sorting efficiency (target: greater than 90% removal of organisms from the sample)
- Have a second taxonomist verify identification of 10% of specimens
- Report QC results with the final dataset

10.3. Metric Calculation

Calculate the California Stream Condition Index (CSCI) if sufficient data are available. CSCI integrates observed vs. expected taxa composition (O/E) and a predictive multi-metric index (pMMI) into a single biological condition score (Mazor et al., 2016).

11. Waste Disposal

Waste Type	Disposal Method
Ethanol-preserved samples (after analysis)	Evaporate ethanol in fume hood; dispose of residual solids in regular trash
Excess 95% ethanol	Flammable waste container; EH&S pickup
Used gloves and paper towels	Regular trash

12. References

See the project-level `references.bib` for full citations. Key references: Ode et al. (2016), Barbour et al. (1999), Mazor et al. (2016), Merritt et al. (2019), Thorp & Rogers (2015).

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13. Training Record

Before collecting BMI samples independently, personnel must:

1. Read this SOP, SOP-F001 Field Safety, and SOP-F304 Stream Physical Habitat
2. Review the SWAMP Bioassessment SOP 004 (BMI collection sections)
3. Demonstrate D-frame kick net technique (proper positioning, 30-second kick, net rinsing) under supervision
4. Participate in at least one complete reach-level BMI collection event
5. Demonstrate proper preservation and labeling
6. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F611

Pollinator Observation Surveys

Standard Operating Procedure

SOP Number	SOP-F611
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standardized methods for surveying pollinator communities at EA Program field sites using timed visual observations and passive pan traps. Pollinator surveys document the diversity and relative abundance of bees, butterflies, flies, beetles, and other flower-visiting insects, providing data for assessing habitat quality, restoration success, and the effects of land use on pollination services (Lebuhn & Fenter, 2013; Potts et al., 2010).

2. Scope

- **Applies to:** Standardized pollinator observation surveys and pan trap sampling at all EA Program terrestrial field sites with flowering vegetation, including the Bernard Field Station, campus landscapes, restoration sites, and agricultural study areas
- **Does not apply to:** Targeted bee nesting surveys; hive management; pollen analysis (palynology); pollinator genetics or molecular identification (though specimens collected here may be used for those purposes); aquatic insect sampling (see SOP-F610)
- **Location:** All EA Program field sites with floral resources

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve survey design and collection permits
Trained Personnel	Conduct surveys and process pan trap samples per this SOP
New Students	Complete training (insect identification and sting safety) before independent work

4. Definitions

- **Diel activity pattern:** The temporal distribution of an organism's activity across a 24-hour period; most bee species are diurnal with peak activity between 09:00 and 15:00 under sunny, warm conditions; surveys outside peak activity hours systematically underestimate community richness (Lebuhn & Fenter, 2013).
- **Floral density:** The number of open flowers per unit area at a survey location; a key covariate for pollinator abundance because visitation scales with resource availability; estimated visually as flowers per m^2 or as qualitative categories (low, medium, high) at each survey event.
- **Focal flower observation:** A survey method in which a single flowering plant or patch is observed for a fixed duration (typically 10 minutes) and all pollinators visiting any flower during that period are recorded; useful for comparing visitation rates between plant species or treatments (Westphal et al., 2008).
- **Functional group:** A coarse taxon category used when species-level identification is not possible in the field; the EA Program uses 10 groups: honey bee (HB), bumble bee (BB),

small native bee (SB), large native bee (LB), butterfly (BF), syrphid fly (SF), other fly (OF), beetle (BT), wasp (WA), and other (OT).

- **Independent detection event:** A single detection of a pollinator at a flower, counted only once per survey period regardless of how long the individual remains; used to standardize counts across surveys of different duration.
- **Pan trap:** A colored bowl (white, fluorescent yellow, fluorescent blue) filled with soapy water; passively captures flying insects attracted by color resemblance to flowers; effective for small- to medium-sized bees but under-represents large-bodied bees and butterflies (Lebuhn & Fenter, 2013; Westphal et al., 2008).
- **Pollinator:** Any animal that moves pollen from the anther to the stigma of a flower, facilitating plant reproduction; includes bees, flies, butterflies and moths, beetles, and hummingbirds; bees are the dominant pollinators at most EA Program field sites (Potts et al., 2010).
- **Pollination service:** An ecosystem service provided by flower-visiting animals; essential for reproduction of approximately 75% of flowering plant species and 35% of global food crop production (Potts et al., 2010).
- **Timed observation transect:** A standardized survey in which the observer walks a fixed route (typically 50 m x 2 m) at a standardized pace (approximately 10 m per minute) and records all pollinators observed on flowers within the survey corridor; produces a count-based estimate of relative pollinator activity (Dicks et al., 2016; Lebuhn & Fenter, 2013).
- **Voucher specimen:** A preserved insect specimen deposited in a reference collection to document the occurrence of a species at a site; required for any first records and difficult identifications; pinned through the right mesopleuron following standard entomological practice.

5. Safety and Hazards

⚠ Hazards

- **Biological:** Bee and wasp stings are the primary hazard. Personnel with known Hymenoptera allergies must carry a prescribed epinephrine auto-injector (EpiPen) and inform the field lead before every outing. Africanized honey bees may be present at some Southern California sites.
- **Chemical:** Soapy water in pan traps (low risk). Ethanol (70%) used for specimen preservation is flammable.
- **Physical:** Heat exposure during mid-day surveys. Uneven terrain. Pan traps may be trampled by people or wildlife.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Light-colored clothing (dark colors attract bees)
☐ Sun protection (hat, sunscreen)
☐ EpiPen (if allergic; inform field lead)
☐ Nitrile gloves (when handling preserved specimens)

5.2. Permits

Insect collection may require a Scientific Collecting Permit from CDFW depending on the site and target taxa. No permit is needed for observation-only surveys.

5.3. Emergency Procedures

For anaphylactic reactions: administer EpiPen, call 911. Refer to SOP-F001 Field Safety. If encountering an aggressive swarm, walk calmly away; do not swat or run erratically.

6. Equipment and Materials

6.1. Timed Observations

Item	Notes
Stopwatch or phone timer	For standardizing observation periods
Hand lens or magnifying glass	For in situ identification
Camera (macro-capable or phone)	For photo-documenting pollinators
Field data sheet	Pre-printed from Appendix A
Insect field guide	e.g., *California Bees and Blooms*
iNaturalist app (smartphone)	For photo-based identification; see SOP-F602
Compass and GPS	For recording transect or plot locations

6.2. Pan Traps

Item	Notes
Plastic bowls (12 oz, 3.25 in rim)	White, fluorescent blue, and fluorescent yellow; one of each color per station
Fluorescent spray paint (blue, yellow)	For painting white bowls; use UV-bright paints
Unscented liquid dish soap	2–3 drops per bowl (breaks surface tension)
Water	To fill bowls approximately 2/3 full
Wooden stakes or wire stands	For elevating bowls to canopy height in tall vegetation
Fine mesh strainer	For removing insects from bowls
Whirl-Pak bags or vials	For storing collected specimens
70% ethanol	For preserving specimens
Forceps (soft-tipped)	For handling specimens
Labels (waterproof, internal)	For vial and bag identification

7. Procedure

7.1. Survey Timing and Conditions

Conduct pollinator surveys during peak pollinator activity:

- **Time of day:** 09:00–15:00 local time (peak flight activity)
- **Weather:** Sunny to partly cloudy; wind speed below Beaufort 4 (< 5.5 m/s); temperature above 15 C. Do not survey during rain, heavy overcast, or high winds, as pollinator activity drops sharply.
- **Season:** During peak bloom at the study site. In Southern California, this is typically March through June for most habitats, with a secondary bloom in fall for some species.
- **Flowering requirement:** Surveys are only meaningful when flowers are present. Record the dominant flowering species and estimate floral density (flowers per m^2) at each survey location.

7.2. Method A: Timed Observation Surveys

Timed observations record pollinator visits to flowers along a fixed route or within a fixed plot during a standardized time period (Dicks et al., 2016; Lebuhn & Fenter, 2013).

Transect walk method:

1. Establish a transect (typically 50 m x 2 m, or a 100 m x 1 m corridor) through the area of interest. Record the transect coordinates per SOP-F006.
2. Walk the transect slowly at a pace of approximately 10 m per minute.
3. Record every pollinator observed visiting a flower within the 2 m band on either side of the transect center line (or 1 m on each side for a 2 m wide transect).
4. For each observation, record:
    - Pollinator group or species (see Section 6.4)
    - Flower species being visited
    - Behavior (nectaring, pollen collecting, resting)
    - Time of observation
5. Complete the transect in 10–15 minutes. Do not stop and wait for pollinators; maintain a steady pace.
6. Repeat the transect 2–3 times per sampling event, spaced at least 30 minutes apart.

Focal flower observation method:

1. Select a flowering plant or patch and observe for a fixed period (typically 10 minutes).
2. Record every pollinator that visits any flower on the focal plant during the observation period.
3. This method is useful for comparing visitation rates among plant species or treatments.

7.3. Method B: Pan Trap Sampling

Pan traps passively capture flying insects attracted to colored bowls (Lebuhn & Fenter, 2013; Westphal et al., 2008).

1. At each sampling station, set out three bowls: one white, one fluorescent blue, one fluorescent yellow. Space bowls approximately 3 m apart.
2. Fill each bowl approximately 2/3 full with water. Add 2–3 drops of unscented dish soap (this breaks the surface tension so insects sink).
3. Place bowls at the height of the surrounding vegetation canopy:
   - In short grass or bare ground: set directly on the ground
   - In tall vegetation (> 30 cm): elevate on stakes or wire stands so the bowl rim is at canopy height
4. Deploy traps in the morning (by 09:00) and retrieve after 24 hours. For shorter deployments (4–6 hours), retrieve in the afternoon.
5. Pour the contents of each bowl through a fine mesh strainer. Transfer captured insects to a labeled Whirl-Pak bag or vial with 70% ethanol.
6. Label each vial with: site, station number, bowl color, date deployed, date retrieved, and collector initials.
7. Return specimens to the lab for sorting, pinning, and identification.

> **Note**
>
> Pan traps are biased toward small- to medium-sized bees and may under-represent large-bodied bees (bumble bees) and butterflies (Westphal et al., 2008). Combine pan traps with timed observations for a more complete picture of the pollinator community.

7.4. Pollinator Identification

Identify pollinators to the finest taxonomic level possible in the field. At a minimum, assign each observation to one of the following functional groups:

Code	Group	Examples
HB	Honey bee	*Apis mellifera*
BB	Bumble bee	*Bombus* spp.
SB	Small native bee	*Halictus*, *Lasioglossum*, *Ceratina*
LB	Large native bee	*Xylocopa*, *Megachile*
BF	Butterfly	All Lepidoptera
SF	Syrphid fly (hover fly)	Syrphidae
OF	Other fly	Bombyliidae, Tachinidae, etc.
BT	Beetle	Flower-visiting Coleoptera
WA	Wasp	Vespidae, Sphecidae, etc.
OT	Other	Hummingbirds, moths, ants

Photograph all specimens that cannot be identified in the field. Upload photos to iNaturalist per SOP-F602 for community-assisted identification.

7.5. Specimen Processing (Lab)

1. Sort pan trap specimens by order under a dissecting scope.
2. Pin bee specimens following standard entomological practice (pin through the right mesopleuron) and label with locality, date, collector, and determination.
3. Store pinned specimens in a labeled unit tray in the insect collection.
4. Specimens can be submitted to a taxonomic specialist or DNA barcoded (COI) for species confirmation.

8. Quality Control

- Record weather conditions (temperature, wind, cloud cover) at the start and end of every survey. Do not compare data collected under different weather conditions without accounting for detectability.
- On 10% of surveys, have two observers independently record pollinators on the same transect to assess inter-observer agreement.
- Record floral density and dominant flowering species at every survey; pollinator abundance is strongly influenced by floral resource availability.
- For pan traps, inspect bowls mid-deployment if wind or wildlife disturbance is likely. Refill or replace tipped bowls and note the interruption.
- Voucher-pin at least 5 representative specimens per functional group per site per season for the reference collection.

9. References

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10. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite; sting response

SOP	Title	Relationship
F006	GPS and Site Navigation	Survey location coordinates
F602	iNaturalist	Photo-based identification
F603	Vegetation Transects	Floral resource documentation
F615	Bird Point Counts	Companion fauna survey method

11. Training Record

Before conducting pollinator surveys independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety in full
2. Disclose any Hymenoptera allergy to the field lead and carry an EpiPen if prescribed
3. Demonstrate identification of the 10 pollinator functional groups (Section 6.4) using pinned specimens or photographs
4. Participate in a supervised transect observation survey and pan trap deployment
5. Be signed off by the PI or field lead

12. Appendix A: Pollinator Observation Data Sheet

Site: _____ **Transect ID:** _____ **Date:** _____

Observer: _____ **Start time:** _____ **End time:** _____

Weather: Temp: _____ C Wind (Beaufort): _____ Cloud: _____ % Sun: Y / N

Dominant flowering species: _____

Time	Pollinator Group/Species	Flower Species	Behavior	Photo #

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F315

Bird Point Count Surveys

Standard Operating Procedure

SOP Number	SOP-F315
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes procedures for conducting standardized bird point count surveys. Point counts are the most widely used method for monitoring bird populations and community composition in

terrestrial habitats. An observer stands at a fixed point for a defined time period and records all birds detected by sight or sound within a specified radius. This SOP follows protocols compatible with the North American Breeding Bird Survey (BBS) and Partners in Flight (PIF) guidelines to enable comparison with regional and national datasets (Ralph et al., 1993; Ralph et al., 1995).

2. Scope

- **Applies to:** Fixed-radius and unlimited-radius bird point count surveys at all EA Program field sites, including the Bernard Field Station, Pomona College campus, restoration areas, and agricultural study sites
- **Does not apply to:** Area searches, nest monitoring, mist-netting, raptor surveys, nocturnal surveys, or aquatic bird surveys (these require separate protocols)
- **Location:** All EA Program terrestrial field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve survey design; ensure permit compliance
Trained Observer	Conduct point counts; identify all species by sight and sound
Data Recorder	Record observations if a second person is available
New Students	Complete training (Section 13) before recording data independently

4. Safety and Hazards

⚠ Hazards

- **Early morning work:** Surveys begin at or before sunrise. Reduced visibility increases trip and fall risk. Use a headlamp while walking to survey points.
- **Biological:** Ticks, rattlesnakes, and stinging insects in brushy and riparian habitats. Poison oak along riparian corridors.
- **Heat:** Summer surveys in southern California can reach high temperatures by mid-morning.

4.1. Required PPE

☐ Closed-toe shoes or field boots
☐ Long pants (tick and brush protection)
☐ Sun protection (hat, sunscreen)
☐ Headlamp (for pre-dawn travel to survey points)

4.2. Permits

Bird surveys do not typically require a Scientific Collecting Permit as long as birds are not captured or handled. However, access to certain sites may require permission from landowners or managing agencies. The PI must confirm access authorization before fieldwork.

4.3. Emergency Procedures

A minimum of two people is recommended for early morning surveys. Carry a cell phone and a first aid kit. Refer to SOP-F001 Field Safety.

5. Definitions

- **Point count:** A survey method in which an observer records all birds detected from a single fixed point during a standardized time period.
- **Fixed-radius point count:** Detections are recorded only within a specified distance from the observer (e.g., 50 m). Provides density estimates without distance estimation models.
- **Unlimited-radius point count:** All detections are recorded regardless of distance, with the estimated distance to each bird noted. Allows distance sampling analysis to estimate detection probability and density.
- **Detection type:** How the bird was detected: singing (S), calling (C), visual (V), flyover (F), or drumming/mechanical sound (D).
- **Breeding Bird Survey (BBS):** A long-running North American monitoring program using roadside point counts; the EA Program protocol is designed to be compatible with BBS methods.

6. Required Materials

Item	Notes
Binoculars (8x42 or 10x42)	Essential for visual identification
Field guide	*Sibley Guide to Birds* or *Kaufman Field Guide*
Rangefinder (laser)	For estimating detection distances in unlimited-radius counts
Stopwatch or timer (phone app)	For standardizing count duration
GPS unit or smartphone	For recording point locations
Bird point count data sheet	Pre-printed (see Appendix A)
Clipboard and pencils	Mechanical pencils; avoid pens that smear
Headlamp	For pre-dawn travel
Audio recorder (optional)	For later verification of song identifications; Merlin or eBird app
Compass	For recording bearing to detections (optional, for distance sampling)

7. Estimated Time

Task	Time
Travel to first point (before sunrise)	Variable
Count period per point	5 or 10 minutes (see Section 8.3)
Travel between points	5–10 minutes (minimum 150 m apart)
Full survey (10 points)	2–3 hours (must finish within 4 hours of sunrise)

8. Procedure

8.1. Survey Design

1. Establish point count locations before the first survey:
    - Points must be at least 150 m apart (250 m preferred) to minimize double-counting of the same individuals (Ralph et al., 1995)
    - Distribute points using a systematic or stratified random design within the study area
    - Permanently mark each point with a GPS waypoint and, where possible, a discreet physical marker (flagging tape, small stake)
2. Record the habitat type at each point (e.g., coastal sage scrub, riparian woodland, grassland, developed)
3. Determine whether fixed-radius (50 m) or unlimited-radius counts will be used. Fixed-radius counts are simpler and require less observer experience. Unlimited-radius counts with distance estimation allow more sophisticated density modeling.

8.2. Survey Timing

- Conduct surveys during the breeding season (mid-March through mid-June in southern California) when singing activity is highest
- Begin surveys at local sunrise and complete all counts within 4 hours of sunrise. Detection rates decline substantially after this window.
- Avoid surveying during rain, sustained wind above 20 mph, or dense fog, as these conditions reduce detectability and violate the assumption of constant detection probability across surveys
- Record weather conditions (wind speed on the Beaufort scale, cloud cover, temperature, precipitation) at the start and end of each survey

8.3. Conducting a Point Count

1. Arrive at the point quietly. Wait 1–2 minutes for birds disturbed by your approach to resume normal activity.
2. Start the timer. The standard count duration is:

- **5 minutes** for fixed-radius counts or BBS-compatible surveys
- **10 minutes** for unlimited-radius distance sampling (often split into 0–3, 3–5, and 5–10 minute intervals to model detection over time)

3. Stand still and record every bird detected within the count period:
   - Species (use the 4-letter AOU alpha code, e.g., HOFI for House Finch, CALT for California Towhee)
   - Number of individuals
   - Detection type: Singing (S), Calling (C), Visual (V), Flyover (F), Drumming (D)
   - Distance band or estimated distance:
     - Fixed-radius: Within 50 m / Beyond 50 m
     - Unlimited-radius: Estimate distance to the nearest 5 m (or use bands: 0–25, 25–50, 50–100, 100–200, >200 m)
   - Direction (compass bearing, optional but useful for distance sampling)
   - Time interval (0–3, 3–5, 5–10 min, if splitting the count)
4. Record flyovers separately. Birds flying over the point without landing or interacting with the habitat are recorded but flagged as flyovers and excluded from density calculations.
5. At the end of the count period, stop recording. Do not add birds detected after the timer ends.

Identification by Sound

The majority of detections during breeding season are auditory (songs and calls). Invest time learning bird songs before the survey season. The Merlin Bird ID app (Cornell Lab of Ornithology) can assist with real-time song identification in the field, but do not rely on it exclusively.

8.4. Between Points

1. Walk to the next point. Record any notable observations along the way as incidental sightings (separate from point count data).
2. Allow a 1–2 minute settling period before starting the next count.

8.5. Repeat Visits

For occupancy modeling or trend analysis, repeat the survey at each point at least 2–3 times during the breeding season, spaced at least 10 days apart. Record the date of each visit.

9. Quality Control

- Each observer should demonstrate proficiency by passing a field identification test (greater than 90% correct identification of common species by sound and sight) before conducting surveys independently

- On 10% of surveys, a second observer independently records detections at the same point to assess inter-observer agreement
- Record the observer's name on every data sheet; observer identity is a variable in analysis
- Flag any uncertain identifications on the data sheet ("?" after the species code) for later review
- If an audio recorder is used, archive recordings for at least one season to allow verification

10. Data Management

1. Enter field data into the project spreadsheet or database within 48 hours
2. Each row represents one detection: point ID, date, time interval, species code, count, detection type, distance, flyover flag
3. Reconcile uncertain identifications using audio recordings or field notes
4. Archive field data sheets (scan if paper)
5. Version-control all analysis scripts per SOP-L801
6. For analyses requiring detection probability correction, use the `unmarked` R package (Fiske & Chandler, 2011) for distance sampling or N-mixture models

11. References

See the project-level `references.bib` for full citations. Key references: Ralph et al. (1993), Ralph et al. (1995), Bibby et al. (2000), Fiske & Chandler (2011).

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12. Training Record

Before conducting bird point counts independently, personnel must:

1. Read this SOP and SOP-F001 Field Safety
2. Demonstrate identification of at least 90% of common species (by sight and sound) at the study site during a supervised field test
3. Participate in at least two supervised point count survey sessions

4. Demonstrate proper data recording (species codes, distance estimation, detection types)
5. Be signed off by the PI or field lead

13. Appendix A: Point Count Field Data Sheet

Print and carry in the field.

Observer: _____ **Date:** _____ **Start time:** _____

Weather: Wind (Beaufort): _____ Cloud cover: _____% Temp: _____ C Precip: Y / N

Point ID	Time Start	Species Code	Count	Detection (S/C/V/F/D)	Distance (m)	Fly-over?	Notes

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F620

Camera Trap Deployment and AI-Assisted Image Analysis

Standard Operating Procedure

SOP Number	SOP-F620
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the deployment, retrieval, data management, and AI-assisted image analysis of camera traps (motion-triggered wildlife cameras) used in the EA Program. Camera traps

provide a non-invasive method for documenting wildlife presence, activity patterns, and species richness at field sites (Thornton et al., 2026). The volume of images produced by even a small number of cameras (thousands to tens of thousands per deployment) makes manual review a major bottleneck. This SOP integrates open-source and cloud-based AI tools to automate blank-image filtering and species identification, reducing processing time by an order of magnitude while maintaining scientific rigor through human-in-the-loop verification (Beery et al., 2019; Wildlife Insights, 2026).

The EA Program currently uses the CamPark T20 trail camera, but the deployment and analysis procedures are applicable to other camera trap models with minor adaptations.

2. Scope

- **Applies to:** Deployment and image analysis of all EA Program camera traps at field sites, including the Bernard Field Station, Pomona College Farm, the Pit, and restoration areas
- **Does not apply to:** Live-view video surveillance systems; drone imagery (see SOP-F630); 360-degree cameras; aquatic camera systems
- **Location:** All EA Program field sites (deployment); EA Program laboratory (data analysis)

3. Responsibilities

Role	Responsibility
Lab PI	Approve deployment plan, oversee data management
Field Lead	Select camera locations, coordinate deployment and retrieval
Trained Personnel	Deploy/retrieve cameras, process images
New Students	Complete training before independent deployment

4. Definitions

- **AI confidence score:** A numerical value (0–1 or 0–100%) output by a classification model indicating the model's certainty in its predicted species identification; predictions below 90% confidence must be manually verified in the EA Program workflow (Beery et al., 2019).
- **Blank image:** A camera trap image with no animal, person, or vehicle visible, typically triggered by vegetation movement or lighting changes; MegaDetector removes these automatically, eliminating the largest portion of review burden.
- **CamPark T20:** The EA Program's primary trail camera; a 16 MP passive infrared (PIR) triggered camera with infrared night flash; detection range approximately 20 m; 8 AA batteries per deployment (CamPark, 2023).
- **Detection rate:** The number of independent detections of a species per 100 camera-trap-nights; the standard metric for comparing wildlife activity across sites or seasons; depends on both actual animal abundance and camera placement (O'Brien et al., 2010).

- **Human-in-the-loop verification:** A quality control protocol in which a human reviewer examines AI predictions, particularly low-confidence ones, to correct errors before results are used for analysis; essential because AI models fail on rare species, unusual poses, and taxa underrepresented in training data (Thornton et al., 2026).
- **Independent detection event:** A single occurrence of a species at a camera during a defined time window (typically 30 minutes); used to prevent over-counting multiple images of the same individual; defined a priori and reported with any analysis (O'Brien et al., 2010).
- **MegaDetector:** An open-source object detection model (Microsoft/Google) that classifies each camera trap image as containing an animal, a human, a vehicle, or being blank; runs as a pre-processing step before species classification; the current version is MegaDetector v5 (Beery et al., 2019).
- **PIR (passive infrared) sensor:** The motion and heat detection sensor in a trail camera; triggers image capture when it detects a warm moving object within the detection zone; sensitivity and detection range vary with temperature, wind, and vegetation.
- **SpeciesNet:** A deep-learning species classification model developed by Google; trained on over 65 million labeled camera trap images from more than 2,000 species and blank categories; used in both the Wildlife Insights platform and the local CameraTrapAI pipeline (Google Research, 2026; Wildlife Insights, 2026).
- **Trap night (camera trap night):** One camera deployed at one location for one 24-hour period; the standard unit of sampling effort; total trap nights = number of cameras × number of days deployed (O'Brien et al., 2010).
- **Wildlife Insights:** A free cloud-based platform for camera trap data management and AI classification, developed by Google, WWF, Conservation International, the Wildlife Conservation Society, and the Smithsonian (Wildlife Insights, 2026).

5. Safety and Hazards

⚠ Hazards

- **Biological:** Tick and insect exposure, poison oak, snakes. Wildlife encounters when checking cameras in remote locations.
- **Physical:** Uneven terrain, climbing to mount cameras on trees or posts.
- **Security:** Cameras left unattended in public areas may be stolen or vandalized.

5.1. Required PPE

☐ Closed-toed shoes or field boots
☐ Long pants (tick and poison oak protection)
☐ Sun protection (hat, sunscreen)
☐ Tick repellent

Refer to SOP-F001 Field Safety. Cameras may inadvertently photograph people; consult the PI regarding institutional requirements for signage and data handling, and delete identifiable images immediately during initial screening.

6. Equipment and Materials

Item	Specifications
CamPark T20 trail camera	16 MP; detection range 20 m; IR night flash (CamPark, 2023)
SD card (Class 10, up to 32 GB)	One per camera
AA batteries (8 per camera)	Lithium recommended for extremes
Mounting strap or bracket	Included with camera
Cable lock (optional)	Theft deterrent for public-access sites
GPS unit or smartphone	Per SOP-F006
Field data sheet	Printed from Appendix A
Pruning shears	For clearing detection zone
Compass	For recording camera bearing
SD card reader (USB)	For lab image download
External hard drive or cloud	For archiving raw images

7. Procedure – Field Deployment

7.1. Camera Setup

1. Install 8 AA batteries and insert a formatted SD card (CamPark, 2023).
2. Power on and enter the settings menu. Configure:

Setting	Recommended Value	Notes
Photo resolution	16 MP	Highest available
Trigger mode	Photo (burst × 3)	Burst increases detection probability
Trigger interval	5 seconds	Balance captures and battery
Time stamp	ON	Required for activity analysis
Date/time	Current local time	Verify at each deployment

3. Take a test photo to verify function and timestamp.

7.2. Site Selection and Mounting

1. Select a location based on study objectives (game trails, water sources, habitat edges, or systematic grid points).
2. Record GPS coordinates and camera bearing (compass direction camera faces) (O'Brien et al., 2010).
3. Clear vegetation within the detection zone (approximately 20 m cone in front of the lens) to reduce false triggers.
4. Mount at 30–50 cm above ground for small-to-medium mammals, or 75–100 cm for larger species; angle slightly downward (5–10°).

5. Secure with mounting strap. Apply a cable lock if at a public site.
6. Walk through the detection zone to verify the trigger functions.
7. Record deployment metadata on the field data sheet (Appendix A).

7.3. Retrieval and Redeployment

1. Power off before removing the SD card.
2. Replace with a fresh, formatted card; check battery level; replace batteries if below 50%.
3. Power on, verify settings, and take a test photo.
4. Record retrieval date, time, and observations on the field data sheet.

8. Procedure – Data Management and AI Analysis

8.1. Image Download and Organization

1. Create a folder: `CamTrap_SiteID_CameraID_YYYYMMDD-YYYYMMDD`.
2. Copy all images from the SD card.
3. Back up immediately to external hard drive or cloud storage.
4. Do not delete images from the SD card until the backup is verified.

8.2. AI-Assisted Analysis Pipeline

The EA Program uses a two-stage AI pipeline following the approach used by Wildlife Insights and the Google CameraTrapAI project (Google Research, 2026; Wildlife Insights, 2026):

Stage 1: Detection (MegaDetector). Identifies whether each image contains an animal, human, vehicle, or is blank; eliminates 50–90% of false-trigger images (Beery et al., 2019).

Stage 2: Classification (SpeciesNet or similar). Identifies detected animals to the lowest possible taxonomic level, trained on over 65 million labeled camera trap images.

Option A: Wildlife Insights (cloud-based). Upload images to wildlifeinsights.org, which runs both stages automatically (Wildlife Insights, 2026). Review AI predictions and export verified data as CSV.

Option B: Local pipeline (Python). For offline or large-volume processing, use the open-source CameraTrapAI package from github.com/google/cameratrapai (Beery et al., 2019; Google Research, 2026).

8.3. Human Verification

1. Sort AI output by confidence score (ascending).
2. Manually verify all predictions below 90% confidence (Thornton et al., 2026).
3. Spot-check a random 10% of predictions above 90% confidence.
4. Correct misclassifications and record reviewer name, date, and total corrections.

8.4. Data Analysis in R

```r
library(tidyverse)
library(lubridate)
library(camtrapR)   # Niedballa et al. 2016

detections <- read_csv("classifications_verified.csv") %>%
  mutate(datetime = ymd_hms(timestamp),
         date = as_date(datetime),
         hour = hour(datetime))

# Independent events (30-min threshold)
independent <- detections %>%
  filter(category == "animal") %>%
  arrange(camera_id, species, datetime) %>%
  group_by(camera_id, species) %>%
  mutate(
    time_diff_min = as.numeric(difftime(datetime, lag(datetime),
                                        units = "mins")),
    independent = is.na(time_diff_min) | time_diff_min >= 30
  ) %>%
  filter(independent) %>%
  ungroup()

# Trap-nights per camera
trap_nights <- detections %>%
  distinct(camera_id, date) %>%
  count(camera_id, name = "trap_nights")

# Detection rate (events per 100 trap-nights)
det_rate <- independent %>%
  count(camera_id, species) %>%
  left_join(trap_nights, by = "camera_id") %>%
  mutate(rate_per_100tn = (n / trap_nights) * 100)

print(det_rate)
```

(Niedballa et al., 2016)

9. Quality Control

- Test-fire each camera before leaving the deployment site.
- Record all deployment metadata on the field data sheet.
- Check cameras every 2–4 weeks to replace batteries and SD cards.
- Report the AI model version, confidence threshold, verification protocol, and correction rate in any publication (Thornton et al., 2026).

10. References

- CamPark (2023)
- Beery et al. (2019)
- Wildlife Insights (2026)
- Google Research (2026)
- Thornton et al. (2026)
- Niedballa et al. (2016)
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Google Research. (2026). *Where wild things roam: Identifying wildlife with SpeciesNet*. https://research.google/blog/where-wild-things-roam-identifying-wildlife-with-speciesnet/

Niedballa, J., Sollmann, R., Courtiol, A., & Wilting, A. (2016). camtrapR: An R package for efficient camera trap data management. *Methods in Ecology and Evolution*, *7*(12), 1457–1462. https://doi.org/10.1111/2041-210X.12600

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Thornton, S. A. et al. (2026). Identification of camera trap images by artificial intelligence and human experts produces similar multi-species occupancy models. *Journal of Applied Ecology*. https://doi.org/10.1111/1365-2664.14862

Wildlife Insights. (2026). *Wildlife insights: About AI*. https://www.wildlifeinsights.org/about-wildlife-insights-ai

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Camera deployment coordinates
F602	iNaturalist	Photo-based species documentation
F630	Drone Operations	Complementary remote sensing
L801	RStudio and GitHub	Data version control

12. Training Record

Before deploying camera traps independently, personnel must:

1. Read this SOP and SOP-F001 in full

2. Demonstrate camera setup under supervision
3. Deploy and retrieve at least one camera with proper metadata recording
4. Process one SD card through the full AI pipeline (download, MegaDetector, SpeciesNet or Wildlife Insights, verification)
5. Produce a verified species list with activity pattern summary
6. Be signed off by the PI or field lead

13. Appendix A: Field Data Sheet

Field	Deployment	Retrieval
Date		
Time		
Site ID		–
Camera serial no.		–
SD card no.		
GPS coordinates		–
Camera bearing (°)		–
Camera height (cm)		–
Batteries	Fresh	_____ %
Test photo taken	Y / N	–
Notes		

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F630

Drone Operations (DJI Mavic and Matrice UAVs)

Standard Operating Procedure

SOP Number	SOP-F630
Revision	1.1
Author(s)	Liz Johnson, Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP details the procedures for safely operating the EA Program's DJI unmanned aerial vehicles (UAVs/drones) for research image collection (DJI, 2015, 2023). It covers pre-flight setup, flight operations, image collection, post-flight procedures, and data transfer. Images obtained from these aircraft are used for photogrammetry (3D modeling), site monitoring, vegetation mapping, and other remote sensing applications.

2. Scope

- **Applies to:** All flights of EA Program drones, including the DJI Mavic 3 Pro and DJI Matrice 100, for research and instructional purposes
- **Does not apply to:** Indoor flights; flights conducted by contracted third-party operators; processing of drone imagery beyond data transfer (see future SOP-L805)
- **Location:** All EA Program field sites and approved flying areas

3. Definitions

- **AGL (Above Ground Level):** Flight altitude measured from the ground surface directly below the aircraft, not from mean sea level; FAA Part 107 limits sUAS to 400 feet AGL (approximately 122 m) in uncontrolled airspace (Federal Aviation Administration, 2024b).
- **Compass calibration:** A pre-flight procedure that orients the drone's internal compass to the local magnetic field; required at each new site or after transporting the aircraft; performed by rotating the aircraft in specific orientations per the DJI app prompts (DJI, 2023).
- **DJI Mavic 3 Pro:** A compact foldable quadcopter with a three-camera Hasselblad system; the EA Program's primary survey aircraft stored in SGM 106; flight time approximately 43 minutes; maximum wind resistance 12 m/s (Beaufort 6) (DJI, 2023).
- **DJI Matrice 100:** A legacy quadcopter platform capable of carrying a research payload of up to 1 kg; stored in SGM 210; suited for multi-sensor work; requires the DJI Pilot app for operation (DJI, 2015).
- **Gimbal:** The motorized, stabilized mount that holds the camera; compensates for drone movement to produce level, stable imagery; always reinstall the gimbal guard during transport.
- **LAANC (Low Altitude Authorization and Notification Capability):** An FAA system that provides near-real-time airspace authorization for UAS operations in controlled airspace (Classes B, C, D, and E surface areas); authorization is obtained through the B4UFLY or Aloft apps before each flight in controlled airspace (Federal Aviation Administration, 2024a, 2024b).
- **LiPo battery (lithium polymer battery):** The rechargeable battery used in DJI drones; stores significant energy and poses a fire hazard if punctured, short-circuited, or charged incorrectly; must be stored at 40–60% charge for long-term storage and kept away from flammable materials.
- **Nadir angle:** A camera gimbal angle of -90°, pointing the camera directly downward toward the ground surface; the standard angle for photogrammetric mapping and orthomosaic generation.

- **Part 107:** Title 14 CFR Part 107 of the Code of Federal Regulations, governing Small Unmanned Aircraft Systems; requires a Remote Pilot Certificate for commercial and research operations; specifies operating limitations including maximum altitude, VLOS requirement, and prohibited airspace (Federal Aviation Administration, 2024b).
- **Photogrammetry:** The science of making measurements from photographs; in the drone context, software such as Reality Capture or Pix4D processes overlapping aerial images to produce 3D models, dense point clouds, digital surface models (DSMs), and georeferenced orthomosaics (Capturing Reality, 2024).
- **RTH (Return to Home):** An automated failsafe function that returns the drone to its recorded home point (takeoff location) if the controller signal is lost or the battery reaches the RTH threshold; the home point must be set before each flight.
- **sUAS (small Unmanned Aircraft System):** FAA classification for unmanned aircraft weighing less than 55 pounds (25 kg); both EA Program drones qualify as sUAS (Federal Aviation Administration, 2024b).
- **TRUST (The Recreational UAS Safety Test):** An FAA-mandated online safety test for recreational flyers; required when operating under the Exception for Limited Recreational Operations (44809) rather than Part 107.
- **UAS (Unmanned Aircraft System):** The complete system including the drone, remote controller, and associated software.
- **VLOS (Visual Line of Sight):** FAA requirement that the Remote Pilot maintain unaided visual contact with the aircraft at all times during flight; flight beyond VLOS requires a Part 107 waiver (Federal Aviation Administration, 2024b).

4. Responsibilities

Role	Responsibility
PI	Approve flight plans; ensure FAA compliance
Certified Pilot	Hold FAA Part 107 certificate; conduct flights
Visual Observer	Maintain visual contact with the drone; alert pilot to hazards
New Students	Complete training before any drone operation

5. Safety and Hazards

5.1. Critical Safety Rules

- Never fly over people who are not directly participating in the operation
- Never fly beyond visual line of sight (VLOS)
- Never fly near other aircraft or in restricted airspace without authorization
- Never touch spinning propellers
- Never fly under the influence of alcohol or drugs

5.2. Hazards

- **Physical:** Spinning propellers can cause lacerations. A drone falling from altitude can cause blunt force injury. LiPo batteries may be hot after flight.
- **Regulatory:** Unauthorized flights can result in FAA fines and criminal penalties (Federal Aviation Administration, 2024b).
- **Property:** Crashes can damage the drone (replacement cost several thousand dollars) and property below.
- **Environmental:** Drones can disturb nesting birds and wildlife. Avoid flying near known nesting sites during breeding season.

5.3. Required PPE

☐ Safety glasses (recommended during takeoff and landing)
☐ Sun protection (hat, sunscreen)
☐ Closed-toe shoes

5.4. Emergency Procedures

If the drone loses connection, activate RTH immediately. If RTH fails, allow the drone to execute its failsafe. Do not attempt to catch a falling drone. Refer to SOP-F001 Field Safety.

6. FAA Regulations

> **!** Legal Requirement
>
> All drone flights must comply with current FAA regulations. The pilot is personally responsible for compliance (Federal Aviation Administration, 2024b).

Research operations require FAA Part 107 Remote Pilot certification. Flights in controlled airspace (near airports) require prior LAANC authorization via the B4UFLY or Aloft apps (Federal Aviation Administration, 2024a). Pomona College campus is within Ontario International Airport (KONT) Class C airspace; LAANC authorization is required for on-campus flights. Both EA Program drones are registered with the FAA; registration numbers are on file with the PI and must be displayed on the aircraft at all times.

6.1. Flight Restrictions under Part 107

- Maximum altitude: 400 feet AGL (Federal Aviation Administration, 2024b)
- Daylight operations only (civil twilight with anti-collision lighting permissible with appropriate equipment)
- Visual line of sight at all times
- No flights over non-participating persons
- Yield right of way to manned aircraft at all times

7. EA Program Fleet

Aircraft	Serial Number	Storage	Notes
DJI Mavic 3 Pro	*TBD*	SGM 106	Primary survey aircraft
DJI Matrice 100	M02DF 19020136	SGM 210	Legacy; multi-sensor
Mavic 3 batteries	*TBD*	SGM 106	3 batteries
Matrice 100 batteries	*TBD*	SGM 210	6 batteries

8. Required Materials

8.1. Pre-Flight Checklist

Item	Check
Drone (inspected, propellers attached)	[ ]
Remote controller (charged)	[ ]
Batteries (charged, minimum 3)	[ ]
Mobile device with DJI app (charged)	[ ]
MicroSD card (formatted, > 2 GB free)	[ ]
FAA Part 107 certificate (pilot)	[ ]
FAA registration number on aircraft	[ ]
LAANC authorization (if controlled airspace)	[ ]
UAV Forecast app check (weather, KP index)	[ ]
Flight plan (altitude determined)	[ ]
Notebook and pen (for flight log)	[ ]

(DJI, 2023; Federal Aviation Administration, 2024b)

8.2. Software

Application	Purpose
DJI Fly or DJI Pilot	Flight control, camera settings, flight planning (DJI, 2015, 2023)
UAV Forecast	Weather, wind, KP index, GPS satellite count
B4UFLY or Aloft	Airspace authorization (LAANC) (Federal Aviation Administration, 2024a)
Reality Capture or Pix4D	Photogrammetry post-processing (Capturing Reality, 2024)

9. Procedure

9.1. Pre-Flight Inspection

1. Inspect the airframe: no cracks, loose propellers, or debris in motors.
2. Charge all batteries to 100% before departure.
3. Check the DJI app for firmware updates; update if prompted (DJI, 2023).

9.2. Pre-Flight Checks at the Site

1. Perform compass calibration per the DJI app (DJI, 2023).
2. Check airspace: open B4UFLY or Aloft and verify authorization (Federal Aviation Administration, 2024a).
3. Record weather conditions in the flight log.

9.3. Flight Operations

1. Announce "Taking off" to all personnel.
2. Hover at 3–5 m to verify stable flight, GPS lock, and camera orientation.
3. Execute the flight plan:
    - For photogrammetry: use automated waypoint or grid mode with 70–80% front overlap and 60–70% side overlap (Capturing Reality, 2024).
    - For manual survey: fly in a systematic pattern at the planned altitude.
4. Monitor battery: begin return at 30%; land immediately at 20%.
5. Announce "Landing" before descent.

9.4. Image Collection Settings

Setting	Recommended Value
Image format	JPEG + RAW (DNG) for photogrammetry
Photo interval	2 seconds (timed shot for grid plans)
Resolution	Maximum available
Gimbal angle	Nadir (-90°) for mapping; oblique (-45°) for 3D models

9.5. Post-Flight Procedures

1. Power off the drone and remote controller.
2. Replace the gimbal cover.
3. Remove the MicroSD card and transfer images:
    - Create a folder: `Drone_SiteID_YYYYMMDD_AltitudeAGL`
    - Copy all images; verify file count
4. Format the MicroSD card after confirming backup.

5. Return all equipment to storage (Mavic to SGM 106; Matrice to SGM 210).
6. Complete the flight log: aircraft, date, location, pilot, duration, battery cycles used, any incidents.

10. Quality Control

- Complete the pre-flight checklist before every flight.
- Verify GPS lock (minimum 8 satellites) before takeoff.
- After each flight, review a sample of images to confirm coverage, focus, and exposure.
- Report any incidents (crashes, near-misses, property damage) to the PI within 24 hours. FAA reporting may be required for incidents involving injury or property damage exceeding $500 (Federal Aviation Administration, 2024b).

11. References

- DJI (2023)
- DJI (2015)
- Federal Aviation Administration (2024b)
- Federal Aviation Administration (2024a)
- Capturing Reality (2024)

Capturing Reality. (2024). *RealityCapture documentation*. https://www.capturingreality.com/realitycapture

DJI. (2015). *DJI Matrice 100 user manual*. https://www.dji.com/matrice100/downloads

DJI. (2023). *DJI Mavic 3 Pro user manual*. https://www.dji.com/mavic-3-pro/downloads

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Federal Aviation Administration. (2024b). *Small unmanned aircraft systems* (14 CFR Part 107). Federal Aviation Administration. https://www.ecfr.gov/current/title-14/chapter-I/subchapter-F/part-107

12. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F006	GPS and Site Navigation	Flight site coordinates
F620	Camera Traps	Complementary wildlife monitoring
L805	Drone Photogrammetry	Post-processing workflow

13. Training Record

Before operating any EA Program drone independently, personnel must:

1. Read this SOP and SOP-F001 in full
2. Hold a current FAA Part 107 Remote Pilot certificate, or have passed TRUST (for recreational operations under direct PI supervision) (Federal Aviation Administration, 2024b)
3. Complete the DJI app tutorial and demonstrate proficiency with simulator mode (DJI, 2023)
4. Demonstrate pre-flight inspection, calibration, takeoff, hover, controlled flight pattern, and landing under supervision
5. Complete at least one supervised photogrammetry data collection flight with acceptable image quality
6. Be signed off by the PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F700

eDNA Field Sampling and Preservation

Standard Operating Procedure

SOP Number	SOP-F700
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes field procedures for collecting, filtering, and preserving environmental DNA (eDNA) samples from surface waters. eDNA consists of DNA shed into the environment by organisms through skin cells, mucus, feces, gametes, and decomposing tissue. Detecting eDNA in

water samples allows non-invasive detection of aquatic species (fish, amphibians, invertebrates, pathogens) without direct observation or capture (Goldberg et al., 2016). Because eDNA is present at extremely low concentrations, preventing contamination during field collection is critical. This SOP covers the field side of the workflow; laboratory processing (filtration, extraction, inhibition testing) is covered in SOP-L700 DNA Extraction.

2. Scope

- **Applies to:** Collection and preservation of water samples for eDNA analysis at all EA Program surface water sites (streams, lakes, ponds, wetlands, stormwater basins)
- **Does not apply to:** Soil eDNA or soil microbial sampling (see SOP-F701); tissue biopsy or organism capture for DNA; airborne eDNA sampling; lab-side filtration, extraction, and molecular analysis
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design; ensure permits are in place
Trained Personnel	Collect and preserve eDNA samples per this SOP
New Students before participating in eDNA sampling	Complete training (including contamination awareness)

4. Definitions

- **Contamination (eDNA):** The introduction of exogenous DNA from sources other than the target organism into a sample or filter; the single largest source of false positives in eDNA surveys; prevented by sterile technique, glove changes between samples, and decontamination of reusable equipment (Goldberg et al., 2016).
- **eDNA (environmental DNA):** Genetic material shed into the environment by organisms through mucus, feces, gametes, skin cells, and other biological material; collected from water without direct capture of the target organism; detectable at extremely low concentrations using PCR-based methods (Goldberg et al., 2016).
- **Equipment blank:** A control sample consisting of deionized water rinsed through decontaminated reusable equipment (e.g., a pump) and collected as a sample; detects carry-over contamination between sites when dedicated equipment is not used.
- **Field blank:** A sterile bottle of molecular-grade deionized water opened and handled at the field site under identical conditions to real samples; filtered and preserved like a field sample; detects airborne or handling contamination during field work; must yield no target amplification (Goldberg et al., 2016).

- **Filter pore size:** The nominal diameter of pores in the filter membrane; 0.45 µm is the standard for eDNA capture from surface water, retaining bacteria, cells, and particulate-bound DNA.
- **Longmire's buffer:** A non-freezing preservation solution (containing EDTA, Tris, NaCl, SDS, and sucrose) used to preserve eDNA on filters in the field when immediate freezing is not possible; inactivates nucleases and inhibits microbial degradation (Renshaw et al., 2015).
- **Open-channel filtration:** A filtration method using a funnel and vacuum pump or syringe; the filter membrane is exposed to the environment during collection, increasing contamination risk compared to closed-capsule methods.
- **PCR inhibitor:** A substance co-extracted with eDNA that suppresses PCR amplification efficiency; common in natural water samples (humic acids, tannins, metal ions); detected by running a dilution series of the extract and checking for concentration-dependent amplification; managed by diluting the extract or using commercial inhibitor removal kits.
- **Sterivex filter:** A commercially available enclosed 0.22 µm polysulfone cartridge filter; preferred for eDNA because the sealed design minimizes contamination, allows direct addition of preservation buffer, and is compatible with standard extraction kits (Renshaw et al., 2015).
- **Target species detection:** The determination of presence or absence of a specific taxon in a water sample based on amplification of taxon-specific eDNA; sensitivity depends on eDNA shedding rate, water volume filtered, PCR method, and inhibitor load in the extract (Goldberg et al., 2016).
- **Volume filtered:** The total volume of water passed through a filter during sample collection; a required reporting variable for calculating eDNA concentration; record for every filter regardless of whether filtration was complete or the filter clogged (Goldberg et al., 2016).

5. Safety and Hazards

⚠ Hazards

- **Physical:** Wading and sampling near water carry slip, fall, and drowning hazards. Work in pairs.
- **Chemical:** DNA preservatives (ethanol, Longmire's buffer) are flammable (ethanol) or contain EDTA and SDS (Longmire's). Handle with gloves.
- **Biological:** Water may contain pathogens. Wear gloves and avoid ingestion.

5.1. Required PPE

☐ Nitrile gloves (change between every sample)
☐ Safety glasses
☐ Wading boots or waders (if entering the water)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety.

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Sterile 1 L HDPE sample bottles	One per sample; single-use
60 mL sterile syringes	For Sterivex filtration
Sterivex 0.22 µm filter capsules	Preferred; one per sample
0.45 µm PES open filter funnels	Alternative to Sterivex
Longmire's buffer (1–2 mL per filter)	For filter preservation without freezing (Renshaw et al., 2015)
Molecular-grade ethanol (95%)	Alternative preservative for Sterivex
Molecular-grade deionized water	For field blanks (1 bottle per event)
Cooler with ice packs (4 °C)	For sample transport if not filtering on-site

7. Procedure

7.1. Pre-Field Preparation

1. Prepare all equipment in the lab using UV-decontaminated surfaces and dedicated pre-PCR equipment.
2. Pre-label all bottles and filters with project ID, site, replicate number, date.
3. Pack a cooler with ice packs or dry ice.
4. Include at least one sterile bottle of molecular-grade DI water per sampling event for field blanks.

7.2. Water Collection

1. Put on fresh nitrile gloves.
2. Open the sterile bottle only when ready to collect.
3. Submerge the bottle to 10–20 cm below the water surface, facing upstream, away from your body (Goldberg et al., 2016).
4. Fill to 1 L and cap immediately.
5. For composite samples: collect 250 mL from each of 4 microhabitats and combine in one bottle.
6. Record the GPS coordinates, collection time, and site conditions.

7.3. Field Filtration (If Filtering On-Site)

Sterivex capsule method:

1. Attach a sterile 60 mL syringe to the Sterivex filter inlet.
2. Draw water from the sample bottle and push through the filter. Repeat until the filter clogs or 1 L has been filtered. Record the total volume filtered.

3. Expel remaining water from the capsule.
4. Add 1–2 mL of Longmire's buffer or fill with molecular-grade ethanol using a syringe (Renshaw et al., 2015). Cap both ends of the Sterivex.
5. Label the capsule and place in a ziplock bag on ice.

Open filter funnel method:

1. Pour the sample through a sterile 0.45 µm PES filter unit.
2. Use sterile forceps to fold the filter membrane in half (sample side inward) and place in a labeled cryovial.
3. Add preservative (ethanol or Longmire's buffer) to cover the filter. Seal.
4. Place on ice immediately.

7.4. If Not Filtering On-Site

Keep whole-water samples on ice (4 °C) and filter in the lab within 6 hours of collection. Exceeding 6 hours significantly increases DNA degradation (Goldberg et al., 2016).

7.5. Field Blanks and Controls

- **Field blank:** Open the sterile DI water bottle at one site per event, filter it using the same method as field samples, and preserve. This blank detects contamination during field handling.
- **Equipment blank:** If any reusable equipment contacts the sample, collect a deionized water rinse blank after decontamination.
- Process blanks through the entire lab workflow alongside samples.

7.6. Decontamination Between Sites

1. Spray or soak all reusable equipment with 10% bleach solution for at least 1 minute.
2. Rinse with clean water.
3. Change gloves.
4. Single-use items (bottles, filters, syringes) are discarded after each sample.

See SOP-F007 for general decontamination procedures.

8. Sample Transport and Storage

1. Keep all samples and preserved filters on ice (4 °C) during transport.
2. Deliver to the lab the same day. If overnight storage is unavoidable, store at -20 °C.
3. For long-term storage (> 1 week before extraction), store preserved filters at -20 °C or -80 °C.
4. Complete the chain of custody form per SOP-F008.

9. Quality Control

- Minimum 1 field blank per sampling event; 1 equipment blank if reusable equipment is used.
- Collect 3 replicate samples per site unless the study design specifies otherwise.
- Record the total volume filtered for each sample.
- Any field blank positive for target DNA invalidates samples from that event unless below a pre-established threshold. Consult the PI.
- Log all glove changes, bottle openings, and deviations in the field notebook.

10. References

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, *15*(1), 168–176. https://doi.org/10.1111/1755-0998.12281

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site decon
F008	Sample Labeling and COC	Sample ID and chain of custody
F308	eDNA Water Collection	Habitat-specific collection strategies
F701	Soil Microbial Sampling	Companion soil DNA protocol
L700	DNA Extraction	Lab-side extraction

12. Training Record

Before collecting eDNA samples independently, personnel must:

1. Read this SOP, SOP-F007, and SOP-L700 in full
2. Demonstrate proper sterile technique (glove changes, bottle handling, filter preservation) during a supervised practice session
3. Successfully process a field blank with a negative result
4. Be signed off by the PI or field lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-F701

Soil Microbial Field Sampling for Molecular Analysis

Standard Operating Procedure

SOP Number	SOP-F701
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes field procedures for collecting soil samples intended for molecular analysis, including DNA extraction, PCR/ddPCR, amplicon sequencing, and metagenomics. Soil microbial communities are sensitive to temperature, moisture, and oxygen exposure, and nucleic acids degrade rapidly in the absence of proper preservation. The procedures in this SOP are designed to minimize DNA and RNA degradation from the moment of collection through transport to the laboratory,

ensuring that molecular results accurately reflect the in situ microbial community (Frosteg aard et al., 1999; Knight et al., 2018).

This SOP supplements the general soil sampling procedures in SOP-F400 with additional precautions specific to molecular work.

2. Scope

- **Applies to:** Field collection of soil and rhizosphere samples for DNA extraction (SOP-L700), ddPCR (SOP-L701), amplicon sequencing, metagenomics, and other molecular analyses
- **Does not apply to:** Soil sampling for chemical or physical analysis only (see SOP-F400); water eDNA sampling (see SOP-F700); laboratory-side DNA extraction and processing
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design; specify target depth and replication
Trained Personnel	Collect and preserve samples per this SOP
New Students before participating in molecular sampling	Complete training (including contamination awareness)

4. Definitions

- **Contamination (molecular soil sampling):** Introduction of exogenous DNA from other samples, the environment, or the researcher's own microbiome into a soil sample; prevented by sterile tool sterilization between samples, frequent glove changes, and use of field blanks (Frosteg aard et al., 1999).
- **Cryogenic freezing (flash-freezing):** Rapid cooling of a soil sample in liquid nitrogen (-196 °C) or on dry ice (-78 °C) immediately after collection; arrests microbial metabolism and nuclease activity; required for RNA work and recommended for optimal DNA preservation (Knight et al., 2018).
- **Dry ice (solid CO_2):** Used for field flash-freezing at -78 °C; causes frostbite on direct skin contact; must be handled with insulated cryogenic gloves and transported in a vented or insulated container.
- **Field blank:** An empty sterile tube or bag opened and closed at the field site during sampling; processed through the full extraction and PCR workflow in the lab; detects airborne or handling contamination; any positive amplification in a field blank invalidates the associated samples unless concentrations fall below a pre-established threshold (Frosteg aard et al., 1999).
- **Flash-freezing:** See cryogenic freezing.

- **LifeGuard Soil Preservation Solution (Qiagen):** A commercially prepared stabilization reagent that arrests nucleic acid degradation at ambient temperature for up to 7 days; an alternative to cryogenic preservation when dry ice is unavailable; add to the sample immediately after collection, invert gently, and store at room temperature or on ice.
- **Microbial community composition:** The relative abundances of different microbial taxa (bacteria, archaea, fungi) in a soil sample; highly sensitive to sample handling time, temperature, and oxygen exposure; shifts within minutes of soil disturbance (Frosteg aard et al., 1999; Knight et al., 2018).
- **Nuclease:** An enzyme that degrades DNA or RNA; active in soil samples at ambient temperatures; inactivated by low temperature (< 4 °C), chemical preservatives (LifeGuard buffer, ethanol), or rapid freezing.
- **Rhizosphere:** The narrow zone of soil immediately surrounding plant roots; has a distinct microbial community influenced by root exudates; sampled by collecting soil adhering directly to roots after gentle removal from the surrounding bulk soil.
- **Sterile technique:** A set of aseptic practices that prevent contamination of molecular samples; in soil microbial sampling, these include autoclaving or 70% ethanol sterilization of all metal tools, use of single-use sterile tubes and gloves, and minimizing exposure time to air (Frosteg aard et al., 1999).
- **Time from extraction to preservation:** A critical metric for molecular soil samples; ideally less than 2 minutes from the moment soil is removed from the ground to placement in ice, dry ice, or preservative; longer times cause measurable shifts in microbial community composition (Knight et al., 2018).

5. Safety and Hazards

⚠ Hazards

- **Biological:** Soil may contain pathogens. Wash hands thoroughly after handling. Treat all samples as potentially infectious.
- **Physical:** Repetitive strain from augering; standard field hazards (heat, ticks, uneven terrain).
- **Chemical:** Liquid nitrogen causes severe cryogenic burns. Dry ice causes frostbite on direct skin contact. Handle with insulated gloves.

5.1. Required PPE

☐ Nitrile gloves (change between samples)
☐ Closed-toed shoes or field boots
☐ Insulated cryogenic gloves (if using liquid nitrogen or dry ice)
☐ Safety glasses (if using liquid nitrogen)
☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to SOP-F001 Field Safety. For liquid nitrogen or dry ice burns, warm the affected area gently with lukewarm water. Do not rub.

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Soil auger (bucket-type, stainless steel)	Autoclaved or ethanol-sterilized before each event
Stainless steel trowel or spatula	Autoclaved or flame-sterilized
Ethanol (70%) in spray bottle	For field sterilization between samples
Lighter or butane torch	For flame-sterilizing metal tools
DI water squeeze bottle	For rinsing tools after ethanol sterilization

6.2. Sample Containers and Preservation

Item	Notes
Sterile 50 mL Falcon tubes or Whirl-Pak bags	Pre-labeled; one per sample
Cooler with ice packs (4 °C)	Minimum preservation for DNA samples
Dry ice (solid CO_2)	For flash-freezing at -78 °C; preferred for RNA work
Insulated container for dry ice	Styrofoam cooler or dewar
LifeGuard Soil Preservation Solution	Optional; stabilizes DNA and RNA at ambient temperature for up to 7 days
Ziplock bags (gallon size)	For double-bagging frozen samples

7. Procedure

7.1. Pre-Field Preparation

1. Autoclave or ethanol-sterilize all metal sampling tools. Wrap in aluminum foil after sterilization.
2. Pre-label all sample tubes or bags: project ID, site, sample number, date, depth.
3. If using LifeGuard, pre-aliquot approximately 5 mL per tube.
4. Pack the cooler. If using dry ice, load the insulated container.
5. Include at least one field blank tube per sampling event.

7.2. Sampling Procedure

1. Navigate to the sampling location; record GPS coordinates.
2. Put on fresh nitrile gloves.
3. Sterilize the auger or trowel: spray with 70% ethanol, allow 30 seconds air-dry, optionally flame. Rinse with DI water.
4. Clear surface litter without disturbing mineral soil.
5. Collect soil from the target depth (typically 0–15 cm). Avoid large roots and rocks.
6. Using the sterilized trowel, transfer approximately 2–5 g of soil directly into the sterile tube. Minimize handling time; do not homogenize in a bucket.

> **!** Speed Matters
>
> Minimize the time between coring and preservation. The soil should be in the tube and on ice within 2 minutes of extraction. Prolonged exposure to air and ambient temperature causes shifts in microbial community composition and DNA degradation (Frosteg aard et al., 1999; Knight et al., 2018).

7. Immediately preserve:
   - **DNA only:** Place capped tube on ice (4 °C). Transport to lab within 6 hours or freeze at -20 °C.
   - **DNA and RNA:** Flash-freeze on dry ice or liquid nitrogen. Transfer to -80 °C freezer on return.
   - **LifeGuard:** Add 5 mL LifeGuard to the soil, cap, invert 3 times, store at ambient temperature up to 7 days.
8. Record in the field notebook: sample ID, GPS, depth, soil temperature, preservation method, time of collection, and any deviations.

7.3. Decontamination Between Samples

1. Spray tools with 70% ethanol; allow 30 seconds air-dry; optionally flame. Rinse with DI water.
2. Change nitrile gloves.
3. Between sites, decontaminate boots and waders per SOP-F007.

8. Sample Transport and Storage

Preservation Method	Field Storage	Lab Storage
Ice (4 °C)	Cooler, ice packs	Process within 24 h or freeze at -20 °C
Dry ice (-78 °C)	Insulated cooler	Transfer to -80 °C freezer immediately

Preservation Method	Field Storage	Lab Storage
Liquid nitrogen (-196 °C)	Dewar	Transfer to -80 °C freezer immediately
LifeGuard buffer	Ambient, up to 7 days	Extract within 7 days; or freeze at -20 °C

Record storage location in the Freezer Inventory and in the lab notebook per SOP-L106.

9. Quality Control

- Include at least one field blank per sampling event.
- Change gloves and sterilize tools between every sample.
- Record the time elapsed between soil extraction and preservation. Flag any sample with elapsed time > 5 minutes.
- Field blanks positive for target DNA invalidate the batch unless concentrations fall below the established threshold.
- Avoid sampling immediately after rainfall when soils are saturated.

10. References

Frosteg aard, Aa., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F. ccois, Jeannin, P., Nesme, X., & Simonet, P. (1999). Quantification of bias related to the extraction of DNA directly from soils. *Applied and Environmental Microbiology*, *65*(12), 5409–5420. https://doi.org/10.1128/AEM.65.12.5409-5420.1999

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolek, T., McCall, L.-I., McDonald, D., Melnik, A. V., Morton, J. T., Navas, J., Quinn, R. A., Sanders, J. G., Swafford, A. D., Thompson, L. R., Tripathi, A., Xu, Z. Z., … Dorrestein, P. C. (2018). Best practices for analysing microbiomes. *Nature Reviews Microbiology*, *16*(7), 410–422. https://doi.org/10.1038/s41579-018-0029-9

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site decon
F008	Sample Labeling and COC	Sample ID scheme
F400	Soil Sampling	General soil collection
F700	eDNA Field Sampling	Water eDNA companion protocol
L700	DNA Extraction	Lab-side extraction
L701	ddPCR Primers	Downstream analysis

12. Training Record

Before collecting soil samples for molecular analysis independently, personnel must:

1. Read this SOP, SOP-F400, and SOP-L700 in full
2. Demonstrate sterile sampling technique (tool sterilization, glove changes, rapid transfer to preservative) during a supervised practice session
3. Successfully process a field blank with a negative extraction and PCR result
4. Be signed off by the PI or field lead



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L002

General Laboratory Safety Plan

Standard Operating Procedure

SOP Number	SOP-L002
Revision	1.1
Author(s)	EA Students, Fall 2015
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

The objective of this SOP is to create a safe operating environment for all researchers in the EA Program laboratories. It establishes general safety rules, emergency procedures, training requirements, and record-keeping procedures.

This Laboratory Safety Plan is a living document and requires ongoing updating, editing, and expansion as incidents occur and lessons are learned.

2. Scope

- **Applies to:** All staff, students, and researchers working in EA Program laboratories and field sites
- **Does not apply to:** Visitors on supervised tours who do not handle materials or equipment
- **Location:** SGM 133 and all associated EA Program spaces

3. Acknowledgements

3.1. Student Contributions

EA030, Fall 2015: Benjamin Schmidt, Kay Garlick-Ott, Marc Los Huertos, Grace Hruska, Juan Jaramillo, Thomas Kelleher, RaSia Khepra, Alexander Landau, Peter Mellinger, Emelia von Saltza, Alexandra Tchaoucheva, Ki'Amber Thompson, Malone Mischke.

4. Responsibilities

Role	Responsibility
Principal Investigator	Ensure all researchers are trained; approve training records
Lab Manager	Conduct training, verify competency, sign off on forms
Chemical Safety Officer	Maintain SOPs, promote safety culture, conduct periodic reviews
All Researchers	Complete required training, follow all SOPs, report hazards

5. Definitions

Laboratory Manager: A student, staff, or faculty member who has been trained and identified as the laboratory manager. The laboratory manager oversees all training and monitors all activities in the laboratory. The current laboratory manager is Assoc. Professor Marc Los Huertos.

Chemical Safety Officer: Assigned annually; responsible for maintaining SOPs, training researchers, and ensuring safe laboratory conditions (see SOP-L003).

PPE (Personal Protective Equipment): Protective clothing, goggles, gloves, or other garments designed to protect the wearer from injury or infection.

SDS (Safety Data Sheet): A document describing the hazards, safe handling, storage, disposal, and first aid for each chemical used in the lab. The SDS binder is located on the counter next to the east entrance to SGM 133. - **Carcinogen:** A substance capable of causing cancer in living tissue; classified by IARC (Group 1, 2A, 2B) or NTP; requires special handling, documentation, and medical surveillance in many jurisdictions. - **Emergency eyewash station:** A plumbed fixture that delivers a continuous low-pressure flow of water to both eyes simultaneously; located within 10 seconds of any area where corrosive chemicals are used; activate immediately after any eye contact with a hazardous substance and flush for a minimum of 15 minutes. - **Fume hood:** A ventilated enclosure that captures and exhausts chemical vapors, dusts, and fumes away from the work area; the sash must be kept at or below the safe working height marked on the hood. - **Personal protective equipment (PPE):** Wearable protection including safety glasses, splash goggles, face shields, lab coats, nitrile or other chemically appropriate gloves, and closed-toe shoes; selected based on the specific hazards of each procedure. - **Safety Data Sheet (SDS):** A standardized 16-section document (GHS format) describing a chemical's identity, physical and health hazards, safe handling, storage, disposal, and emergency response; must be accessible for every hazardous chemical in the lab. - **Secondary containment:** A tray, cabinet, or outer container that holds spilled liquid if the primary container fails; required under all liquid chemicals stored above bench height and all corrosives.

6. Health and Safety

6.1. General Safety Rules

- Developing a safe and productive environment is key to sustaining a strong scientific ethic; reflect on how you and your colleagues can promote safe conditions
- All personnel must always employ a buddy system, whether working within or outside traditional work hours (8 AM – 5 PM), and on weekends
- You must obtain written permission to work in the lab outside traditional hours
- No work is allowed in the laboratory without expressed permission from the Laboratory Manager
- No food, drink, or cosmetics in the laboratory
- No headphones or earbuds that block auditory hazard warnings
- Long hair must be tied back; loose clothing and dangling jewelry must be secured
- Wash hands thoroughly before leaving the laboratory
- Report all accidents, injuries, and near-misses to the lab manager immediately

6.2. Personal Protective Equipment (PPE)

When entering the lab:

- ☐ Your legs should be covered
- ☐ Wear closed-toed shoes (no sandals)
- ☐ Put on a lab coat as you enter
- ☐ Safety glasses or goggles (when working with chemicals)
- ☐ Nitrile gloves (when handling chemicals or biological materials)
- ☐ Wear a mask when working in the lab with another person (to protect against infectious diseases)

Additional PPE may be required depending on the procedure. Consult the specific SOP and the SDS for each chemical.

6.3. Emergency Equipment Locations

Know the location of the following before beginning any work:

- ☐ Fire extinguisher (near the front entrance)
- ☐ Safety shower (right and left sides of the lab)
- ☐ Eyewash station (right and left sides of the lab)
- ☐ First aid kit
- ☐ Spill kit (mounted on the wall near the PPE shelves, entrance to SGM 133)
- ☐ SDS binder (counter next to the east entrance)
- ☐ Emergency exits
- ☐ Lab contact list (posted in SGM 133; notify the lab manager if it seems out of date)

7. Emergency Procedures

! Emergency Numbers

- **Campus Safety:** (909) 607-2000 (from a campus phone, dial x72000) – open 24/7
- **Emergency:** 911
- **Poison Control:** 1-800-222-1222
- **CUC Student Health Services:** 909-621-8222
- **CUC Environmental Health and Safety:** 909-621-8538

When calling Campus Safety, be ready to provide your name, the location of the emergency, and your contact phone number. Campus Safety will assess the situation and call any necessary services (ambulance, fire department, etc.).
Pomona College On-Call Deans and the Monsour On-Call Counselor are reachable via Campus Safety's emergency number.

7.1. Earthquakes

Earthquakes are common in southern California. Developing an effective strategy before an earthquake strikes is key to laboratory safety.

Indoor response:

1. Move away from windows, filing cabinets, shelves, chemicals, and other heavy objects that may fall
2. "Duck, Cover, and Hold" until shaking stops
3. If not near a table or sturdy desk, crouch against an interior wall and cover your neck and head
4. Once shaking stops, take any necessary items (phone, wallet, medication) and leave the building
5. Go to the nearest Pomona College evacuation site and wait for further instructions
6. Put on shoes as there could be broken debris
7. DO NOT USE ELEVATORS

Outdoor response:

1. Move away from buildings, trees, signs, electrical poles, and wires
2. Protect your head and neck
3. After shaking stops, go to the nearest evacuation site

Aftershocks may occur. Be prepared and follow appropriate evacuation plans if they do.

7.2. Fires and Evacuation

In the case of a small fire, you may attempt to use a fire extinguisher. Remember:

1. Remove people from the area
2. Activate the closest fire alarm
3. Call Campus Safety
4. Extinguish the fire only if safe to do so:
    - Place yourself between the fire and the exit
    - **P**ull the pin
    - **A**im the nozzle toward the base of the fire
    - **S**queeze the handle to release the extinguishing agent
    - **S**weep the base of the fire from side to side
5. Use only one fire extinguisher; if the fire is not controlled after one, leave the room, close the door, and evacuate

If trapped in a building:

1. Call Campus Safety
2. Close all windows and doors
3. Wet and place cloth material around and under the door to prevent smoke from entering

7.3. Medical Emergencies

1. Call Campus Safety; let them know the victim's condition
2. Provide necessary first aid until Campus Safety and/or emergency services arrive (only if trained)
3. Do not move the injured person unless necessary
4. See the campus evacuation map for AED locations

7.4. Chemical Spills

If there is a hazardous chemical spill in the laboratory, do not attempt to clean it up unless trained and equipped to do so. Refer to SOP-L003 Hazardous Materials Handling for spill response procedures.

- If chemicals get in your eye, flush for 15 minutes at the eyewash station
- If toxic materials are spilled on your body, remove all clothing and stand beneath the lab shower for 15 minutes
- In the event of inhalation, move to fresh air
- If someone is at risk, protect yourself first; only provide assistance if you can do so safely

7.5. Crime on Campus

Active shooter: Exit the building if safe to do so. If not, close and lock the laboratory door, turn off lights, pull down window shades, keep quiet, silence phones, and call Campus Safety.

Bomb threat and utilities failure: Review the Emergency Brochure posted near laboratory exits for detailed procedures.

8. Training Requirements

8.1. Required Training for All Researchers

A researcher is not permitted to use lab equipment until he or she has been appropriately trained. Training is complete only after the Training Documentation Form (Appendix A) has been signed by both the researcher and the lab manager.

Every researcher must complete the following before beginning independent work:

Order	Training Item	SOP Reference
1	General Laboratory Safety (this SOP)	L01
2	Hazardous Materials Handling	L003
3	Foreign Soil Handling (all users, even if not using foreign soils)	L015
4	Field Safety (if conducting field work)	F001

Order	Training Item	SOP Reference
5	Pomona College Lab Safety Course	Annual requirement
6	Pomona College Handbook on Sexual Harassment	Required reading
7	Project-specific SOPs	As assigned by PI

8.2. Training Procedure

1. Read the assigned SOP thoroughly
2. Discuss the SOP with the lab manager, including hazards, mitigation strategies, and emergency procedures
3. Demonstrate competency for any hands-on procedures (if applicable)
4. Sign and date the SOP Training Documentation Form (Appendix A)
5. Obtain the lab manager's signature verifying your training
6. Place the completed form in the SOP Training Binder in the lab

8.3. Annual Requirements

- All researchers must attend the Pomona College Lab Safety Course each year they are working in the lab
- Training sign-offs must be renewed when SOPs are revised to a new version
- The lab manager documents the completion date of the annual safety course

8.4. Estimated Time

Initial safety training (this SOP plus discussion) generally requires approximately 45 minutes.

9. Housekeeping

- When you arrive, look around to identify any safety concerns and report them immediately to the Lab Manager
- Keep work areas clean and organized
- Return all chemicals and equipment to their designated storage locations after use
- Clean up spills immediately using appropriate procedures
- Dispose of waste in the correct containers (chemical waste, sharps, biohazard, general trash)
- Do not block emergency exits, safety showers, or eyewash stations
- Report broken or malfunctioning equipment to the lab manager

10. Ethical Efforts and Contributions

Working in the lab requires thinking carefully about what you are contributing and how others using the lab will find it. Each researcher is expected to leave the lab in better condition than they found it and to contribute to the ongoing improvement of safety documentation.

11. References

12. References

13. Appendix A: SOP Training Documentation Form

Print this form for each researcher. Once completed and signed, place it in the SOP Training Binder in the lab.

13.1. SOP Training Documentation

Researcher Name: _____

Email Address: _____

Expected Graduation Year (NA for non-students): _____

13.1.1. Agreement

By signing each row below, I confirm that I have read and understood the SOP, including all safety hazards, operating procedures, and emergency protocols. I understand that any injuries or equipment damage that occur while I am using laboratory equipment after being trained will be my responsibility. I agree to follow the procedures as written and to report any deviations, incidents, or unsafe conditions to the lab manager.

Initials: _____

13.1.2. SOP Sign-Off Record

SOP No.	SOP Title	Version	Researcher Signature	Date	Lab Manager Signature	Date
L01	General Laboratory Safety					
L02	Hazardous Materials Handling					

SOP No.	SOP Title	Version	Researcher Signature	Date	Lab Manager Signature	Date
F01	Field Safety					
F41	Foreign Soil Handling					

13.1.3. Pomona College Lab Safety Course (Annual Requirement)

Academic Year	Date Completed	Lab Manager Signature

13.1.4. Sexual Harassment Policy Acknowledgment

Date Read	Researcher Signature

13.1.5. Additional Notes

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L003

Hazardous Materials Handling

Standard Operating Procedure

SOP Number	SOP-L003
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Author(s)	EA30-Fall 2015, Virginia Paschal
Date	2026-06-26
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1. Purpose

This SOP covers the safe use, storage, disposal, and spill treatment of hazardous materials. It applies to staff, students, and researchers who use acid, base, explosive, caustic, and poisonous materials in EA Program laboratories.

This document does not explicitly cover radioactive materials.

2. Scope

- **Applies to:** All staff, students, and researchers handling hazardous chemicals in EA Program laboratories
- **Does not apply to:** Radioactive materials (see separate procedures)
- **Location:** SGM 133 and associated EA Program laboratory spaces

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Caustic chemicals:** Chemicals such as ammonium hydroxide, potassium hydroxide, and sodium hydroxide that are able to burn or corrode organic tissue by chemical action.
- **Combustible liquid:** A liquid with a flashpoint at or above 60 °C (140 °F); can burn at temperatures usually above normal working temperatures.
- **Corrosive chemicals:** Strong acids and bases, dehydrating agents, nonmetal chlorides, and halogens that are health hazards and can damage skin, eyes, and respiratory tissue on contact. Store corrosives under the fume hood on low shelving or in approved storage cabinets. Gas cylinders should not be stored in the same cabinet with corrosive liquids. Segregate acids from bases and oxidizing acids from organic acids; separate nitric acid from all other acids.
- **Flammable liquid:** A liquid with a flashpoint below 60 °C (140 °F) that can ignite easily at normal working temperatures.
- **Hazardous material:** Any substance posing a physical or health hazard as defined by OSHA 29 CFR 1910.1200, including flammables, corrosives, oxidizers, toxins, and carcinogens (Occupational Safety and Health Administration, 1990).
- **Safety Data Sheet (SDS):** A standardized document describing a chemical's hazards, safe handling, storage, disposal, and emergency response procedures; located on the counter near the east entrance and at the PPE shelves in SGM 133.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Acids, bases, flammables, oxidizers, and toxic reagents are used routinely in EA Program labs.
- **Physical:** Glassware breakage, heat from hot plates and ovens, compressed gas cylinders.
- **Biological:** Soil and water samples may contain pathogens.

5.1. Required PPE

☐ Safety glasses / goggles
☐ Lab coat
☐ Nitrile gloves
☐ Face shield (concentrated acids and bases)

5.2. Required Training

- SOP-L002 Laboratory Safety before handling any hazardous chemical

6. General Safe Handling Procedures

6.1. Working with Hazardous Materials

- Read the SDS for every chemical before first use.
- Never work alone when handling concentrated acids, bases, or flammables.
- Use the fume hood when working with volatile, toxic, or corrosive chemicals.
- Cap unused containers immediately; return chemicals to designated storage after use.
- Purchase the smallest quantity needed; order corrosives in plastic-coated containers where available.

6.2. Physical Hazards

Glassware: Inspect glassware for cracks before use. Use extreme care when inserting glass tubing into stoppers. Use heat-resistant gloves near hot glassware.

Burns: Use hot plates or water baths instead of open flames. Tie up loose hair and wear snug-fitting, non-synthetic clothing when possible. Always use a fume hood with volatile organic compounds.

Chemical spills: Read the SDS before working with any hazardous chemical. Always wear PPE, cap unused chemical containers, and use fume hoods. Never work alone.

Mercury spills: Follow the college's emergency instructions for mercury spill response.

Explosions: Develop written protocols for explosive chemicals. Use fume hoods; avoid experiments in closed containers; always wear PPE.

Chemical inhalation: Never breathe deeply from a chemical mixture. Use a fume hood; consider gas monitoring equipment for experiments that generate toxic gases.

7. Chemical Storage

7.1. General Rules

- Store chemicals by hazard class, not alphabetically.
- Never store chemicals on the floor; use low shelves or cabinets with front lips.
- Secure shelves to the wall. Ensure shelves have lips to prevent containers from falling.

7.2. Storage by Hazard Class

Hazard Class	Storage Location
Flammable liquids	Flammable-storage cabinet (yellow, self-closing)
Corrosive acids	Acid cabinet under fume hood (separate from bases)
Corrosive bases	Base cabinet under fume hood (separate from acids)
Oxidizers	Separate from flammables and organics
Toxics / reagents	General chemical shelves, secured
Compressed gases	Chained to wall; away from corrosives
Refrigerated	Lab refrigerator (no food); door labeled

8. Waste Disposal

See SOP-L006 Waste Disposal for the complete waste classification and disposal procedures. Key rules:

- Segregate waste by chemical compatibility (acids, bases, halogenated solvents, non-halogenated solvents, heavy metals).
- Label every waste container with the chemical name, hazard class, and accumulation start date.
- Never pour hazardous chemicals down the drain without verifying local discharge limits.
- Schedule EH&S pickup when a container reaches 75% capacity or after 270 days of accumulation, whichever comes first.

9. References

Occupational Safety and Health Administration. (1990). *Occupational exposure to hazardous chemicals in laboratories, 29 CFR 1910.1450*. US Department of Labor. https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1450

10. Training Record

Training is documented in EA Program files. Before working independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review SDS forms for all chemicals to be handled
4. Demonstrate understanding of spill response procedures
5. Be signed off by the PI or Chemical Safety Officer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L004

Chemical Spill Response

Standard Operating Procedure

SOP Number	SOP-L004
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP provides step-by-step procedures for responding to chemical spills in EA Program laboratories. It covers how to classify a spill, when to clean it up yourself vs. when to evacuate, how to clean up minor spills by chemical class, and how to document and report the incident. This SOP is designed to be a quick reference during an emergency; read it in advance so the procedures are familiar before you need them (National Research Council, 2011; Occupational Safety and Health Administration, 2012).

For general chemical safety, storage, and waste disposal, see SOP-L003 Hazardous Materials Handling. For general lab emergency procedures (fire, earthquake, medical), see SOP-L002 Laboratory Safety.

2. Scope

- **Applies to:** All chemical spills in EA Program laboratories (SGM 133, 134, 135)
- **Does not apply to:** Radioactive material spills; biological spills (BSL-2 organisms); field spills (handle per SOP-F001 and local regulations)
- **Location:** All EA Program laboratory spaces

3. Responsibilities

Role	Responsibility
All Lab Personnel	Assess the spill, protect themselves, initiate response
Lab Manager	Oversee cleanup of minor spills; report major spills
Lab PI	Ensure training; review incident reports
Campus Safety	Respond to major spills; coordinate with fire department

4. Emergency Contact Information

Post this information near every laboratory phone and exit.

Contact	Number / Method
Campus Safety	x72000 (from campus phone) or 909-621-8170
Pomona College Facilities	x18383
Poison Control	1-800-222-1222
911	For life-threatening emergencies
Lab Manager	*TBD – post current contact*
Lab PI (Marc Los Huertos)	*TBD – post current contact*

Refer also to (Pomona College, 2024).

5. Definitions

- **Carcinogen:** A substance capable of causing cancer with repeated or prolonged exposure; minor spills of carcinogens (e.g., acrylamide, formaldehyde, chloroform, benzene) require extra precautions including respiratory protection and disposal as hazardous waste regardless of volume (National Research Council, 2011).
- **Corrosive:** A chemical that causes visible destruction or irreversible alteration of skin, eye, or mucous membrane tissue on contact, or that corrodes steel or aluminum; includes strong acids (pH < 2) and strong bases (pH > 12); neutralized before disposal with appropriate Spill-X material (Occupational Safety and Health Administration, 2012).
- **Emergency eyewash station:** A plumbed or pressurized portable fixture that delivers a continuous flow of clean water to both eyes simultaneously; required to be within 10 seconds of unobstructed travel from any hazardous chemical work area; must be activated for at least 15 minutes for any eye chemical contact (Occupational Safety and Health Administration, 2012).
- **Flammable liquid:** A liquid with a flash point below 60 °C (140 °F); includes common laboratory solvents such as ethanol, methanol, acetone, hexane, and isopropanol; minor spills of flammables must be cleaned up with absorbent pads, not paper towels, and all ignition sources must be extinguished immediately (National Research Council, 2011).
- **Flash point:** The minimum temperature at which a liquid produces sufficient vapor to ignite momentarily in the presence of a flame or spark; the key property for classifying a spilled liquid as a flammable hazard.
- **Major spill:** A spill that cannot be safely managed by lab personnel; any spill meeting the criteria in §5.2 – including spills involving injury, unknown chemicals, large volumes, or highly toxic/reactive materials – requires evacuation and a call to Campus Safety; do not attempt to clean up a major spill.
- **Minor spill:** A small spill of a known chemical with known hazards that trained lab personnel can safely clean up using available PPE and spill control materials; all criteria in §5.1 must be met before treating a spill as minor.
- **Safety Data Sheet (SDS):** A standardized 16-section document describing a chemical's identity, hazards, safe handling, first aid, spill response, and disposal; Section 6 (Accidental Release Measures) is the primary reference for spill response; must be accessible in the laboratory at all times (Occupational Safety and Health Administration, 2012).
- **Secondary containment:** A tray, cabinet, or other physical barrier that holds spilled or leaked chemical if the primary container fails; required under all liquid chemical containers in storage; prevents spills from spreading and simplifies cleanup.
- **Spill-X-A / Spill-X-C / Spill-X-S:** Commercial spill control kits manufactured by Ansul (Johnson Controls): A = acid neutralizer (for strong acids), C = caustic/base neutralizer (for strong bases), S = solvent absorbent (for flammable organic solvents); each kit changes color when neutralization is complete.
- **Universal absorbent:** An inert granular or pad material (e.g., diatomaceous earth, Zorb-All pads) that absorbs chemical spills without reacting; suitable for a wide range of chemicals but does not neutralize acids or bases.

6. Spill Classification

Before responding, classify the spill. Your response depends on the classification (Occupational Safety and Health Administration, 2012).

6.1. Minor Spill

A spill is **minor** if ALL of the following are true:

- The chemical and its hazards are known (you have read the SDS)
- The volume is small (less than approximately 500 mL of liquid or 100 g of solid)
- No one is injured or exposed
- The spill is confined to a small area (benchtop or floor, not spreading)
- You have the proper PPE and spill control materials on hand
- The chemical is not highly toxic, volatile, flammable, or reactive at the spilled quantity

Action: Clean it up yourself using the procedures in Section 7.

6.2. Major Spill

A spill is **major** if ANY of the following are true:

- Someone is injured, contaminated, or experiencing symptoms
- The chemical is unknown
- The volume is large (more than approximately 500 mL or 100 g)
- The spill involves a highly toxic, volatile, flammable, or reactive material at any quantity
- The spill is spreading and cannot be easily contained
- You do not have proper PPE or cleanup materials
- You are unsure whether you can handle it safely

Action: Evacuate and call for help (Section 6).

7. Major Spill Response

7.1. If in Doubt, Get Out

If you are unsure whether a spill is minor or major, treat it as major. Your safety is more important than preventing property damage.

1. **Protect yourself and others.**
    - Alert everyone in the lab. Help anyone who is injured or contaminated only if you can do so without endangering yourself.

- If chemicals are on someone's skin or in their eyes, begin flushing with water immediately (safety shower for body, eyewash for eyes). Continue flushing for at least 15 minutes (Occupational Safety and Health Administration, 2012).
- Remove contaminated clothing while flushing.

2. **Evacuate.**
   - Clear all personnel from the room and close (do not lock) doors.
   - Activate the building fire alarm if there is a fire risk or if toxic vapors require building evacuation.
3. **Call for help.**
   - Call Campus Safety: x72000 or 909-621-8170.
   - For life-threatening emergencies: 911.
   - Notify the lab manager and PI.
4. **Stay available.**
   - Wait near the lab entrance (not inside) to brief first responders.
   - Bring the SDS for the spilled chemical to the responders.

8. Minor Spill Response

8.1. General Procedure

1. Put on appropriate PPE before approaching the spill: gloves, safety glasses, lab coat at minimum; add face shield for large acid/base spills (National Research Council, 2011).
2. Inform others in the lab that you are cleaning up a spill.
3. Select the correct cleanup material (Section 7.2–7.4).
4. Apply cleanup material from the outside edge of the spill toward the center to prevent spreading.
5. Allow sufficient time for neutralization (Spill-X-A and Spill-X-C change color when complete).
6. Scoop up the spent absorbent and place in a labeled waste container (hazardous waste).
7. Wipe the area with a damp paper towel; dispose of used paper towels as hazardous waste.
8. Remove PPE carefully; wash hands.
9. Document the spill (Section 9).

8.2. Acid Spills

Use **Spill-X-A** (acid neutralizer). Apply the powder to the spill and mix gently with a spatula. When the color indicator shows neutralization is complete, sweep or scoop into a waste container. For spills involving HF (hydrofluoric acid): treat as a major spill regardless of volume; HF causes deep, potentially lethal burns with delayed onset.

8.3. Base Spills

Use **Spill-X-C** (caustic neutralizer). Apply and mix as for acid spills. Monitor for heat generation; some base reactions with neutralizer can be exothermic.

8.4. Flammable Organic Solvent Spills

1. Extinguish all ignition sources immediately (no open flames, turn off hot plates, do not use electrical switches).
2. Increase ventilation (open sash of fume hood, turn on exhaust fan).
3. Use **Spill-X-S** absorbent pads or granular universal absorbent. Do not use paper towels (can generate static or wick the solvent).
4. Collect all absorbent material in a waste container.
5. If vapors persist, evacuate and call Campus Safety (National Research Council, 2011).

8.5. Toxic or Unknown Chemicals

If the chemical is toxic (acutely hazardous, carcinogenic, or unknown): treat as a major spill and call Campus Safety. Do not attempt to clean up acutely toxic or carcinogenic spills without specific training and proper respiratory protection.

9. Spill Kits

The following spill kits are maintained in each laboratory. Inspect each kit at the start of every semester and after any use. Verify that all items are present and not expired. Replenish as needed and record the check date on the spill kit inspection log.

Kit Type	Location	Contents
Spill-X-A (acid)	SGM 133, fume hood	Neutralizing powder, scoop, bag, PPE
Spill-X-C (base)	SGM 133, fume hood	Neutralizing powder, scoop, bag, PPE
Spill-X-S (solvents)	SGM 133, bench shelf	Absorbent pads, bag, PPE
Universal absorbent	SGM 134	Granular absorbent, scoop, bags

10. Body and Eye Exposure Response

10.1. Skin Contact

1. Remove contaminated clothing immediately.
2. Flush the affected area with large volumes of water for at least 15 minutes (Occupational Safety and Health Administration, 2012).
3. Do not apply neutralizing agents directly to skin.
4. Seek medical attention.

10.2. Eye Contact

1. Immediately go to the eyewash station.
2. Hold eyelids open and flush both eyes continuously for at least 15 minutes.
3. Call Campus Safety or 911; all chemical eye exposures require medical evaluation.

11. Documentation and Reporting

After every spill, including minor ones that were cleaned up successfully:

1. Complete the EA Program Spill/Incident Report Form (posted on the lab shared drive).
2. Record: date, time, location, chemical name and CAS number, volume spilled, who was involved, any exposures or injuries, actions taken, waste disposal method.
3. Submit the form to the PI within 24 hours.
4. The PI submits a report to Pomona College EH&S for any spill that resulted in an exposure or injury, required evacuation, or involved a reportable quantity of a regulated chemical (Pomona College, 2024).
5. Review the incident with the lab group within one week to identify how to prevent recurrence.

12. References

- Occupational Safety and Health Administration (2012)
- National Research Council (2011)
- Pomona College (2024)

National Research Council. (2011). *Prudent practices in the laboratory: Handling and management of chemical hazards* (Updated). National Academies Press. https://doi.org/10.17226/12654

Occupational Safety and Health Administration. (2012). *Laboratory safety guidance* (OSHA 3404-11R). US Department of Labor.

Pomona College. (2024). *Emergency instructions*. http://www.pomona.edu/sites/default/files/emergency-instructions.pdf

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General emergency procedures
L003	Hazardous Materials Handling	Chemical safety, storage, waste
L005	Chemical Inventory Management	Inventory affected by spills

14. Training Record

Before working independently in the lab, all personnel must:

1. Read this SOP in full
2. Complete SOP-L002 and SOP-L003 training
3. Locate all spill kits, the safety shower, and the eyewash station in their assigned lab
4. Walk through a simulated minor spill cleanup with the lab manager
5. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L005

Chemical Inventory Management

Standard Operating Procedure

SOP Number	SOP-L005
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP establishes the procedures for managing the chemical inventory in EA Program laboratories. A current, accurate inventory is essential for safety (knowing what is on hand in an emergency), regulatory compliance (fire code limits on flammable storage, Pomona College Chemical Hygiene Plan), waste minimization (avoiding duplicate purchases and expired stockpiles), and research efficiency (knowing what is available before ordering) (Occupational Safety and Health Administration, 2012; Standard on Fire Protection for Laboratories Using Chemicals, 2019).

2. Scope

- **Applies to:** All chemicals stored in EA Program laboratories (SGM 133, 134, 135), including reagents, standards, solvents, acids, bases, compressed gases, and prepared solutions
- **Does not apply to:** Common consumables that are not hazardous (Kimwipes, gloves, filter paper, etc.), or chemicals stored exclusively in other departments' spaces
- **Location:** All EA Program laboratory spaces

3. Definitions

- **CAS number (Chemical Abstracts Service Registry Number):** A unique numerical identifier assigned to every chemical substance by CAS; the most reliable identifier for chemicals because, unlike common names, it is unambiguous; required on every inventory spreadsheet row.
- **Chemical Hygiene Plan (CHP):** A written program required by OSHA's Laboratory Standard (29 CFR 1910.1450) describing the safety procedures, equipment, and work practices in place to protect laboratory workers from hazardous chemicals; Pomona College's CHP is maintained by EH&S (Occupational Safety and Health Administration, 2012).
- **Expiration date:** The manufacturer-recommended date after which a chemical's stated purity, concentration, or safety characteristics may no longer be guaranteed; after this date, the chemical must be tested or disposed of.
- **GHS (Globally Harmonized System of Classification and Labelling of Chemicals):** The United Nations system for standardizing chemical hazard communication; governs the content and format of Safety Data Sheets and container labels in the United States under the OSHA Hazard Communication Standard (Occupational Safety and Health Administration, 2012).
- **Hazard class:** A classification of a chemical's primary physical or health hazard (flammable, corrosive, oxidizer, toxic, carcinogen, etc.) per OSHA/GHS criteria; used to assign compatible storage locations and set fire code quantity limits (Standard on Fire Protection for Laboratories Using Chemicals, 2019).
- **NFPA 45:** The National Fire Protection Association standard governing fire protection for laboratories using chemicals; sets maximum allowable quantities (MAQs) of flammable and combustible liquids that may be stored in different lab occupancy types (Standard on Fire Protection for Laboratories Using Chemicals, 2019).
- **Peroxide-forming solvent:** A solvent that can spontaneously form explosive organic peroxides during storage (e.g., diethyl ether, THF, isopropanol); must be tested periodically with peroxide test strips and disposed of before the safe shelf life is exceeded; open-date label required on every container (National Research Council, 2011).
- **Safety Data Sheet (SDS):** A standardized 16-section document (GHS format) describing a chemical's identity, hazards, safe handling, storage, disposal, and emergency response; legally required to be accessible in the workplace for all hazardous chemicals; Section 7 (Handling and Storage) and Section 13 (Disposal) are most relevant to inventory management (Occupational Safety and Health Administration, 2012).
- **Small Quantity Generator (SQG):** A US EPA RCRA designation for facilities generating 100–1,000 kg of hazardous waste per month; Pomona College's classification, which sets specific accumulation time and storage requirements.

- **Surrogate chemical:** A non-hazardous chemical used for training and demonstration purposes in place of a hazardous substance.

4. Responsibilities

Role	Responsibility
Lab PI	Approve purchases; ensure compliance with fire code limits
Lab Manager	Maintain the inventory database; conduct audits
All Lab Personnel	Log new chemicals upon receipt; update inventory when chemicals are consumed or disposed
Chemical Safety Officer	Review inventory for incompatibilities and compliance

5. Safety Considerations

Accurate inventory management is a safety measure (Occupational Safety and Health Administration, 2012; Standard on Fire Protection for Laboratories Using Chemicals, 2019):

- First responders and the fire department use the chemical inventory to assess hazards during emergencies.
- NFPA 45 fire code limits the total quantity of flammable liquids that may be stored outside of approved flammable-storage cabinets (Standard on Fire Protection for Laboratories Using Chemicals, 2019).
- Knowing what is stored and where prevents accidental mixing of incompatible chemicals.
- Expired chemicals (especially peroxide-forming solvents) can become hazardous over time (National Research Council, 2011).

See SOP-L003 for chemical storage, compatibility, and labeling requirements.

6. The Inventory System

6.1. Electronic Inventory

The EA Program maintains a shared Google Sheet (or equivalent spreadsheet) as the master chemical inventory. The spreadsheet is stored on the lab shared drive and accessible to all lab personnel.

Required columns:

Column	Description
Chemical name	Full name (no abbreviations or formulas only)
CAS number	Chemical Abstracts Service registry number
Manufacturer	Supplier name
Catalog number	Supplier catalog/part number

Column	Description
Lot number	Manufacturer lot number
Date received	YYYY-MM-DD
Quantity received	Amount and units (e.g., 500 mL, 100 g)
Current quantity	Updated when significantly depleted
Concentration/purity	If applicable (e.g., "ACS reagent grade, 37%")
Expiration date	From manufacturer label, or calculated shelf life
Hazard class	Flammable, corrosive, oxidizer, toxic, etc.
Storage location	Room, cabinet/shelf code (e.g., SGM 133, Flam-1)
SDS on file	Yes/No
Owner/project	Who ordered it and for what project
Status	In use / Sealed / Expired / Disposed

6.2. Physical Labels

Every chemical container in the lab must have a label that includes:

- Chemical name (spelled out)
- Concentration (if a prepared solution)
- Hazard warning(s) per GHS
- Date received or date prepared
- Researcher name
- Expiration date (if applicable)

See SOP-L003, Section 6.4 for detailed labeling requirements.

7. Procedures

7.1. Receiving New Chemicals

1. Inspect the container for damage, leaks, or missing labels when the shipment arrives.
2. If the container is damaged or leaking, do not accept it into the lab. Notify the lab manager. Follow spill procedures in SOP-L004 if needed.
3. Add the chemical to the inventory spreadsheet immediately, completing all required columns.
4. Verify that the SDS is on file. If not, download it from the manufacturer's website and add it to the SDS binder and the electronic SDS folder.
5. Label the container with the date received and the researcher's name if not already present.
6. Store the chemical in the correct location based on its hazard class (see §5.3).

7.2. Ordering Chemicals

Before ordering a new chemical:

1. Check the inventory spreadsheet to see if the chemical is already in stock.

2. Check with other lab users to see if someone has an open container that can be shared.
3. Order only the quantity needed for the project. Purchasing excess chemicals generates waste and occupies storage space.
4. For highly hazardous chemicals, consult the PI and review the SDS before ordering (National Research Council, 2011).

7.3. Storage Assignment

Assign storage based on hazard class. Chemicals must be segregated by compatibility (Standard on Fire Protection for Laboratories Using Chemicals, 2019):

Hazard Class	Storage Location
Flammable liquids	Flammable-storage cabinet (yellow, self-closing)
Corrosive acids	Acid cabinet (under fume hood) – separate from bases
Corrosive bases	Base cabinet (under fume hood) – separate from acids
Oxidizers	Separate from flammables and organics
Toxics / reagents	General chemical shelves, secured
Compressed gases	Chained to wall or bench; away from corrosives
Refrigerated/frozen	Lab refrigerator or freezer (no food)

! Important

Never store chemicals on the floor. Use low shelves or cabinets with front lips. Ensure shelves are fastened to the wall. See SOP-L003, Section 6.3 for detailed storage rules.

7.4. Using and Depleting Chemicals

- When you open a sealed container for the first time, write the date opened on the label.
- When a container is nearly empty or fully consumed, update the "Current quantity" and "Status" columns in the inventory spreadsheet.
- Return every chemical to its designated storage location after use.

7.5. Disposing of Chemicals

When a chemical is expired, no longer needed, or in poor condition:

1. Update the inventory spreadsheet: change Status to "Disposed" and record the disposal date.
2. Follow the waste disposal procedures in SOP-L003, Section 8: fill out a waste tag, complete the packing manifest, and schedule pickup.
3. Do not pour chemicals down the drain unless explicitly permitted by the SDS and local regulations for that specific chemical.

7.6. Periodic Audits

Conduct a full physical audit of the chemical inventory at least once per year (typically at the start of the fall semester) (National Research Council, 2011):

1. Print the current inventory spreadsheet.
2. Walk through every storage location and physically verify each item.
3. Note discrepancies between the spreadsheet and actual stock.
4. Remove expired chemicals for disposal.
5. Update the spreadsheet to reflect the audit results.
6. File the annotated printout as the audit record.

8. Waste Minimization

- Order the smallest quantity that will meet your needs.
- Share chemicals across projects when possible.
- Prepare only the volume of working solutions needed for an analytical session.
- Substitute less hazardous chemicals when feasible (e.g., replace a halogenated solvent with a non-halogenated alternative).
- Dispose of expired chemicals promptly rather than letting them accumulate.

See SOP-L003, Section 8.3 for additional waste minimization guidance.

9. References

- Occupational Safety and Health Administration (2012)
- National Research Council (2011)
- (Standard on Fire Protection for Laboratories Using Chemicals, 2019)

National Research Council. (2011). *Prudent practices in the laboratory: Handling and management of chemical hazards* (Updated). National Academies Press. https://doi.org/10.17226/12654

Occupational Safety and Health Administration. (2012). *Laboratory safety guidance* (OSHA 3404-11R). US Department of Labor.

Standard on Fire Protection for Laboratories Using Chemicals, Pub. L. Nos. NFPA 45 (2019). https://www.nfpa.org/codes-and-standards/nfpa-45-standard-development/45

10. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L003	Hazardous Materials Handling	Storage, labeling, waste disposal

SOP	Title	Relationship
L004	Chemical Spill Response	Spill procedures for inventoried chemicals

11. Training Record

Before ordering, receiving, or storing chemicals independently, personnel must:

1. Read this SOP and SOP-L003 in full
2. Demonstrate how to add a new chemical to the inventory spreadsheet
3. Locate SDS binders and the electronic SDS folder
4. Identify the correct storage location for a given hazard class
5. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L006

Waste Disposal and Hazardous Waste Pickup

Standard Operating Procedure

SOP Number	SOP-L006
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Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the classification, segregation, labeling, storage, and disposal of all laboratory waste generated in EA Program laboratories, with emphasis on hazardous chemical waste

requiring EH&S pickup. It consolidates and expands the waste disposal sections of SOP-L003 Hazardous Materials Handling (Sections 8.1–8.6) into a standalone reference that can be posted in the waste accumulation area and used during training.

2. Scope

- **Applies to:** All laboratory waste generated in EA Program laboratories (SGM 133, 134, 135), including chemical waste, biological waste, sharps, and non-hazardous solid waste
- **Does not apply to:** Radioactive waste (not generated in EA Program labs); field waste (see SOP-F001); e-waste and surplus equipment (coordinate with campus surplus property office)
- **Location:** All EA Program laboratory spaces

3. Responsibilities

Role	Responsibility
Lab PI	Ensure compliance with campus and regulatory waste policies; sign manifests as generator
Lab Manager	Coordinate EH&S pickups, maintain satellite accumulation areas, train personnel
All Lab Personnel	Segregate and label waste correctly per this SOP
Campus EH&S	Collect hazardous waste, prepare manifests, arrange off-site disposal

4. Definitions

- **Characteristic hazardous waste:** Waste that exhibits ignitability, corrosivity, reactivity, or toxicity as defined by 40 CFR Part 261; must be managed as RCRA hazardous waste regardless of quantity.
- **EH&S (Environmental Health and Safety):** The Pomona College department responsible for regulatory compliance, hazardous waste pickup scheduling, safety training, and chemical spill response coordination.
- **Hazardous waste manifest:** A shipping document that tracks hazardous waste from the point of generation to the final disposal facility; prepared by EH&S and signed by the PI as generator.
- **Listed hazardous waste:** Waste from specific industrial processes or containing specific chemicals appearing on EPA lists (F, K, P, U lists under 40 CFR Part 261); managed as RCRA hazardous waste.
- **Satellite accumulation area (SAA):** A designated location at or near the point of waste generation where up to 55 gallons of hazardous waste (or 1 quart of acutely hazardous waste) may be accumulated without a time limit; must be under the control of the operator who generates the waste.

- **Small Quantity Generator (SQG):** A US EPA RCRA classification for facilities generating 100–1,000 kg of hazardous waste per month; Pomona College's status, which sets 270-day accumulation limits and specific storage, training, and emergency planning requirements.
- **Waste stream:** A category of hazardous waste defined by chemical composition and hazard class (e.g., halogenated solvents, non-halogenated solvents, corrosive acids, heavy metal solutions); wastes must be segregated by compatible stream to prevent dangerous reactions during storage and transport.

5. Regulatory Context

Pomona College is classified as a Small Quantity Generator (SQG) of hazardous waste under RCRA (40 CFR Part 262) and California DTSC regulations. Key requirements:

- Hazardous waste may be accumulated in satellite accumulation areas (SAAs) near the point of generation in containers of 55 gallons or less (1 quart for acutely hazardous waste)
- Once full, containers must be moved to the central accumulation area and picked up by EH&S within 3 days
- All containers must be labeled with the words "Hazardous Waste," the contents, the hazard(s), and the accumulation start date
- The generator (Lab PI) must sign the packing manifest

6. Waste Classification

6.1. Non-Hazardous Solid Waste

Waste Type	Disposal
Paper, packaging, cardboard	Recycling bins
Uncontaminated gloves and wipes	Standard trash
Clean glassware (broken)	Sharps box or wrapped cardboard, then trash
Uncontaminated soil (dried)	Standard trash or return to field site
Used aluminum tins (clean)	Recycle or reuse

6.2. Hazardous Chemical Waste

Any waste that is ignitable, corrosive, reactive, or toxic (per RCRA D-list), or that is a listed waste (F, K, P, or U list), must be collected and disposed as hazardous waste. Common EA Program hazardous waste streams:

Waste Stream	Typical Source SOPs	Container
Acid waste (HNO_3, HCl, H_2SO_4)	L410, L411, L403	Poly carboy, labeled "Acid Waste"
Base waste (NaOH, KOH)	Various	Separate poly carboy

Waste Stream	Typical Source SOPs	Container
Halogenated solvent waste (chloroform, DCM)	L409	Amber glass, labeled "Halogenated Solvent Waste"
Non-halogenated solvent waste (acetone, ethanol, hexane)	Various	Separate glass or poly container
Heavy metal solutions (Pb, Cd, Cr, Hg)	L410, L411	Poly carboy, labeled by metal
Formaldehyde solutions	L305 (preservatives)	Glass, labeled "Formaldehyde Waste"
Oxidizer solutions (H_2O_2, $NaClO_3$)	L412	Collect separately; do not mix with organic waste
Used oil (pump oil)	Vacuum pumps	Labeled container

Critical Segregation Rules

- **Never mix** acids with bases, oxidizers with organics, or halogenated with non-halogenated solvents.
- **Never pour** hazardous waste down the drain unless explicitly approved by EH&S for that specific waste stream.
- When in doubt, treat as hazardous and collect for EH&S pickup.

6.3. Biological Waste

Waste Type	Disposal
Non-pathogenic cultures (soil microbes)	Autoclave, then standard trash
Colilert/Quanti-Tray materials (L305)	Standard trash after UV deactivation or autoclave
eDNA extraction waste (bead tubes, filters)	Standard trash (non-infectious after extraction)
Foreign soil waste (L015)	Autoclave per SOP-L015 before disposal

6.4. Sharps

Waste Type	Disposal
Needles, syringes with needles	Sharps container (red)
Broken glass (contaminated)	Sharps container
Razor blades, scalpels	Sharps container
Broken glass (uncontaminated)	Cardboard box labeled "Broken Glass," then trash
Intact glass vials (uncontaminated)	Rinse and recycle, or trash

6.5. Drain-Disposable Waste

A limited set of aqueous, non-hazardous solutions may be poured down the drain with copious water, **only if approved by EH&S**:

- Dilute (less than 1%) salt solutions (NaCl, KCl, K_2SO_4, Na_2SO_4) at neutral pH
- Dilute sugar or buffer solutions at neutral pH
- Neutralized acid or base solutions (pH 5–9) that contain no metals or other hazardous constituents

> **!** Important
>
> If you are unsure whether a waste can go down the drain, collect it as chemical waste. The default is always to collect, never to drain.

7. Procedure

7.1. Labeling Waste Containers

1. Every waste container in the SAA must have a hazardous waste label (available from EH&S or the lab manager) before any waste is added
2. Fill in: waste description (chemical names and approximate percentages), hazard(s) (corrosive, flammable, toxic, etc.), accumulation start date, and laboratory location
3. Update the label if new constituents are added to the container

7.2. Accumulating Waste

1. Add waste to the appropriate labeled container in the SAA
2. Keep containers closed when not actively adding waste
3. Do not fill containers beyond 80% capacity (leave headspace for thermal expansion and safe handling)
4. Inspect containers weekly for leaks, corrosion, or label degradation

7.3. Requesting EH&S Pickup

1. When a container is full (or the study is complete), notify the lab manager
2. The lab manager contacts campus EH&S to schedule a pickup
3. Prepare a packing manifest listing: container number, contents (chemical names and approximate percentages), volume, hazard class, and generator (PI) name
4. The PI reviews and signs the manifest
5. Move the container to the central pickup area designated by EH&S
6. EH&S collects the waste, provides a manifest copy, and arranges off-site treatment or disposal

7.4. Empty Container Management

1. Triple-rinse empty reagent bottles with the appropriate solvent (water for aqueous chemicals, the same solvent for organic chemicals)
2. Remove or deface the original label
3. Collect rinse liquid as waste (add to the corresponding waste stream)
4. Clean, delabeled glass bottles may be reused for non-critical purposes or recycled
5. Do not reuse containers that held acutely hazardous (P-listed) chemicals; dispose as hazardous waste

8. Satellite Accumulation Area (SAA) Management

- Each SAA is located at or near the point of waste generation (e.g., the fume hood where digestions are performed, the bench where extractions occur)
- Maximum volume in an SAA: 55 gallons of hazardous waste (or 1 quart of acutely hazardous waste)
- Containers in the SAA must be in good condition, compatible with the waste, and kept closed except when adding waste
- Spill containment (secondary tray) is required under all liquid waste containers
- The lab manager inspects SAAs monthly and logs the inspection on the SAA checklist posted in each area

9. Emergency Spill Response

For waste container spills, follow SOP-L004 Chemical Spill Response. Key points:

- Contain the spill using absorbent materials appropriate to the waste type (do not use paper towels for solvent spills; use chemical absorbent pads)
- Contaminated absorbent becomes hazardous waste; bag, label, and include in the next EH&S pickup
- Report all spills to the lab manager and PI; document in the incident log

10. References

- 40 CFR Part 262. *Standards Applicable to Generators of Hazardous Waste.* US EPA.
- California Code of Regulations, Title 22, Division 4.5. *Hazardous Waste Control Law.* California DTSC.
- Pomona College EH&S. *Hazardous Waste Management Guide.* Available from the Campus Safety Office.
- National Research Council. (2011). *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards* (updated ed.). National Academies Press.

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L003	Hazardous Materials Handling	Chemical safety and storage
L004	Chemical Spill Response	Spill cleanup procedures
L005	Chemical Inventory	Tracking chemicals and SDS
L015	Foreign Soil Handling	Autoclave requirements for imported soil

12. Training Record

Before handling laboratory waste independently, personnel must:

1. Read this SOP and SOP-L003 in full
2. Locate the SAAs in each laboratory room and identify the waste streams currently in use
3. Demonstrate proper waste labeling (fill out a hazardous waste label correctly)
4. Demonstrate correct segregation by assigning 5 sample waste items to the correct container
5. Review the spill response procedure in SOP-L004
6. Be signed off by the lab manager or PI



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L007

EA Program Equipment and Instrument Inventory

Standard Operating Procedure

SOP Number	SOP-L007
Revision	1.1
Author(s)	Neha Vandemeer
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This document is a living inventory of instruments, equipment, and major supplies maintained by the EA Program. It serves as the central reference for locating equipment, tracking ID numbers, and linking each item to its governing SOP. All personnel should consult this inventory before purchasing duplicate items and should update it whenever equipment is acquired, moved, or decommissioned.

2. Scope

- **Applies to:** All EA Program equipment in SGM 105, 106, 131, 132, 133, 134, and associated field storage
- **Does not apply to:** Consumable supplies (reagents, glassware under $50 replacement cost), personal equipment, or equipment belonging to other departments
- **Maintained by:** Lab manager, with updates from all trained personnel

3. Definitions

- **Asset tag:** A numbered label affixed to a piece of equipment to uniquely identify it within the EA Program inventory; the number should match the ID# field in this document.
- **Decommissioned:** Status assigned to equipment that has been permanently removed from service (broken beyond repair, surplused, or disposed of); must be logged with the date and reason in the inventory and reported to the lab manager.
- **Equipment:** In this SOP, a durable item with a replacement value above approximately $50 and an expected service life of more than one year; includes vehicles, instruments, balances, power tools, pumps, and major field gear.
- **ID# (inventory number):** A unique alphanumeric code assigned to each piece of equipment; the EA Program convention is `EA-[CATEGORY]-[NNN]` (e.g., EA-FLD-001 for field equipment, EA-LAB-001 for laboratory equipment, EA-INS-001 for analytical instruments); items marked *TBD* need numbers assigned at the next audit.
- **Related SOP:** The primary operating procedure associated with a piece of equipment; a dash (–) indicates no dedicated SOP exists yet.
- **TBD (to be determined):** Placeholder in the ID# or Location column indicating that the information needs to be confirmed during the next physical audit.

4. Responsibilities

Role	Responsibility
Lab Manager	Maintain this document; conduct annual audits; assign ID numbers
PI	Approve major equipment purchases; authorize surplus or disposal
All Lab Personnel	Report new acquisitions, moves, or damage to the lab manager promptly

5. How to Use This Inventory

- Items marked *TBD* in the ID# column need to be assigned an inventory number during the next audit.
- Items marked *TBD* in the Location column need to be physically located and recorded.
- The Related SOP column links to the operating procedure for that item; a dash indicates no dedicated SOP exists yet.
- Report discrepancies (missing items, wrong locations) to the lab manager immediately.

6. Vehicles

Item	ID#	Qty	Location	Related SOP
2016 Ford Explorer #1	*TBD*	1	Garage, EA Slot	F100
2016 Ford Explorer #2	*TBD*	1	Garage, EA Slot	F100

7. Field Equipment

Item	ID#	Qty	Location	Related SOP
Cooler #1	*TBD*	1	SGM 132	–
Cooler #2	*TBD*	1	SGM 132	–
TopCon AT-G4 Survey Scope w/ Tripod & Stadia	*TBD*	3	SGM 131	–
Meter Tape (10 m)	*TBD*	2	SGM 131	–
Meter Tape (25 m)	*TBD*	1	SGM 131	–
Meter Tape (100 m)	*TBD*	1	SGM 131	–
Infiltrometer	*TBD*	3	SGM 131	–
VanDorn Bottle (Horizontal)	*TBD*	1	SGM 133	F300
VanDorn Bottle (Vertical)	*TBD*	1	SGM 133	F300
LiCor Underwater PAR Sensor	*TBD*	1	SGM 133	–
Walkie Talkies	*TBD*	3	SGM 131	–
Honda Field Generator	*TBD*	1	SGM 132	F101
Ryobi Field Generator	*TBD*	1	SGM 132	F101
Eureka Manta2 WQ Sonde	*TBD*	2	*TBD*	F200
Pygmy Velocity Meter	*TBD*	1	*TBD*	F201
Type AA Velocity Meter	*TBD*	1	*TBD*	F201
Stop Watches	*TBD*	10	*TBD*	–
Alkalinity Test Kit	*TBD*	3	*TBD*	L301

8. Remote Sensing and Drones

Item	ID#	Qty	Location	Related SOP
DJI Mavic 3 Pro	*TBD*	1	SGM 106	–
DJI Mavic 3 Batteries	*TBD*	3	SGM 106	–
DJI Matrice 100	*TBD*	1	SGM 210	–
DJI Matrice 100 Batteries	*TBD*	6	SGM 210	–

9. Raspberry Pi and Air Quality Sensors

Item	ID#	Qty	Location	Related SOP
Raspberry Pi Zero+	NA	16	SGM *TBD*	–
Raspberry Pi 3+	NA	2	SGM *TBD*	–
Raspberry Pi 4+	NA	2	SGM *TBD*	–

10. Analytical Instruments

Item	ID# / Serial	Qty	Location	Related SOP
Thermo Scientific Delta V IRMS	*TBD*	1	SGM 135	L208
Thermo Scientific Flash IRMS EA	*TBD*	1	SGM 135	L475
Thermo Scientific GasBench II	*TBD*	1	SGM 135	L415
PANalytical Axios XRF Spectrometer	*TBD*	1	SGM 135	L205
Shimadzu TOC-L w/ ASI-L Autosampler	*TBD*	1	SGM 133	L204, L407
Shimadzu TNM-L TN Module	*TBD*	1	SGM 133	L204
Hach DR3900 Spectrophotometer	*TBD*	1	SGM 133	L303
Hach DR900 Portable Colorimeter	*TBD*	1	SGM 133	L303
Turner Designs Trilogy Fluorometer	*TBD*	1	SGM 133	L203
Bio-Rad QX200 ddPCR System	*TBD*	1	*TBD*	L701
Picarro G2508 CRDS Analyzer	S/N 2143-JFAADS2048	1	*TBD*	F504
Eosense eosMX-P Multiplexer	*TBD*	1	*TBD*	F504

11. Balances and Measurement

Item	ID# / Serial	Qty	Location	Related SOP
Mettler Toledo XPE205 Delta Range Microbalance	*TBD*	1	*TBD*	L102, L200
Mettler Toledo Balance	*TBD*	3	*TBD*	L102
High Capacity Portable Scale (24 x 24 in)	*TBD*	1	*TBD*	–
Digital Calipers	*TBD*	7	*TBD*	–
Hydrometer	*TBD*	5	*TBD*	L401

12. Laboratory Equipment

Item	ID# / Serial	Qty	Location	Related SOP
MilliQ Elix Water Purification System	*TBD*	1	SGM 134	–

Item	ID# / Serial	Qty	Location	Related SOP
ESCO Laminar Flow Cabinet	*TBD*	1	SGM 134	L113
Drying Oven	*TBD*	1	SGM 134	L105
Thermo Fisher Sorvall ST 8R Centrifuge	*TBD*	1	SGM 133	L109
Thermo Fisher Legend Micro 21 Centrifuge	SN41972616	1	SGM 133	–
Fisher Isotemp Muffle Furnace	*TBD*	1	SGM 133	L111
WTW OxiTop BOD System	*TBD*	8	SGM 133	L306
Echo Labs Revolve Microscope	*TBD*	1	SGM 133	L211

13. Annual Audit Procedure

1. Walk through every room listed in this inventory (SGM 105, 106, 131, 132, 133, 134, 210) and physically verify the presence and condition of each item.
2. Assign an inventory ID number to any item currently marked *TBD*.
3. Update the Location column if any item has been moved.
4. Note any items that are missing, damaged, or decommissioned.
5. Add any newly acquired equipment.
6. Commit the updated file to the GitHub repository with a descriptive commit message.

14. References

This SOP is an internal administrative document. No peer-reviewed external references apply. For related guidance on laboratory inventory and chemical tracking, see SOP-L005 Chemical Inventory.

15. Related SOPs

SOP	Title	Relationship
L005	Chemical Inventory	Companion inventory for reagents
F002	Field Equipment Checklist	Uses this inventory for storage locations
L801	RStudio and GitHub	Version control for this document

16. Training Record

All lab personnel should review this inventory document when joining the EA Program and whenever they are assigned responsibility for a new class of equipment. No formal sign-off is required for this SOP.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L010

Laboratory Notebook Recording

Standard Operating Procedure

SOP Number	SOP-L010
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP establishes the physical documentation standards for laboratory notebooks in the EA Program. A properly maintained notebook is a legal record that establishes what was done, when, by whom, and what was observed. These standards follow Good Documentation Practice (GDP) principles used in regulated laboratories and ensure that EA Program data are traceable, contemporaneous, and defensible (Kanare, 1985; U.S. Environmental Protection Agency, 2002).

This SOP covers *how* to record. For *what* to record during an analytical session, see SOP-L802 Quality Control, Section 7.1. For field notebook protocols, see SOP-F003 Field Notebook Protocols.

2. Scope

- **Applies to:** All laboratory work performed under EA Program SOPs, including sample preparation, instrument operation, calibration, data reduction, and reagent/standard preparation
- **Does not apply to:** Field notebooks (see SOP-F003) or purely electronic records in R scripts and Git (see SOP-L801), though electronic records should meet the same traceability standards (US Food and Drug Administration, 2004)
- **Location:** All EA Program laboratories (SGM 133, 134, 135)

3. Responsibilities

Role	Responsibility
Lab PI	Set documentation standards; review notebooks
Lab Manager	Issue notebooks; maintain archive; verify compliance
All Lab Personnel	Maintain a notebook per this SOP
New Students	Read this SOP before beginning any lab work

4. Definitions

- **Archival ink:** Ink formulated to resist fading, water, and chemical degradation over decades; required for all permanent laboratory notebook entries; ballpoint or pigment-based pens are preferred over roller-ball gel inks (Kanare, 1985).

- **Carbon-copy notebook:** A notebook with interleaved carbonless copy pages that produce a duplicate of each entry; the copy is torn out and stored separately as a backup, while the original remains bound in the notebook.
- **Contemporaneous record:** An entry made at the time the observation or action occurs, not reconstructed from memory afterward; the defining characteristic of a legally defensible laboratory record (Kanare, 1985).
- **GDP (Good Documentation Practice):** A set of principles for creating accurate, legible, contemporaneous, original, and attributable records; the foundation of data integrity in regulated and research laboratories (U.S. Environmental Protection Agency, 2002).
- **Single-strikethrough correction:** The required method for correcting an error in a laboratory notebook: draw a single line through the incorrect entry so it remains legible, write the correct entry nearby, and sign and date the correction; erasing or using white-out is prohibited (Kanare, 1985).
- **Table of Contents (TOC):** A running index of entries, maintained on the first 2 pages of every notebook, linking dates and topics to page numbers; enables rapid retrieval of specific entries.
- **Witness signature:** A second person's signature and date on a notebook entry confirming that they observed or reviewed the work; required for patent-related records and recommended for any significant observation.

5. Required Notebook Type

Use a bound, carbon-copy laboratory notebook with numbered pages (e.g., a 200-page carbonless copy notebook). Do not use spiral-bound notebooks, loose-leaf paper, or stapled pages as primary records. The carbon-copy notebook provides a built-in backup: the copy page is removed and stored separately, and the original remains permanently bound (Kanare, 1985).

Acceptable notebooks are available from the lab manager. Notify the lab manager when you are on the last 20 pages so a replacement can be ordered.

6. Setting Up a New Notebook

1. Write your name, contact information, the notebook serial number (if present), and the start date on the outside spine.
2. On the inside front cover, record: your name, contact information, project name, and the date the notebook was put into service.
3. Reserve the first two pages for a Table of Contents. Update the TOC as you fill the notebook.

7. Writing Instruments

7.1. Required

Write **all** entries in permanent, non-erasable ink (Kanare, 1985). Use a ballpoint pen that produces a clear impression on both the original and the carbon-copy page. Black or dark blue ink is preferred.

7.2. Not Permitted

- Pencil (can be erased)
- Gel ink or felt-tip pens that do not transfer reliably to the carbon copy
- Erasable ink pens

> **Tip**
>
> Test your pen on a spare carbon-copy page before starting a new notebook. Press firmly enough to produce a legible duplicate, but not so hard that you tear the paper.

8. Entry Formatting

8.1. Every Entry Begins With

At the top of each new entry (or new day), record:

1. **Date** in ISO format: YYYY-MM-DD (e.g., 2026-05-18)
2. **Your name** (or initials, if your full name is on the inside cover and you are the sole user)
3. **Purpose** of the work session in one sentence (e.g., "TOC-L calibration and K_2SO_4 extract analysis, Project: BFS Soils")
4. **SOP(s) in use** with revision numbers

8.2. Contemporaneous Recording

Write entries **at the time the work is performed**, not from memory afterward (Kanare, 1985). If you record a result on a printout, sticky note, or scrap paper during an urgent moment, tape or staple the original into the notebook and initial and date it at the earliest opportunity. Never discard the original note.

8.3. Level of Detail

Write enough detail so that another trained researcher could reproduce your work using only the notebook and the referenced SOP. At minimum, include all items listed in SOP-L802, Section 7.1 (U.S. Environmental Protection Agency, 2002).

8.4. Printouts, Graphs, and Attachments

Tape or staple instrument printouts, chromatograms, calibration reports, or other documents directly into the notebook. Write across the border of the attachment and the notebook page so that it is clear the attachment was placed at that time. Initial and date each attachment.

8.5. Tables and Calculations

- Draw tables with a ruler if practical.
- Show all calculations, including units and intermediate steps, so that results can be verified.
- Circle or box final results for easy identification.

8.6. Blank Space and Unused Pages

- Do not leave large blank areas within an entry. If you finish an entry partway down a page, draw a diagonal line through the remaining blank space and initial and date it. This prevents later additions.
- Never skip pages. If you accidentally skip a page, draw a diagonal line through it, write "INTENTIONALLY LEFT BLANK," and initial and date it (Kanare, 1985).

9. Error Correction

If you make a mistake in the notebook (Kanare, 1985):

1. Draw a **single horizontal line** through the incorrect text so it remains legible.
2. Write the correct information directly above, below, or beside the crossed-out entry.
3. Write your initials and the date next to the correction.
4. If the error is extensive (e.g., an entire wrong entry), strike through the full section, write "Error – see page XX," and rewrite the correct entry on the next available page.

! Prohibited Correction Methods

- White-out, correction tape, or liquid paper
- Erasing or scratching out (even when using pencil)
- Tearing out or cutting out pages
- Writing over an existing entry

Any of these actions raises data integrity concerns and may invalidate the record (Kanare, 1985).

10. Electronic Data References

When analysis is performed electronically (R scripts, instrument software, spreadsheets), record in the notebook:

- The name and location of the electronic file (e.g., full file path or GitHub repository URL)
- The date and time the file was created or the analysis was run
- The software version or R version used

Cross-referencing electronic records ensures that the notebook and the digital record are linked and neither stands alone (U.S. Environmental Protection Agency, 2002; US Food and Drug Administration, 2004).

11. Carbon-Copy Management

1. After completing each session, remove the carbon-copy page.
2. Review the copy for legibility. If the copy is not readable, note this on the original and on the copy.
3. Place the copy in the project's designated folder on the lab shared drive or in the physical project file.
4. The original stays bound in the notebook permanently.

12. Signature and Witness

- Sign and date each entry when you complete it.
- If your work produces a significant new observation or result (a new datum, a method validation, a reproducibility test), have a witness (a labmate or the PI) sign and date the entry as well.
- For IP-sensitive work, all entries should be witnessed at the time of writing, not retroactively.

13. Carbon Copy Storage and Notebook Archiving

13.1. Carbon Copies

File carbon copies chronologically in the project file within 48 hours of each session. Label the folder clearly with the project name, PI, and date range.

13.2. Full Notebooks

When a notebook is full:

1. Record the completion date on the outside spine.
2. Update the Table of Contents on the first two pages.
3. File the completed notebook in the **Notebook Archive** (SGM 133, labeled shelf), organized by project and date.
4. Update the Notebook Registry spreadsheet with the completion date.
5. Notebooks are retained for a minimum of **7 years** after the end of the associated project, or longer if required by a funding agency, IRB, or regulatory body.
6. Notebooks are the property of the EA Program and Pomona College. They must not be taken home, discarded, or removed from the archive without PI approval (Kanare, 1985).

14. Common Mistakes

Mistake	Consequence
Using pencil	Entries can be erased; not a permanent record

Mistake	Consequence
Recording data on scratch paper first	Scratch paper gets lost; transcription adds errors
Leaving entries unsigned	Cannot confirm who performed the work
Leaving blank space without striking through	Appearance that data could be inserted later
Using white-out or scribbling over errors	Raises data integrity concerns
Not removing carbon-copy duplicates	Backup is only useful if stored separately
Forgetting to reference electronic files	Data become disconnected from context
Skipping the Table of Contents	Information is hard to locate later

15. References

- Kanare (1985)
- U.S. Environmental Protection Agency (2002)
- US Food and Drug Administration (2004)

Kanare, H. M. (1985). *Writing the laboratory notebook*. American Chemical Society.

U.S. Environmental Protection Agency. (2002). *Guidance for quality assurance project plans (EPA QA/G-5)* (EPA/240/R-02/009). U.S. Environmental Protection Agency. https://www.epa.gov/sites/default/files/2015-06/documents/g5-final.pdf

US Food and Drug Administration. (2004). *Guidance for industry: Part 11, electronic records; electronic signatures – scope and application*. US FDA.

16. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Training documentation forms
L801	RStudio Server and GitHub	Electronic data management
L802	Quality Control and QA	What to record (Section 7.1)
F003	Field Notebook Protocols	Field counterpart

17. Training Record

Before beginning independent laboratory work, all personnel must:

1. Read this SOP in full
2. Set up a new notebook correctly (inside cover, Table of Contents, registry entry)
3. Demonstrate a properly formatted entry including: header, data recording, attached printout, error correction, blank-space management, and signature
4. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L015

Foreign Soil Handling and Import Permits

Standard Operating Procedure

SOP Number	SOP-L015
Revision	1.1
Author(s)	Kathryn Hargan
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP outlines the mandatory practices for securely and safely handling, analyzing, and disposing of soils and sediments collected from outside the United States. The importation and movement of untreated foreign soil is classified by the USDA Animal and Plant Health Inspection Service (APHIS) as an extremely high-risk activity (USDA Animal and Plant Health Inspection Service, 2024b). Several levels of review and approval are required before a permit can be issued. This SOP has been written to fulfill the conditions of the EA Program's APHIS foreign soil permit.

These practices are mandatory for all personnel working with foreign soils in the EA Teaching and Research Laboratory at Pomona College. Additionally, all persons working in the laboratory, even those not directly handling foreign soils, must read this SOP and be aware of standard operations involving quarantine materials so that a safe and secure laboratory environment is maintained.

2. Scope

- **Applies to:** All soils and sediments collected outside the United States and its territories that are imported to or processed in the EA Program laboratory
- **Does not apply to:** Domestic soils (including soils from Hawaii, Puerto Rico, and the U.S. Virgin Islands, which are not foreign countries under APHIS regulations); fully sterilized or commercially prepared soil reference materials
- **Analyses covered:** ICP-MS, EA-IRMS (total C, N, S and stable isotope ratios), texture, organic matter content, and other parameters where soils can be disposed of properly after analysis
- **Location:** Foreign soil storage in SGM 133 (designated cabinet); processing in SGM 134 (dedicated area)

3. Responsibilities

Role	Responsibility
PI (Permit Holder)	Maintain active APHIS permit; ensure compliance
Lab Manager	Oversee quarantine storage; verify sterilization
Trained Personnel	Handle soils per this SOP; log all activity
All Lab Personnel	Read this SOP; understand quarantine protocols even if not handling foreign soils

4. Definitions

- **APHIS (Animal and Plant Health Inspection Service):** The USDA agency responsible for protecting US agriculture from pests and diseases; regulates genetically engineered organisms and administers quarantine regulations; issues permits for importing soil and biological materials that could harbor plant pests or pathogens (USDA Animal and Plant Health Inspection Service, 2024b).
- **Autoclave sterilization:** Treatment of soil at 121 °C and 15 psi for at least 30–60 minutes to inactivate plant pathogens, weed seeds, nematodes, and other regulated organisms; required by USDA APHIS before foreign soil may be used in experiments outside a permitted facility.
- **CBP (U.S. Customs and Border Protection):** The federal agency that clears imported soils and plant material for entry into the United States, working alongside APHIS inspectors at Plant Inspection Stations.
- **Controlled import permit (PPQ Form 588):** A USDA APHIS document authorizing the importation of specified soil or biological material under defined containment and handling conditions; the PI must obtain this before any foreign soil enters the United States (USDA Animal and Plant Health Inspection Service, 2024b).
- **Decontamination:** A procedure that eliminates or reduces microbial contamination to a safe level with respect to transmission of infection; for foreign soils, typically achieved by autoclave or dry-heat sterilization.
- **Foreign soil:** Soil or sediment originating outside the United States and its territories; regulated by USDA APHIS under 7 CFR Part 330 because of the risk of introducing exotic plant pests, pathogens, and invasive species (USDA Animal and Plant Health Inspection Service, 2024b).
- **Permitted facility:** A laboratory or greenhouse that has been inspected and approved by USDA APHIS for work with imported soil or regulated biological materials; must meet physical containment requirements specified in the permit.
- **Plant Inspection Station (PIS):** A USDA/CBP facility at a port of entry where imported shipments are inspected and cleared by APHIS and CBP inspectors before release.
- **PPQ Form 508:** The green and yellow APHIS shipping label required for shipments of 3 lb or less that will be treated at the Plant Inspection Station.
- **PPQ Form 550:** The black and white APHIS shipping label required for shipments routed directly to a USDA-approved permitted facility.
- **Regulated article:** Any soil, plant material, or organism whose importation is controlled by USDA APHIS because of its potential to harbor or transmit plant pests or diseases.
- **ePermits:** The USDA APHIS online system for applying for and managing import permits and requesting shipping labels (USDA Animal and Plant Health Inspection Service, 2024a).

5. Safety and Hazards

5.1. APHIS Quarantine Requirement

Soil is strictly controlled under APHIS quarantine regulations 7 CFR Part 330 because it can readily provide a pathway for introducing dangerous organisms into the United States. Importation of soil from foreign sources is prohibited unless authorized by APHIS under specific conditions, safeguards, and controlled circumstances described in a valid permit and/or compliance agreement.

Noncompliance can result in permit revocation, fines, and legal action (USDA Animal and Plant Health Inspection Service, 2024b).

5.2. Required PPE

- ☐ Standard laboratory coat
- ☐ Nitrile gloves
- ☐ Lab safety goggles
- ☐ Closed-toe shoes

5.3. Storage Restrictions

- Foreign soils must be stored only in the designated quarantine cabinet in SGM 133
- Foreign soils must be processed only in the dedicated area in SGM 134
- Soils must never be opened or processed outside these designated areas

5.4. Chemical Hazards

If chemicals are used during analysis (e.g., acids for digestion), review the Safety Data Sheets before beginning. For isopropyl alcohol used in decontamination, work in a well-ventilated area and keep away from ignition sources.

5.5. Emergency Procedures

Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling. For spills of foreign soil, see §11 (Spill Cleanup).

6. Personnel and Training Requirements

All personnel working with foreign soils must complete the following before handling any quarantine material:

1. Complete SOP-L002 Laboratory Safety training, including the annual Pomona College lab safety course
2. Read the USDA/APHIS Compliance Agreement (current agreement number on file with the PI)
3. Read the current Foreign Soil Permit (permit number on file with the PI) (USDA Animal and Plant Health Inspection Service, 2024b)
4. Read this SOP in full
5. Complete SOP-L105 Drying Ovens training (required for sterilization procedures)
6. Be signed off by the PI or lab manager

All Lab Users

Even personnel who do not work directly with foreign soils must read this SOP so they understand the quarantine protocols in effect in SGM 133 and SGM 134. This is a condition of the APHIS permit.

APHIS Contact

For questions about the permit, shipping, or compliance, contact the local APHIS Plant Protection and Quarantine office. Verify current contact information with the PI before each import event, as personnel assignments may change.

7. Required Materials

Item	Location	Notes
Drying oven (capable of 250 °C)	SGM 134	See SOP-L105
Foreign Soils Log Book	SGM 133	Stored with quarantine cabinet
Quarantine cabinet (labeled)	SGM 133	Lockable; red "Quarantine Soil" tags
Refrigerator or freezer (if required)	SGM 133	Designated area for foreign soils
Whirl-Pak bags and Ziplock bags	SGM 133	For sample containment
Isopropyl alcohol	SGM 134	For decontamination of surfaces and equipment
Red tags ("Quarantine Soil – Unsterilized")	SGM 133	
Green tags ("Quarantine Soil – Sterilized")	SGM 133	
PPQ Form 508 and/or PPQ Form 550 labels	On file	Requested through ePermits (USDA Animal and Plant Health Inspection Service, 2024a) or by email

8. Sample Collection and Import

8.1. Equipment Decontamination at the Collection Site

All field equipment must be thoroughly cleaned and disinfected of all soil residues at the collection site before packing for transport. Acceptable methods:

- Soak in isopropyl alcohol for one hour, or
- Soak and wash in hot water with detergent for 30 minutes

8.2. Shipping Foreign Soils

1. Pack soil and sediment samples in sturdy, leak-proof containers. Declare contents as "Soil samples."
2. The primary container must be securely closed and watertight.
3. Enclose the primary container in a second, durable, watertight secondary container. Each container must be capable of containing the soil independently.
4. Attach the appropriate APHIS shipping label to the exterior of each shipping package (USDA Animal and Plant Health Inspection Service, 2024b):
   - **PPQ Form 508** (green and yellow): For shipments of 3 lbs or less that will be treated at the PIS
   - **PPQ Form 550** (black and white): For shipments routed directly to the USDA-approved facility (Pomona College)

> ⚠ Label Placement
>
> Packages without APHIS shipping labels on the exterior may be refused entry even if labels are enclosed inside the package.

5. Each label is individually numbered with a distinctive barcode. The permit holder must request the appropriate labels at the time of permit issuance.
6. Additional labels can be obtained through the "My Shipments/Labels" feature in ePermits (USDA Animal and Plant Health Inspection Service, 2024a) or by emailing `BlackWhiteGreenYellowlabelrequest@aphis.usda.gov`.

8.3. Waybills

- **PPQ Form 508 shipments** require two waybills: one international waybill to direct the shipment to the PIS, and one domestic waybill (placed inside the package) to direct the shipment from the PIS to the permit holder.
- **PPQ Form 550 shipments** require one waybill to direct the shipment to the permit holder.

The waybill must include: carrier account number or postage, permit holder name, permit number, label number, delivery address, and telephone number. If the waybill address does not match the permit address, the package may be refused entry by CBP.

8.4. Hand-Carrying Soils into the United States

Permitted articles may be hand-carried into the U.S. only if (USDA Animal and Plant Health Inspection Service, 2024b):

1. Hand carrying was requested in the permit application and authorized in the permit conditions
2. The permit conditions list the specific steps that must be followed
3. If someone other than the permit holder will hand-carry, that person must be identified on the permit
4. The authorized hand-carrier must follow all permit conditions pertaining to hand-carried shipments

9. Receiving, Storage, and Logging

9.1. Receiving

1. Transport soils directly to the Biogeochemistry Lab quarantine cabinet in SGM 133. Under no circumstances may soils be opened or processed until they reach the quarantine cabinet.
2. Open containers only inside the designated closed room area in SGM 133 or SGM 134. Take all precautions to prevent the escape of any potential pest that may be in the soil.

9.2. Logging

Upon arrival at Pomona College, log every sample in the Foreign Soils Log Book. Each entry must include:

Field	Description
Sample number	Year and consecutive number (e.g., 18-01)
Date of arrival	YYYY-MM-DD
Origin (provenance)	Country, region, site name
Collector name/initials	Who collected the sample in the field
Mass (grams)	As received
Reason for collection	Research project or study name
Storage location	SGM 133 cabinet shelf, refrigerator, or freezer
Sterilization status	Unsterilized (red tag) on arrival

9.3. Labeling and Storage

1. Samples remain sealed in original Whirl-Pak bags within Ziplock bags until processed.
2. Store bags on shelf trays within the closed quarantine cabinet. Samples must not be left on tables or elsewhere when not in use.
3. All stored, unsterilized soils must be labeled with red tags stating “Quarantine Soil – Unsterilized.”
4. Sterilized samples must be labeled with green tags stating “Quarantine Soil – Sterilized.”
5. Any individual sample being processed outside its storage cabinet must also be clearly labeled to indicate sterilization status.

10. Treatment, Sterilization, and Disposal

10.1. Sterilization Method

Soil samples must be sterilized by dry heat treatment at 250 °C for 24 hours, or by one of the other approved methods documented in the Foreign Soils Log Book.

> **!** All Residues Must Be Sterilized
>
> All residues from processing (including ash, rinse water filter residues, and equipment rinse material) must be sterilized using the same methods as the soil itself.

10.2. Disposal

After sterilization, soils may be disposed of in standard solid waste. Document disposal date, mass, and method in the Foreign Soils Log Book.

11. Mass Accounting and Audit

Periodically verify that the sum of all samples used in analyses and the remaining inventory agrees with the original imported mass within 10% (APHA et al., 2012).

> **i** Note
>
> Discrepancies exceeding 10% must be investigated and documented.

12. Quality Control

- Verify that the APHIS Foreign Soil Permit is current and has not expired before any import activity.
- Confirm that APHIS shipping labels match the current permit number.
- Audit the Foreign Soils Log Book at least once per semester to verify all entries are complete and mass accountability is within 10% (APHA et al., 2012).
- Report any anomalies (missing samples, mass discrepancies, permit questions) to the PI immediately.

13. References

- USDA Animal and Plant Health Inspection Service (2024b)
- USDA Animal and Plant Health Inspection Service (2024a)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

USDA Animal and Plant Health Inspection Service. (2024a). *ePermits: Permits for importing regulated articles*. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/permits/epermits

USDA Animal and Plant Health Inspection Service. (2024b). *Importing soil and earth: Regulations under 7 CFR part 330*. https://www.aphis.usda.gov/plant-pests-diseases/permits/soil

14. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Chemical spill response
L105	Drying Ovens	Sterilization at 250 °C
L112	Autoclave Sterilization	Alternative sterilization method

15. Training Record

Before handling foreign soils independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Complete SOP-L105 Drying Ovens training
4. Read the current APHIS Compliance Agreement and Foreign Soil Permit (copies on file with the PI) (USDA Animal and Plant Health Inspection Service, 2024b)
5. Complete the annual Pomona College lab safety course
6. Demonstrate proper logging, labeling (red/green tags), and sterilization procedures under supervision
7. Be signed off by the PI

16. Appendix A: Sample Numbering Convention

Each sample receives a number consisting of the two-digit year and a consecutive number for that year. For example, 18-34 indicates the 34th sample logged in 2018. The numbering resets to 01 at the start of each calendar year.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L020

Reagent and Standard Preparation

Standard Operating Procedure

SOP Number	SOP-L020
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes general best practices for preparing, labeling, storing, and documenting laboratory reagents and calibration standards used across EA Program analytical methods. Individual analytical SOPs specify the exact reagents and concentrations required for each method; this SOP provides the overarching framework for solution preparation that ensures traceability, accuracy, and safety.

2. Scope

- **Applies to:** Preparation of aqueous reagent solutions, extraction solutions, calibration standards, buffer solutions, and indicator solutions used in EA Program laboratory analyses
- **Does not apply to:** Preparation of biological growth media (covered in microbiology SOPs); preparation of molecular biology reagents (master mixes, primers, probes; see SOP-L701 and related SOPs); field reagent preparation (covered in field SOPs)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve reagent-grade specifications
Lab Manager	Maintain chemical inventory, order reagents, prepare shared stock solutions
Trained Personnel	Prepare working solutions per this SOP and the relevant analytical SOP
New Students	Complete training before preparing solutions independently

4. Definitions

- **Blank solution:** A solution prepared identically to calibration standards and samples but containing no analyte; used to correct for background signal and verify the absence of contamination in reagents and glassware.
- **Calibration standard:** A solution of known analyte concentration prepared from a primary or secondary standard; used to establish the relationship between instrument signal and concentration.
- **Matrix-matched standard:** A calibration standard prepared in the same solvent or extract matrix as the samples (e.g., 2 M KCl for soil extracts); minimizes matrix effects on instrument response.
- **Primary standard:** A highly pure, chemically stable substance whose mass can be accurately weighed to prepare a solution of precisely known concentration without prior standardization; examples include potassium hydrogen phthalate (KHP) and anhydrous sodium carbonate.

- **Reagent grade:** A chemical purity classification (also called ACS grade) meeting minimum purity specifications set by the American Chemical Society; appropriate for most analytical work; trace-metal-grade or ultrapure-grade reagents are required for trace analysis.
- **Secondary standard (working standard):** A solution prepared from a primary standard or a certified reference material (CRM) and used as the working calibration solution; must be verified against the primary standard periodically.
- **Stock solution:** A concentrated solution of a reagent or standard prepared in bulk and used to prepare working solutions by dilution; labeled with concentration, preparation date, expiration date, preparer's name, and any hazard information.
- **Volumetric dilution:** Dilution performed using Class A volumetric glassware (volumetric flasks, pipettes) to achieve accurate, traceable concentrations; pipette to volume, mix thoroughly, then bring to final volume.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Concentrated acids, bases, and organic solvents used in reagent preparation are corrosive, toxic, and/or flammable. Review the SDS for every chemical before use.
- **Thermal:** Dissolving some solutes (e.g., NaOH, H_2SO_4 dilution) is exothermic. Add solute to solvent slowly, never the reverse for acid dilutions.
- **Glassware:** Volumetric flasks and graduated cylinders can break during mixing. Inspect for cracks before use.

5.1. Required PPE

☐ Safety glasses or splash goggles (for concentrated acids/bases)
☐ Lab coat
☐ Nitrile gloves
☐ Fume hood (for volatile or fuming reagents)

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes. For spills, follow SOP-L004 Chemical Spill Response.

6. Water Quality

All reagent and standard preparation must use water of appropriate purity:

Water Grade	Source	Use
Type I (18.2 MOhm, ultrapure)	Milli-Q system (see future SOP-L100)	Calibration standards, trace metal work, DOC analysis, molecular biology
Type II (deionized)	Central DI supply	General reagent preparation, rinsing
Tap water	Building supply	Initial glassware rinse only; never for reagent preparation

> **Note**
>
> Verify Milli-Q resistivity (should read 18.2 MOhm-cm at 25 C) before preparing trace-level standards. If the reading is below 18.0, replace the cartridge or consult the lab manager.

7. Procedure

7.1. Planning

1. Consult the relevant analytical SOP for the exact reagent name, concentration, and grade required
2. Calculate the mass or volume of solute needed using the target concentration and final volume. Document the calculation in the lab notebook.
3. Verify that the chemical in inventory matches the required grade (ACS reagent grade, trace metal grade, molecular biology grade, etc.)

7.2. Weighing Solids

1. Calibrate or verify the analytical balance per SOP-L200
2. Use a clean, dry weighing vessel (weigh boat or beaker)
3. Tare the vessel on the balance
4. Transfer the required mass of solid reagent using a clean spatula. Record the actual mass to the precision of the balance.
5. Return the reagent bottle cap immediately to prevent contamination and moisture uptake

7.3. Dissolving and Diluting

1. Transfer the weighed solid to a clean beaker and add approximately 75% of the final volume of water. Stir with a magnetic stir bar or glass rod until dissolved.
2. For acid dilutions: always add acid to water ("Do as you oughta, add acid to water"). Stir continuously and allow the solution to cool before adjusting to final volume.
3. Transfer the solution quantitatively to a volumetric flask of the target volume
4. Rinse the beaker and stir bar with small portions of water and add the rinses to the flask
5. Bring to the mark on the volumetric flask at room temperature. Read the meniscus at eye level.
6. Stopper the flask and invert 10 times to mix

7.4. Preparing Calibration Standards by Serial Dilution

1. Prepare or obtain a stock standard of known concentration (certified reference material or gravimetrically prepared stock)
2. Using calibrated volumetric pipettes or micropipettes (SOP-L103), transfer the calculated aliquot of stock solution into a volumetric flask
3. Dilute to the mark with the appropriate matrix (water, extractant solution, or acid matrix matching the samples)
4. Prepare the full calibration curve (typically 5–7 points plus a blank) in order from lowest to highest concentration to minimize carryover contamination
5. Record all dilution steps, pipette volumes, and flask sizes in the lab notebook

> **Tip**
>
> For trace-level calibration standards (ppb range), prepare fresh daily or per the stability guidance in the analytical SOP. Dilute standards degrade faster than concentrated stocks due to adsorption to container walls.

7.5. pH Adjustment (When Required)

1. After dissolving, measure the pH with a calibrated pH meter per SOP-L300
2. Adjust to the target pH using the appropriate acid or base (as specified in the analytical SOP), added dropwise with stirring
3. Record the final pH and the volume of acid/base added

7.6. Labeling

Every prepared solution must be labeled with the following information before it leaves the preparation bench:

- Solution name and concentration (e.g., "0.5 M K_2SO_4")
- Solvent or matrix (e.g., "in DI water")
- Date prepared
- Expiration date or shelf life (per the analytical SOP or manufacturer recommendation)
- Preparer's initials
- Hazard warnings if applicable (e.g., "Corrosive," "Flammable")

> **Important**
>
> Never use an unlabeled solution. If you find an unlabeled container, do not use it. Dispose per SOP-L006 Waste Disposal.

7.7. Storage

Solution Type	Storage Conditions
Aqueous inorganic standards	Room temperature, dark; amber bottles for light-sensitive analytes
Organic solvent solutions	Flammable cabinet; fume hood if volatile
Buffer solutions	Room temperature or 4 C per the analytical SOP
Concentrated acids and bases	Acid cabinet (acids) or base shelf (bases); never store together
Molecular biology reagents	-20 C (primers, probes, enzymes) or 4 C (buffers) per manufacturer

7.8. Documentation

Record the following in the lab notebook for every prepared solution:

- Reagent name, lot number, manufacturer, and grade
- Calculation showing target concentration and volumes/masses
- Actual mass weighed (to balance precision)
- Final volume and flask size
- Date prepared, preparer's initials
- Expiration date
- Any deviations from the standard procedure

8. Quality Control

- Verify the concentration of each new stock standard by analyzing it against the previous batch or against a certified reference material (CRM). Agreement should be within 5%.
- For critical calibration standards, prepare from a second independent source (different manufacturer or lot) and compare
- Discard solutions past their expiration date; do not extend shelf life without re-verification
- Check volumetric glassware calibration (Class A tolerance) annually by gravimetric verification
- Verify micropipette accuracy quarterly per SOP-L103

9. Common Reagent Recipes

The table below lists frequently used reagent solutions across EA Program methods. Refer to the source SOP for complete preparation details.

Solution	Concentration	Source SOP	Shelf Life
KCl extraction solution	2 M	L403	6 months
K_2SO_4 extraction solution	0.5 M	L409	6 months
Sodium hexametaphosphate (HMP)	50 g/L	L401	1 month
HNO_3 (for sample acidification)	2%	L410	Prepare fresh
KHP stock standard (DOC)	1,000 mg C/L	L407	1 month (refrigerated)
KNO_3 stock standard (TN)	1,000 mg N/L	L407	6 months
NaOH (for pH adjustment)	1 M	Various	6 months
HCl (for pH adjustment)	1 M	Various	6 months
Longmire's buffer (eDNA preservation)	As formulated	F700	6 months (refrigerated)

10. Waste Disposal

Dispose of expired or unwanted reagent solutions per SOP-L006 Waste Disposal. Key points:

- Hazardous reagents (acids, bases, solvents, metal solutions) go to the appropriate waste stream in the SAA
- Dilute, non-hazardous salt solutions at neutral pH may be drained with copious water if approved by EH&S
- Never pour an unknown or unlabeled solution down the drain

11. References

- National Research Council. (2011). *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards* (updated ed.). National Academies Press.
- Harris, D.C. (2016). *Quantitative Chemical Analysis*. 9th ed. W.H. Freeman.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. Section 1080: Reagent Water.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L003	Hazardous Materials Handling	Chemical safety
L005	Chemical Inventory	Reagent sourcing and tracking
L006	Waste Disposal	Disposing of expired reagents
L102	Using Balances	Weighing technique
L103	Micropipettes	Volumetric transfers
L200	Balance Calibration	Ensuring mass accuracy

SOP	Title	Relationship
L300	pH and Conductivity	pH verification of prepared solutions

13. Training Record

Before preparing reagents or standards independently, personnel must:

1. Read this SOP and SOP-L003 in full
2. Demonstrate proper use of the analytical balance (SOP-L102/L200) and micropipettes (SOP-L103)
3. Prepare one reagent solution and one set of calibration standards under supervision, demonstrating correct calculation, weighing, dissolving, volumetric dilution, labeling, and documentation
4. Demonstrate correct acid dilution technique (acid to water)
5. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L101

Glassware Cleaning and Preparation

Standard Operating Procedure

SOP Number	SOP-L101
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the inspection, routine cleaning, acid washing, drying, and storage of laboratory glassware used in the EA Program laboratory. Properly cleaned glassware is essential for reliable analytical results; residual detergent, organic films, or trace metals can introduce systematic bias into water quality, soil extraction, and instrumental analyses. The cleaning tier required depends on the downstream analysis, ranging from routine detergent washing for general use to trace-metal-grade acid washing for ICP-MS and other sensitive techniques.

2. Scope

- **Applies to:** All reusable borosilicate glass labware (beakers, Erlenmeyer flasks, graduated cylinders, volumetric flasks, watch glasses, funnels, and media bottles) used in the EA Program laboratory
- **Does not apply to:** Disposable glassware (e.g., serological pipettes), plastic labware, quartz cuvettes, or specialty items requiring manufacturer-specific cleaning (e.g., NMR tubes, electrochemistry cells)
- **Location:** Glassware washing station, EA Program laboratory, SGM 133

3. Definitions

- **Acid bath:** A polyethylene tub containing 10% (v/v) HCl or HNO_3 in deionized water, used to remove trace-metal contamination from glassware; kept in the fume hood at all times.
- **Acid washing:** A multi-step cleaning procedure in which glassware is soaked in dilute acid after routine detergent washing to remove adsorbed trace metals; required for ICP-MS, ICP-OES, and nutrient analyses.
- **Borosilicate glass:** A glass formulation with low thermal expansion, high chemical resistance, and suitability for laboratory use; the standard material for EA Program glassware.
- **Deionized (DI) water:** Water from which ions have been removed by ion-exchange resin; used for all rinsing steps. Type I (18.2 MOhm) water from the Milli-Q system is required for final rinses of trace-metal-grade glassware.
- **Routine cleaning tier:** Washing with Alconox detergent and tap water, followed by DI water rinses; appropriate for general-use glassware not destined for trace-metal or nutrient analysis.
- **Trace-metal grade:** A cleaning standard requiring acid washing with HNO_3 (Trace Metal grade) and Type I water rinses; used for glassware that contacts samples destined for ICP-MS or similar sensitive analyses.
- **Volumetric glassware:** Glassware calibrated to contain or deliver a precise volume (volumetric flasks, graduated cylinders, pipettes); must not be oven-dried because thermal expansion permanently alters calibration.

- **Water-break test:** A visual check for cleanliness in which DI water is rinsed through glassware; a continuous, uniform film (no beading) indicates adequate cleaning.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure supplies are stocked
Lab Manager	Maintain acid bath schedule, monitor DI water quality
Trained Personnel	Clean glassware after each use, report damaged items
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Broken or chipped glassware can cause lacerations. Hot glassware from the drying oven (105 C) causes burns on contact.
- **Chemical:** Concentrated acids (HCl, HNO_3) used in acid baths are corrosive and emit irritating fumes. Alconox detergent is a mild irritant.

5.1. Required PPE

☐ Safety glasses or splash goggles (goggles required for acid bath work)
☐ Lab coat
☐ Nitrile gloves (heavy-duty acid-resistant gloves for acid bath handling)
☐ Closed-toed shoes

5.2. Emergency Procedures

- **Acid splash to skin or eyes:** Flush immediately with water at the eyewash station or safety shower for at least 15 minutes. Refer to SOP-L003 Hazardous Materials Handling.
- **Broken glass injury:** Apply pressure with a clean cloth. If the cut is deep or contaminated with chemicals, seek medical attention.
- **Acid spill:** Neutralize small spills with sodium bicarbonate; for large spills, use the spill kit and evacuate the area. See SOP-L003.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Glassware washing sink	With hot and cold water supply
Drying oven	Set to 105 C; see SOP-L105
Drying rack (peg-style)	Wall-mounted or countertop
Acid bath (polyethylene tub with lid)	Dedicated to acid washing; kept in fume hood
Fume hood	Required for all acid bath work

6.2. Glassware Inventory

Common Fisherbrand borosilicate glassware used in the EA Program laboratory:

Item	Fisherbrand Cat. No.	Sizes in Lab
Low-Form Griffin Beakers	FB100050 – FB1002000	50 – 2000 mL
Narrow-Mouth Erlenmeyer Flasks	FB500125 – FB5001000	125 – 1000 mL
Wide-Mouth Erlenmeyer Flasks	FB501125 – FB501500	125 – 500 mL
Class A Volumetric Flasks	FB400100 – FB4002000	100 – 2000 mL
Graduated Cylinders	S63457 – S63461	50 – 1000 mL
Glass Media Bottles with Cap	FB800100 – FB8001000	100 – 1000 mL

6.3. Cleaning Reagents and Consumables

Item	Cat. No. / Source	Storage
Alconox powdered detergent	Fisher 04-320-1	Shelf above wash sink
Hydrochloric acid, ACS grade (for general acid bath)	Fisher A144-212	Acid cabinet, fume hood
Nitric acid, Trace Metal grade (for trace-metal acid bath)	Fisher A509P500	Acid cabinet, fume hood
Deionized water (Type I or II)	Milli-Q system; see *SOP-L11*	Dispenser at wash station
Glassware brushes (assorted sizes)	Fisher 03-578 series	Drawer at wash station
Lint-free wipes (Kimwipes)	Fisher 06-666 series	Benchtop dispenser

7. Procedure

7.1. Inspection Before Use

1. Inspect every piece of glassware before use. Check for chips, cracks, stars, or deep scratches, especially around rims, spouts, and the base
2. Discard any piece that is broken, chipped, starred, or badly scratched. Place it in the designated broken-glass box (rigid cardboard container labeled “BROKEN GLASS”), not in the regular trash

3. Check volumetric glassware (flasks, graduated cylinders) for legible graduation marks. Do not use glassware with faded or unreadable scales.

> **Tip**
>
> Run your fingernail gently around the rim of a beaker or flask. If you feel a nick or irregularity, the piece should be discarded. Chipped rims create stress points that can propagate into cracks during heating.

7.2. Routine Cleaning (General Laboratory Use)

Use this procedure for glassware that will be used for general chemistry, soil extractions (KCl, K_2SO_4), gravimetric analyses, and other procedures that do not require trace-metal cleanliness.

1. Rinse the glassware with tap water immediately after use to prevent residues from drying
2. Prepare a warm solution of Alconox detergent (approximately 10 g per liter of warm tap water) in the wash basin
3. Submerge the glassware and scrub all interior and exterior surfaces with an appropriately sized brush. Use brushes with wooden or plastic handles only; metal-handled brushes and worn brushes where the spine contacts the glass will scratch the surface
4. Pay special attention to the interior bottom of beakers and flasks, where residues accumulate
5. Rinse thoroughly under running tap water, inverting and filling each piece at least three times to remove all detergent
6. Perform a final rinse with three changes of deionized (DI) water
7. Invert the glassware on a peg-style drying rack or place upright in the drying oven at 105 C for 2–4 hours (see SOP-L105)

> **Critical**
>
> Do not allow acid to contact glassware before detergent is thoroughly removed. Acid reacting with residual soap creates a hydrophobic film that is difficult to remove and will interfere with subsequent analyses.

7.3. Acid Washing (Trace-Metal and Nutrient Analysis)

Use this procedure for glassware destined for trace-metal analysis (ICP-MS, ICP-OES, XRF digestions), nutrient analysis, or any procedure where the SOP specifies "acid-washed glassware." All acid bath work must be performed in the fume hood.

1. Complete the routine cleaning procedure (Section 6.2) first. Glassware must be detergent-free before entering the acid bath.
2. Prepare the acid bath in a dedicated polyethylene tub inside the fume hood:
   - **General acid bath:** 10% (v/v) HCl in DI water
   - **Trace-metal acid bath:** 10% (v/v) HNO_3 (Trace Metal grade) in DI water

3. Submerge the clean glassware completely in the acid bath, ensuring no air bubbles are trapped inside
4. Cover the tub with its lid and soak for a minimum of 4 hours; overnight soaking (12–24 hours) is preferred
5. Using acid-resistant gloves and splash goggles, remove the glassware from the acid bath
6. Rinse each piece with at least five changes of DI water. For trace-metal work, use Type I (18.2 M-ohm) water from the Milli-Q system for the final three rinses.
7. Place the rinsed glassware on a clean drying rack or in the drying oven. Do not use paper towels or Kimwipes to dry the interior of acid-washed glassware, as fibers introduce contamination.
8. Once dry, cover the opening of each piece with a clean watch glass, aluminum foil, or parafilm and label it "ACID WASHED" with the date

Note

The acid bath should be replaced when it becomes visibly discolored or after approximately 20 uses, whichever comes first. Record replacement dates in the acid bath log on the fume hood.

7.4. New Glassware

New borosilicate glassware is slightly alkaline from the manufacturing process. Before first use in quantitative analyses:

1. Soak the new glassware in a 1% HCl or 1% HNO_3 solution for several hours
2. Proceed with the routine cleaning procedure (Section 6.2)
3. For trace-metal applications, also complete the acid-washing procedure (Section 6.3)

7.5. Stubborn Residues and Special Cases

Residue Type	Cleaning Method
Dried organic matter	Soak overnight in Alconox; scrub with brush
Mineral scale or precipitates	Soak in 10% HCl for 4+ hours
Grease or stopcock lubricant	Rinse with acetone in the fume hood, then routine wash
Biological residues (agar, culture media)	Autoclave before washing; see SOP-L002
Unknown or heavily stained	Soak in 10% HNO_3 overnight; if persistent, contact the lab manager

Do Not Use Chromic Acid

Chromic acid (potassium dichromate in sulfuric acid) was historically used as a glassware cleaner but is a potent human carcinogen and is prohibited in this laboratory. Use the acid-wash procedures described above instead.

7.6. Drying and Storage

1. Air-dry inverted on a peg rack, or oven-dry at 105 C for 2–4 hours (see SOP-L105)
2. Do not oven-dry volumetric glassware (volumetric flasks, graduated cylinders). Thermal expansion can permanently alter the calibration. Air-dry volumetric glassware only.
3. Store clean, dry glassware in designated cabinets with shelf liner
4. Store glassware inverted or covered to prevent dust accumulation
5. Keep acid-washed glassware in a separate, labeled section of the cabinet or on a dedicated shelf to prevent accidental use for non-critical applications

8. Quality Control and Acceptance Criteria

8.1. Water-Break Test

The water-break test is a simple visual check for cleanliness:

1. Rinse the interior of the glassware with DI water
2. Observe the drainage pattern as water sheets off the surface
3. **Pass:** Water forms a uniform, unbroken film across the entire interior surface
4. **Fail:** Water beads up, forms droplets, or breaks into rivulets, indicating residual organic contamination or grease

If the water-break test fails, repeat the cleaning procedure. If it fails again after acid washing, discard the piece, as surface damage may be preventing adequate cleaning.

8.2. Routine Verification

- Perform the water-break test on a representative sample (1 in 10 pieces) from each cleaning batch
- For acid-washed glassware, run a DI water blank through the glassware and analyze it on the target instrument (e.g., ICP-MS) to confirm background levels are below method detection limits
- Record cleaning batch dates and water-break test results in the glassware cleaning log

9. Waste Disposal

Waste Type	Disposal Method
Spent acid bath (HCl or HNO_3)	Neutralize with sodium bicarbonate to pH 6–8, then dispose down the drain with copious water, or collect for EH&S pickup if volume is large
Broken glass	Place in the rigid "BROKEN GLASS" disposal box; do not place in regular trash or recycling
Detergent wash water	Drain; Alconox is biodegradable

Waste Type	Disposal Method
Acetone rinse waste	Collect in organic waste carboy in fume hood; EH&S pickup

10. References

- Corning Life Sciences. *Suggestions for Cleaning Glassware*. Application Note CLS-AN-112. Corning, NY.
- Sigma-Aldrich / MilliporeSigma. *Cleaning Laboratory Glassware*. Technical Document.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

11. Training Record

Before cleaning glassware independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Demonstrate proper use of brushes, detergent preparation, and the rinse sequence under supervision
4. For acid-washed glassware: demonstrate proper fume hood use, acid-resistant PPE donning, and acid bath handling under supervision
5. Perform a water-break test and have results reviewed by the lab manager
6. Be signed off by the PI or lab manager

12. Appendix A: Quick-Reference Cleaning Decision Guide

Downstream Analysis	Cleaning Tier Required
Gravimetric soil moisture (SOP-L400)	Routine (Section 6.2)
Loss on ignition (SOP-L406)	Routine (Section 6.2)
KCl extraction for NH_4^+/NO_3^- (SOP-L403)	Routine (Section 6.2)
TOC / TC analysis (SOP-L407)	Routine + combust ceramic boats at 900 C
pH and conductivity (SOP-L402	Routine (Section 6.2)
Hach colorimetric methods (SOP-L303)	Routine (Section 6.2)
ICP-MS / ICP-OES (*SOP-L31*)	Acid wash with 10% HNO_3 (Section 6.3)
XRF acid digestions (*SOP-L30*)	Acid wash with 10% HNO_3 (Section 6.3)
DNA extraction (SOP-L700)	Autoclave; routine wash
Microbial biomass carbon (SOP-L409)	Acid wash with 10% HCl (Section 6.3)

13. Appendix B: Acid Bath Preparation

13.1. 10% HCl Acid Bath

1. Place the clean polyethylene tub in the fume hood
2. Fill the tub approximately 90% full with DI water
3. Slowly add concentrated HCl (approximately 37%) to reach a final concentration of 10% (v/v). For a 20 L bath, add approximately 2 L of concentrated HCl to approximately 18 L of DI water.
4. Always add acid to water, never water to acid
5. Label the tub with: acid type, concentration, preparation date, and a hazard warning
6. Cover with the fitted lid when not in use

13.2. 10% HNO_3 Acid Bath (Trace Metal Grade)

1. Follow the same steps as above, substituting Trace Metal grade HNO_3 (Fisher A509P500) for HCl
2. Use Type I DI water from the Milli-Q system
3. This bath is reserved for trace-metal glassware only; do not cross-contaminate with the general HCl bath

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L102

Using Balances

Standard Operating Procedure

SOP Number	SOP-L102
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the proper use, zeroing, taring, and routine quality checks for the laboratory balances in the EA Program. Correct weighing technique is fundamental to virtually every quantitative laboratory procedure. For calibration and verification protocols, see SOP-L200 Analytical Balance Calibration.

2. Scope

- **Applies to:** All Mettler Toledo balances in the EA Program laboratory, including the basic balances (MS1602TS/00) and the precision balance (XPE205 DeltaRange) (Mettler Toledo, 2015a, 2015b)
- **Does not apply to:** Portable field balances or calibration procedures (see SOP-L200)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Capacity (maximum load):** The maximum mass that can be placed on the weighing pan without risking damage to the weighing cell; exceeding the rated capacity will damage the load cell and require expensive repair; the MS1602TS capacity is 1620 g and the XPE205 capacity is 220 g / 81 g (DeltaRange) (Mettler Toledo, 2015a, 2015b).
- **Draft shield:** The glass or acrylic enclosure surrounding the weighing pan on the XPE205 precision balance; reduces air current disturbances that cause reading instability; always keep closed except when placing or removing items.
- **Drift:** Gradual, directional change in the balance reading under constant load; caused by temperature changes, vibration, or electronic instability; the balance should be allowed to warm up for at least 30 minutes before critical weighing.
- **Linearity:** The degree to which balance readings are proportional to applied mass across the full weighing range; verified during calibration per SOP-L200.
- **Net weight:** The mass of sample only, after the tare weight of the container has been subtracted; what is displayed after pressing the Tare key with the container on the pan.
- **Readability (resolution):** The smallest increment the balance can display; the MS1602TS reads to 0.01 g and the XPE205 reads to 0.1 mg (0.0001 g) (Mettler Toledo, 2015a, 2015b).
- **Repeatability:** The standard deviation of repeated measurements of the same weight under identical conditions; a key short-term precision indicator; affected by vibration, draft, and sample placement position.

- **Spirit level (bubble level):** A small bubble vial mounted on the balance base; the bubble must be centered in the target circle before weighing; off-level readings can introduce systematic errors.
- **Tare:** The function that zeroes the displayed mass when a container is on the pan, so subsequent readings show only the mass of material added (Mettler Toledo, 2023).
- **Weighing cell:** The internal transducer (typically an electromagnetic force compensation cell) that converts the mechanical load on the pan to an electrical signal; the most sensitive and expensive component of the balance; protect it from impact, overloading, and corrosive chemicals.
- **Zeroing:** Setting the balance display to exactly zero with an empty pan and no load; performed at the start of each session and whenever the balance is moved; distinct from taring, which zeroes the balance with a container already on the pan.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Dropping heavy items onto the balance pan can damage the weighing cell. Handle objects carefully and never exceed the rated capacity.
- **Chemical:** Some materials weighed on the balance may be hazardous. Refer to the relevant SOP for the material being weighed. Never place chemicals directly on the weighing pan; always use a sample vessel.

5.1. Required PPE

☐ Lab coat
☐ Nitrile gloves (when handling chemical samples)
☐ Safety glasses (when weighing chemical reagents)

5.2. Emergency Procedures

If a chemical spill occurs on or near the balance, clean the spill per SOP-L003 Hazardous Materials Handling before resuming use.

6. Equipment and Materials

6.1. EA Program Balances

Balance	Model	Capacity	Readability	Location
Precision balance (1)	XPE205	220 g / 81 g	0.1 mg	SGM 133
Basic balance (1–3)	MS1602TS	1620 g	0.01 g	SGM 133

(Mettler Toledo, 2015b, 2015a)

6.2. Weighing Supplies

Item	Notes
Weighing paper	Single use; keeps chemicals off the pan
Sample vessels	Weighing boats, crucibles, or tared containers
Forceps or tweezers	For handling calibration weights or small containers without contamination
Lint-free wipes (Kimwipes)	For cleaning the pan and draft shield
Anti-static brush	For removing powder adhering to the draft shield interior

7. Procedure

7.1. Startup and Warm-Up

Allow the balance to warm up for at least 30 minutes before making critical weighings (< 0.001 g significance) (Mettler Toledo, 2015b). This allows the internal components to reach thermal equilibrium.

7.2. Leveling the Balance

1. Check the spirit level before each use session.
2. If the bubble is not centered, adjust the leveling feet at the base of the balance.
3. For the XPE205, use the built-in leveling aid: **Balance menu > Leveling aid** (Mettler Toledo, 2015b).

7.3. Zeroing

1. Verify the balance is empty and the draft shield is closed.
2. Press **Zero** (or **On/Zero**). The display should read 0.000 g (for the MS1602TS) or 0.0000 g (for the XPE205).
3. If the display does not settle, check that the balance is level, no air currents are present, and the pan is clean and dry.

7.4. Taring

1. Zero the balance (§[sec-zeroing]).
2. Open the draft shield.
3. Place the sample vessel on the center of the weighing pan.
4. Close the draft shield.
5. Press the **Tare** key. The display reads zero and the **Net** indicator appears.

7.5. Performing a Weighing

1. Open the draft shield (if applicable).
2. Place the weighing object into the tared sample vessel.
3. Close the draft shield and wait for the stability indicator.
4. Record the displayed mass in the lab notebook.
5. If using the XPE205 electronic results feature, tap **Add result** to append the value to the results list.

7.6. Completing and Saving Results (XPE205)

1. Tap **Complete** to open the task completion dialog.
2. Select an option to save or print the results list.
3. Follow the on-screen instructions.
4. Tap **Complete** to finalize; the results list is saved or printed and then cleared.

7.7. Shutdown

1. Remove all samples and vessels from the weighing pan.
2. Clean the pan and draft shield interior with a lint-free wipe.
3. Close the draft shield.
4. To enter standby mode, hold the power/standby key. The display goes dark but the balance remains powered on and thermally stable.
5. To switch the balance off completely, disconnect from the power supply. Note that the balance will require a full warm-up before its next use.

8. Quality Control and Acceptance Criteria

8.1. Leveling the Balance

Exact horizontal positioning is essential for repeatable and accurate results (Mettler Toledo, 2015b):

1. Check the spirit level before each use session.
2. If the message “Balance is out of level” appears, tap **Level the balance** to open the leveling aid.
3. Adjust the leveling feet following the on-screen instructions until the bubble is centered.

8.2. Internal Adjustment (XPE205)

1. Navigate to **Methods > Adjustments**.
2. Verify that the adjustment strategy is set to **Internal adjustment**.
3. Select the adjustment and tap **Start**.
4. Do not touch the balance during the adjustment.
5. When complete, review the adjustment results.
6. Tap **Print** if a record is needed, then tap **Finish adjustment** (Mettler Toledo, 2015b).

For external calibration verification using traceable reference weights, see SOP-L200.

9. Waste Disposal

No waste is generated by this procedure. Cleaning materials (Kimwipes) can be disposed of in standard trash unless they contacted hazardous chemicals, in which case follow SOP-L003.

10. References

Mettler Toledo. (2015a). *MS1602TS basic balance: Operating instructions*. https://www.mt.com

Mettler Toledo. (2015b). *XPE205 DeltaRange precision balance: Operating instructions*. https://www.mt.com

Mettler Toledo. (2023). *Good weighing practice: The SOP for balances and scales*. https://www.mt.com/us/en/home/library/guides/laboratory-weighing/good-weighing-practice.html

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Chemical spills on/near balance
L200	Balance Calibration	Calibration and external verification
L400	Soil Moisture	Weighing application
L406	Loss on Ignition	Weighing application

12. Training Record

Before using the laboratory balances independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate proper leveling, zeroing, taring, and weighing technique under supervision
4. For the XPE205 precision balance, also demonstrate use of the draft shield and internal adjustment procedure
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L103

Using Micropipettes

Standard Operating Procedure

SOP Number	SOP-L103
Revision	0.3
Author(s)	Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the proper use, volume setting, tip loading, sample aspiration and dispensing, and care of Gilson Pipetman micropipettes. Micropipettes are precision instruments used to transfer small volumes of liquid (less than 1 mL) with high accuracy. Correct technique is essential for reproducible results in chemical, biological, and environmental analyses.

2. Scope

- **Applies to:** All Gilson Pipetman micropipettes (P10, P20, P200, P1000) in the EA Program laboratory
- **Does not apply to:** Serological pipettes, Pasteur pipettes, bottle- top dispensers, or Repipet-type dispensers
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure annual calibration
Lab Manager	Schedule calibration, maintain tip inventory
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Aerosol-resistant (filter) tip:** A pipette tip containing a hydrophobic filter that prevents aerosols and liquid from entering the pipette barrel; required when pipetting volatile, radioactive, infectious, or PCR-sensitive samples.
- **Air-displacement pipette:** The standard laboratory micropipette in which a piston displaces air in the tip, which in turn displaces liquid; the most common type; accuracy depends on tip seal quality and pipetting technique.
- **Dead volume:** The small volume of liquid remaining in the tip after dispensing; varies by tip type and affects accuracy at low volumes.
- **Forward pipetting:** The standard technique in which the plunger is depressed to the first stop before immersing the tip, then liquid is aspirated by releasing the plunger; used for aqueous solutions.
- **Reverse pipetting:** A technique in which the plunger is depressed past the second stop before aspirating; liquid is dispensed only to the first stop, leaving excess in the tip; used for viscous or volatile liquids to improve accuracy.

- **Systematic error:** A consistent, directional bias in pipette delivery; detected by gravimetric calibration; caused by worn seals, incorrect technique, or using tips not matched to the pipette model.
- **Volume range:** The minimum and maximum volume a given pipette model is designed to deliver accurately; pipetting below 10% of the maximum volume significantly increases relative error.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Liquids being pipetted may be hazardous. Consult the relevant SOP and Safety Data Sheet for the material being transferred.
- **Physical:** Repetitive pipetting can cause repetitive strain injuries. Take breaks during extended pipetting sessions.
- **Biological:** When pipetting biological samples, use filter tips to prevent aerosol contamination of the pipette barrel.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

If hazardous liquid enters the pipette barrel, stop immediately. Decontaminate the pipette per the manufacturer's instructions and refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Volume Range	Notes
Pipetman P10	1.0 – 10.0 μL	Yellow or white tip
Pipetman P20	2.0 – 20.0 μL	Yellow tip
Pipetman P200	20 – 200 μL	Yellow tip
Pipetman P1000	200 – 1000 μL	Blue tip

6.2. Consumables

Item	Notes
Disposable pipette tips	Match tip size to pipette; see Section 6.3
Filter pipette tips	Required for biological or volatile samples
Tip waste container	Benchtop receptacle for used tips

7. Procedure

7.1. Selecting the Correct Pipette

Always select the smallest pipette that covers the target volume. using an unnecessarily large pipette for a small volume reduces accuracy. The volume ranges are:

Pipette	Range	Smallest Graduation
P10	1.0 – 10.0 μL	0.02 μL
P20	2.0 – 20.0 μL	0.02 μL
P200	20 – 200 μL	0.2 μL
P1000	200 – 1000 μL	2 μL

! Important

Never set the pipette below the minimum or above the maximum of its range. Exceeding these limits will damage the piston seal and put the pipette out of calibration.

7.2. Setting the Volume

1. Identify the volume display window on the front face of the pipette. The three-digit display reads differently for each pipette size:
   - **P10:** digits represent ones, tenths, and hundredths of uL
   - **P20:** digits represent tens, ones, and tenths of uL
   - **P200:** digits represent hundreds, tens, and ones of uL
   - **P1000:** digits represent thousands, hundreds, and tens of uL
2. Turn the volume adjustment ring clockwise to increase volume or counterclockwise to decrease volume
3. If you overshoot the target while dialing down, dial below the desired setting and then dial back up to the correct value. This avoids mechanical backlash in the piston mechanism.

7.3. Loading a Tip

1. Select the correct tip size for the pipette being used. Tip boxes are labeled with the compatible pipette sizes. When in doubt, confirm with the lab manager.
2. Press the pipette shaft firmly into a tip in the tip box. A slight twist while pressing helps ensure a secure, airtight seal.

3. Do not touch the tip with your hands.
4. Always use a fresh tip for each different reagent or sample to prevent cross-contamination.

7.4. Aspirating (Loading) a Sample

1. Depress the plunger slowly to the **first stop** (the point of initial resistance). This is the calibrated load volume.
2. While holding the plunger at the first stop, immerse the tip into the liquid just enough to cover the opening (3–4 mm). Do not plunge the tip to the bottom of the vessel.
3. Slowly and steadily release the plunger to draw liquid into the tip. Do not let the plunger snap back.
4. Wait one to two seconds before removing the tip from the liquid, especially with viscous solutions. This allows the full volume to enter the tip and prevents air bubbles.
5. Visually inspect the tip: there should be no air gap or bubble at the distal end. If there is, dispense the liquid back and repeat.

> **Tip**
>
> For viscous liquids (glycerol, concentrated DNA solutions), use the "reverse pipetting" technique: depress the plunger to the **second stop** before immersing the tip, draw up the liquid by releasing to the rest position, and then dispense by pressing only to the **first stop**. The small amount of liquid remaining in the tip is discarded.

7.5. Dispensing a Sample

1. Touch the tip to the inner wall of the receiving vessel at a slight angle
2. Depress the plunger slowly to the **first stop** to dispense most of the liquid
3. Continue pressing to the **second stop** to expel the final drop
4. Hold the plunger at the second stop while withdrawing the tip from the vessel
5. Release the plunger only after the tip is clear of the liquid

7.6. Ejecting the Tip

1. Hold the pipette over the tip waste container
2. Press the tip ejector button on the back of the pipette
3. Never reuse a tip between different solutions

7.7. Important Precautions

- Never point a pipette upward with liquid in the tip. Liquid can flow back into the barrel and damage the piston and seals.
- Never lay a pipette down with a loaded tip.
- When not in use, set the pipette to its maximum volume and hang it vertically on the pipette stand. This relaxes the piston spring and extends the instrument's life.
- Do not use a pipette as a stirring rod.

- If the plunger action feels rough, sticky, or inconsistent, stop using the pipette and report the issue to the lab manager.

8. Quality Control and Acceptance Criteria

8.1. Gravimetric Accuracy Check

Verify pipette accuracy by weighing dispensed volumes of distilled water on the analytical balance (see SOP-L200):

1. Tare a clean microcentrifuge tube on the analytical balance
2. Pipette the test volume of distilled water into the tube
3. Record the mass. At 20 C, 1 μL of water weighs approximately 1.00 mg.
4. Repeat for a total of 10 replicates
5. Calculate the mean and coefficient of variation (CV)

Pipette	Test Volume	Acceptable CV
P10	10 μL	less than 3%
P20	20 μL	less than 2%
P200	200 μL	less than 1%
P1000	1000 μL	less than 0.5%

If the CV exceeds the acceptable threshold, the pipette should be removed from service and sent for professional calibration.

8.2. Calibration Schedule

All micropipettes must be professionally calibrated at least once per year. Calibration stickers on each pipette show the date of last calibration and the next due date. Do not use a pipette with an expired calibration.

9. Waste Disposal

Waste Type	Disposal Method
used pipette tips (non-hazardous)	Standard tip waste box; dispose in regular trash when full
used tips contaminated with hazardous chemicals	Chemical waste per SOP-L003
used tips contaminated with biological material	Biohazard bag; autoclave before disposal

10. References

- Gilson. *Pipetman Classic: user's Guide*. Gilson, Inc., Middleton, WI.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

11. Training Record

Before using micropipettes independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate correct volume setting, tip loading, aspiration, and dispensing technique under supervision
4. Perform a gravimetric accuracy check (Section 7.1) with acceptable results for at least two pipette sizes
5. Be signed off by the PI or lab manager

12. Appendix A: Volume Display Reading Guide

The three-digit volume display reads differently for each Pipetman size. The table below shows the maximum display setting and the place value of each digit:

Pipette	Max Display	Digit 1 (top)	Digit 2 (middle)	Digit 3 (bottom)
P10	1 0 . 0 0	10 μL	1 μL	0.1 μL
P20	2 0 . 0	10 μL	1 μL	0.1 μL
P200	2 0 0	100 μL	10 μL	1 μL
P1000	1 0 0 0	1000 μL	100 μL	10 μL

13. Appendix B: Troubleshooting

Symptom	Probable Cause	Corrective Action
Liquid drips from tip after aspiration	Tip not seated properly; damaged tip	Reseat or replace tip
Air bubble in tip after aspiration	Plunger released too quickly; tip not immersed deep enough	Re-aspirate slowly with tip 3–4 mm below surface
Dispensed volume too low	Pipette out of calibration; wrong tip size	Perform gravimetric check; verify tip compatibility
Plunger feels sticky or rough	Liquid entered barrel; worn piston seal	Clean barrel; send for service

Symptom	Probable Cause	Corrective Action
Volume display will not turn	Mechanical jam	Do not force; contact lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L104

Using a Vortex Mixer

Standard Operating Procedure

SOP Number	SOP-L104
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation and care of vortex mixers used in the EA Program laboratory. Vortex mixers produce a rapid orbital or oscillating motion that creates a vortex inside a tube or small container, providing fast and thorough mixing of liquids, suspensions, and reagents. They are used routinely in DNA extraction (SOP-L700), Qubit sample preparation (SOP-L213), chemical extractions, and general reagent mixing.

2. Scope

- **Applies to:** All vortex mixers in the EA Program laboratory, including the Fisher Scientific vortex mixers (SGM 134, qty 3) and the Vortex-Genie 2 (SGM 133)
- **Does not apply to:** Orbital shakers (for extended, gentle agitation of multiple samples); magnetic stir plates; sonicators (see SOP-L115)
- **Location:** SGM 133 and SGM 134

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain equipment, replace cup attachments
Trained Personnel	Operate the vortex mixer per this SOP
New Students	Complete training before independent use

4. Definitions

- **Continuous mode:** A vortex mixer operating mode in which the platform spins continuously when the power switch is on; used when mixing many tubes in sequence without holding each one.
- **Touch mode:** A vortex mixer operating mode in which the platform spins only when downward pressure is applied to the platform; allows the user to control mixing duration for each tube individually.
- **Vortex:** A spiral or whirling motion of liquid produced when a tube's closed end is pressed off-center against a spinning platform; efficient for resuspending pellets, dissolving solids, and homogenizing small volumes.
- **Vortex mixer (Vortex-Genie):** A benchtop mixer with an eccentric rotating cup or platform that creates a vortex in tubes pressed against it; operated at variable speeds (typically 0–3,200 rpm).

5. Safety and Hazards

⚠ Hazards

- **Aerosol generation:** Vortexing uncapped or loosely capped tubes can spray liquid and generate aerosols. Always cap tubes securely before vortexing. If the sample contains hazardous biological or chemical material, vortex inside a fume hood or biological safety cabinet.
- **Tube ejection:** At high speed, improperly held tubes can slip from the cup head and be thrown from the mixer. Hold tubes firmly against the cup or use the hands-free cup attachment.

- **Breakage:** Thin-walled glass tubes may shatter under vigorous vortexing. Use polypropylene or thick-walled borosilicate tubes whenever possible.
- **Entanglement:** Keep hands, hair, jewelry, and loose clothing away from the spinning cup head.
- **Electrical:** Do not operate the mixer with a damaged power cord or loose components. Inspect the cord before use; report damage to the lab manager.

5.1. Required PPE

☐ Safety glasses
☐ Nitrile gloves (standard lab PPE)
☐ Lab coat

6. Equipment

Item	Location	Notes
Fisher Scientific vortex mixer	SGM 134	Qty 3; touch or continuous mode
Vortex-Genie 2 (Scientific Industries)	SGM 133	Variable speed; touch or continuous mode
Cup head (standard)	Attached	For single tubes (1.5 mL to 50 mL)
Flat head or platform head (optional)	SGM 134	For microplates or multiple small tubes held in a rack

7. Procedure

7.1. Pre-Use Inspection

1. Verify the vortex mixer is clean and free from visible damage
2. Ensure the power cord is intact and properly connected
3. Check that the rubber cup head or attachment is securely installed and not cracked or deformed

7.2. Setup

1. Verify that the vortex mixer is on a stable, flat bench surface and that the rubber feet are intact (prevents the unit from walking during operation)
2. Confirm that the appropriate head is installed:
    - **Cup head:** for individual tubes (microcentrifuge tubes, 15 mL and 50 mL centrifuge tubes, glass test tubes)
    - **Platform head:** for microplates or tube racks (if available)

3. Set the speed dial to the desired setting. For most routine mixing, a medium speed (setting 5–7 on the Vortex-Genie 2 dial) is sufficient. Use lower speeds for gentle mixing and higher speeds for vigorous vortexing (e.g., bead disruption steps in DNA extraction).

7.3. Operation – Touch Mode

Touch mode is the default for most applications. The mixer runs only while the tube is pressed against the cup head.

1. Cap the tube securely
2. Hold the tube at a slight angle (approximately 15–30 degrees from vertical) and press the bottom of the tube firmly into the center of the cup head
3. The motor engages automatically. A visible vortex should form in the liquid.
4. Vortex for the duration specified in the analytical SOP (typically 2–5 seconds for brief mixing, 10–30 seconds for thorough resuspension)
5. Release the tube from the cup to stop

7.4. Operation – Continuous Mode

Use continuous mode for hands-free operation or when extended vortexing is required.

1. Switch the mode selector to **Continuous** (or **On**)
2. Adjust the speed dial to the desired setting
3. Place the capped tube in the cup head or in a tube holder secured to the platform head
4. The motor runs continuously at the set speed
5. After the prescribed duration, switch back to **Touch** mode or turn off the unit
6. Remove the tube

7.5. Vortexing Multiple Tubes

For rapid sequential vortexing of many tubes (e.g., a rack of Qubit assay tubes):

1. Set the mixer to touch mode at the desired speed
2. Work through the rack systematically, pressing each tube against the cup for 2–3 seconds
3. Return each tube to the rack before picking up the next

For simultaneous vortexing of a plate or rack, attach the platform head and secure the plate or rack with the provided clamp or tape. Run in continuous mode for the prescribed duration.

7.6. Special Applications

Application	Speed Setting	Duration	Notes
Brief reagent mixing	Medium (5–7)	2–5 s	Touch mode; just to mix

Application	Speed Setting	Duration	Notes
Qubit assay tube mixing (L213)	Medium (5–7)	2–3 s	Brief vortex after adding sample to working solution
DNA extraction bead disruption (L700)	Maximum (10)	Per kit protocol (often 10 min on adapter)	Use continuous mode with horizontal adapter if available
Resuspending pellets after centrifugation	High (8–10)	10–30 s	Until pellet is fully resuspended
Mixing soil extracts (L403, L409)	Medium (5–7)	5–10 s	Ensure homogeneous suspension before filtering

8. Maintenance

Task	Frequency
Wipe the cup head and body with 70% ethanol	After each use session
For eDNA/PCR work: wipe with 10% bleach, then ethanol	After each use session
Check rubber feet for wear	Monthly
Inspect cup head for cracks or deformation	Monthly
Replace cup head	When cracked or deformed

> **Note**
>
> If liquid spills into the cup head or body of the mixer, unplug the unit immediately, wipe up the spill, and allow to dry completely before re-use. Liquid inside the motor housing can cause electrical failure.

9. Waste Disposal

No waste is generated by the vortex mixer itself. Dispose of sample waste per the relevant analytical SOP and SOP-L006 Waste Disposal.

10. References

- Scientific Industries. *Vortex-Genie 2 Instruction Manual.* Scientific Industries, Bohemia, NY.

11. Related SOPs

SOP	Title	Relationship
L115	Sonicator	Alternative mixing/disruption
L213	Qubit Fluorometer	Sample preparation requires vortexing
L403	KCl Extraction	Extract mixing
L700	DNA Extraction	Bead disruption step

##Training Record

Before using the vortex mixer independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper tube holding technique in touch mode (cap secured, slight angle, firm contact with cup)
3. Demonstrate switching between touch and continuous mode
4. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Mixer does not start	Power disconnected or cord damaged	Check power supply; inspect cord; try another outlet
Excessive vibration or walking	Unit on uneven surface; rubber feet worn	Reposition on a flat surface; replace rubber feet
Poor or incomplete mixing	Speed too low; tube not centered on cup	Increase speed; press tube firmly into center of cup
Sample leakage during vortexing	Tube cap not secured; tube overfilled	Cap tightly; reduce fill volume to leave headspace
Abnormal noise or grinding	Cup head loose or damaged; internal bearing wear	Tighten or replace cup head; report to lab manager for service
Liquid entered motor housing	Spill from uncapped tube	Unplug immediately; wipe and dry thoroughly; do not use until fully dry

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L105

Using Drying Ovens

Standard Operating Procedure

SOP Number	SOP-L105
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe operation of gravity convection and forced-air drying ovens used in the EA Program laboratory. Drying ovens are used to determine gravimetric moisture content of soil, sediment, and plant tissue samples (see SOP-L400 Soil Moisture), to pre-dry samples before loss on ignition (see SOP-L406 Loss on Ignition), to dry glassware, and to regenerate desiccants. Consistent oven operation ensures accurate and reproducible mass-loss measurements across the laboratory (Gardner, 1986; Robertson et al., 1999).

2. Scope

- **Applies to:** All gravity convection and forced-air drying ovens in the EA Program laboratory, operated at temperatures up to 250 C
- **Does not apply to:** Muffle furnaces (550+ C; see SOP-L50), vacuum ovens, microwave drying, or autoclaves
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Desiccator:** An airtight container holding a hygroscopic drying agent (typically silica gel or Drierite) used to cool crucibles or dried samples to room temperature without re-absorbing atmospheric moisture.
- **Forced-air oven (mechanical convection):** A drying oven with a fan that circulates hot air to produce uniform temperature and faster drying; preferred when rapid or even drying is required.
- **Gravity convection oven:** A drying oven without a fan; relies on natural convection. Slightly less temperature-uniform than forced-air but adequate for most soil and sediment drying.
- **Gravimetric moisture content:** The mass of water lost on drying expressed as a proportion of the dry mass: $\theta_g = (M_{wet} - M_{dry})/M_{dry}$; expressed as g g^{-1} or as a percentage.
- **Oven-dry basis:** The convention of expressing soil or sediment concentrations relative to the mass of material dried at 105 °C to constant mass; the standard reference state for soil analysis.
- **Set point:** The target temperature programmed into the oven controller; the actual chamber temperature should be verified with a calibrated thermometer and may differ from the set point by a few degrees.
- **Tare mass:** The mass of an empty, pre-dried container (crucible, tin, or pan) recorded before adding sample; subtracted from the gross mass to obtain sample mass.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain ovens, schedule annual calibration checks
Trained Personnel	Operate ovens following this SOP, report malfunctions
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Oven surfaces and contents reach temperatures up to 250 C. Severe burns can result from touching hot surfaces, shelves, or sample containers without proper PPE. Steam may escape when opening the door if drying wet samples.
- **Chemical:** Do not dry samples containing volatile organic solvents, flammable materials, or corrosive chemicals in these ovens. Drying ovens are not explosion-proof. Volatile fumes can accumulate inside the oven chamber and pose fire or explosion risks.
- **Electrical:** Do not operate ovens with damaged power cords or plugs. Ensure the oven is properly grounded.

5.1. Required PPE

☐ Heat-resistant gloves or oven mitts (required when loading/unloading)
☐ Safety glasses
☐ Lab coat
☐ Closed-toed shoes

5.2. Emergency Procedures

For burns, cool the affected area under cool running water for at least 10 minutes. Seek medical attention for burns with blistering. If smoke or unusual odors are detected, turn off the oven, unplug it, and ventilate the area. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Drying oven	Lab bench, SGM 133	*TBD – confirm manufacturer and model number*
Calibrated thermometer or thermocouple	Inside oven	For independent temperature verification

Item	Location	Notes
Desiccator with indicating desiccant	Lab bench	For cooling samples after removal from oven
Metal tongs or oven mitts	Near oven	For handling hot containers

6.2. Consumables

Item	Notes
Aluminum weighing tins	Pre-labeled or numbered
Ceramic crucibles	For higher-temperature applications
Indicating desiccant (silica gel or Drierite)	Replace or regenerate when color changes

7. Procedure

7.1. Pre-Use Checks

1. Verify the oven is clean and free of residue from previous use
2. Check that the oven vent is unobstructed (gravity convection ovens rely on passive airflow; blocked vents cause uneven heating)
3. Confirm the temperature setpoint using the oven's built-in display or controller
4. Verify the temperature against an independent thermometer or thermocouple placed inside the oven. The reading should agree within +/- 2 C of the setpoint. If it does not, contact the lab manager.

7.2. Setting the Temperature

Common drying temperatures used in the EA Program laboratory:

Application	Temperature	Duration	Reference SOP
Gravimetric soil moisture	105 C	24+ hours	SOP-L41
Pre-drying soil for LOI	105 C	24+ hours	SOP-L50
Drying glassware	105 C	2–4 hours	–
Drying plant tissue	65–70 C	48–72 hours	–
Regenerating Drierite desiccant	210 C	1–2 hours	–
Acid-treated soil (IC removal)	105 C	Until dry	SOP-L51

1. Set the temperature using the oven controller dial or digital display
2. Allow the oven to reach the target temperature and stabilize before loading samples (typically 30–60 minutes)
3. Record the setpoint and actual temperature in your lab notebook

7.3. Loading Samples

1. Arrange samples on oven shelves with adequate spacing to allow air circulation. Do not stack containers or block vents.
2. Place containers with the lids removed or loosely resting to allow moisture to escape
3. For large batches, distribute samples evenly across shelves to minimize temperature gradients
4. Close the oven door promptly after loading

> **Tip**
>
> Place a small label or sign on the oven door indicating: your name, the setpoint temperature, the date and time samples were loaded, and the expected removal time. This prevents others from accidentally changing the temperature or disturbing your samples.

7.4. Monitoring

1. Check the oven temperature at the start and end of each drying cycle using the independent thermometer
2. Do not open the oven door unnecessarily during drying, as this introduces cool air and extends drying time
3. For multi-day drying, check the oven temperature at least once daily

7.5. Removing Samples

1. Wear heat-resistant gloves or oven mitts
2. Use metal tongs for small containers (aluminum tins, crucibles)
3. Transfer hot containers directly into a desiccator to cool to room temperature. Do not weigh hot samples; thermal convection currents around hot objects cause unstable balance readings (Harris & Lucy, 2020).
4. Allow samples to cool in the desiccator for 20–30 minutes before weighing
5. Close the oven door after removing samples

7.6. Verifying Dryness (Constant Mass)

For analytical applications (soil moisture, LOI), drying is complete only when the sample reaches constant mass:

1. After the initial drying period, remove the sample, cool in the desiccator, and weigh
2. Return the sample to the oven for an additional 2–4 hours
3. Cool and re-weigh
4. If the mass has changed by less than 0.01 g between successive weighings, constant mass has been achieved
5. If the mass has decreased by more than 0.01 g, return to the oven and repeat until constant mass is reached

7.7. Shutdown

1. If the oven will not be used for several days, turn it off using the power switch or controller
2. Leave the door slightly ajar to prevent moisture buildup and odors inside the chamber
3. Ensure no samples remain in the oven before shutting down

8. Quality Control and Acceptance Criteria

- Verify oven temperature against an independent thermometer at least monthly; record in the oven calibration log
- Acceptable temperature accuracy: +/- 2 C of setpoint at 105 C
- If the oven temperature drifts beyond +/- 5 C, remove the oven from service and contact the lab manager
- Ensure the desiccator contains active (indicating) desiccant; regenerate or replace when the indicator changes color
- For analytical drying, always confirm constant mass (Section 6.6) rather than relying on a fixed drying time alone

8.1. Interferences

- Overcrowding the oven can create temperature gradients and uneven drying, leading to inconsistent results
- Opening the oven door frequently extends drying time and introduces moisture
- Drying samples containing volatile compounds at 105 C will cause mass loss beyond water content, biasing moisture determinations

9. Waste Disposal

Waste Type	Disposal Method
Dried soil samples	Standard trash or return to field site
Aluminum tins	Clean and reuse, or recycle
Spent desiccant	Regenerate at 210 C or dispose as non-hazardous solid waste

10. References

Gardner, W. H. (1986). Water content. In A. Klute (Ed.), *Methods of soil analysis, part 1: Physical and mineralogical methods* (2nd ed., pp. 493–544). Soil Science Society of America.

Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research.* Oxford University Press.

11. Training Record

Before using the drying oven independently, personnel must:

1. Read this SOP and SOP-L002 Laboratory Safety in full
2. Demonstrate proper use of heat-resistant gloves and tongs
3. Demonstrate setting the oven temperature and verifying it with an independent thermometer
4. Demonstrate loading and unloading samples, including transfer to the desiccator
5. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Oven does not heat	Power switch off, unplugged, or blown fuse	Check power connections; replace fuse if needed
Temperature overshoots setpoint	Faulty thermostat or controller	Remove from service; contact lab manager
Uneven drying across samples	Overcrowded shelves or blocked vent	Redistribute samples; check vent is clear
Samples not reaching constant mass after 48 hours	Very wet or clay-rich samples; temperature too low	Verify setpoint is 105 C; extend drying time; check oven seal
Unusual odor during drying	Contaminated samples or residue from previous use	Turn off oven; ventilate; clean interior

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L106

Refrigerators and Freezers

Standard Operating Procedure

SOP Number	SOP-L106
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the proper use, sample storage protocols, and inventory management for the laboratory refrigerator (4 C), freezer (approximately –30 C), and ultra-low freezer (approximately –80 C). Proper cold storage is essential for preserving sample integrity for chemical, biological, and molecular analyses (APHA et al., 2012).

2. Scope

- **Applies to:** All use of the laboratory refrigerator (4 C), freezer (–30 C), and ultra-low freezer (–80 C) for sample preservation and reagent storage
- **Does not apply to:** Food storage (prohibited), field coolers, or cryogenic storage (liquid nitrogen)
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Cold chain:** The unbroken sequence of refrigerated storage and transport that maintains samples at the required temperature from collection through analysis; a break in the cold chain may compromise sample integrity.
- **Cryogenic storage:** Storage at temperatures below -80 °C, achieved with ultra-low freezers or liquid nitrogen dewars; required for DNA, RNA, enzymes, and some biological samples.
- **Freezer burn:** Dehydration and oxidation of samples stored improperly in a freezer, caused by sublimation of ice crystals; prevented by airtight sealing of containers.
- **Holding temperature:** The target storage temperature specified by the analytical method or regulatory protocol for a given sample type (e.g., 4 °C for water samples, -20 °C for soil extracts, -80 °C for nucleic acids).
- **Temperature excursion:** An event in which the storage temperature falls outside the acceptable range; must be documented and the potential impact on sample integrity evaluated.
- **Ultra-low freezer (-80 °C):** A mechanical freezer capable of maintaining temperatures between -70 °C and -86 °C; used for long- term storage of DNA, RNA, microbial cultures, and enzyme preparations.

4. Responsibilities

Role	Responsibility
Lab PI	Approve storage assignments, ensure training compliance
Lab Manager	Monitor temperatures, coordinate power outage response
Trained Personnel	Store and retrieve samples following this SOP, maintain inventory records
New Students	Complete training before independent use

4.1. Acknowledgements

We thank Aparna Chintapalli for setting up the –80 C ultra-freezer map and inventory system.

5. Safety and Hazards

⚠ Hazards

- **Physical:** The –80 C freezer poses a risk of cold burns (frostbite) upon contact with surfaces or samples. Heavy drawers and racks can cause crush injuries if dropped.
- **Chemical:** No flammable materials may be stored in the laboratory refrigerator or freezers. These units are not explosion-proof and can generate significant static electricity, which can cause an explosion in the presence of flammable fumes.
- **Electrical:** Power outages can compromise stored samples (see Section 7).

❗ Important

No food or beverages shall be stored in the laboratory refrigerator or freezers under any circumstances.

5.1. Required PPE

☐ Insulated cryogenic mittens or gloves (required for –80 C freezer)
☐ Lab coat
☐ Safety glasses (when handling chemical reagents from cold storage)
☐ Closed-toed shoes

5.2. Emergency Procedures

For cold burns, warm the affected area gradually with lukewarm water; do not rub. Seek medical attention if blistering occurs. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Laboratory refrigerator	Lab, SGM 133	Set to 4 C
Laboratory freezer	Lab, SGM 133	Set to approximately –30 C
Ultra-low freezer (–80 C)	Lab, SGM 133	*TBD – confirm manufacturer and model number*
Thermometer / temperature logger	Inside each unit	For monitoring; check daily

6.2. Consumables

Item	Notes
Cryoboxes (cardboard or plastic)	For organizing samples in the –80 C freezer
Cryogenic labels / markers	Must withstand –80 C without peeling
Sample tubes (cryovials, microcentrifuge tubes)	Must be rated for the target storage temperature

7. Procedure

7.1. Refrigerator (4 C)

1. Store samples that require short-term cold storage (soil extracts, water samples awaiting analysis, reagents with refrigeration requirements)
2. Label all containers with: sample ID, date, researcher name, and project
3. Do not overcrowd the refrigerator; allow air circulation around samples
4. Remove expired samples and reagents during monthly cleanouts
5. *TBD – define shelf assignment system for the refrigerator*

7.2. Freezer (–30 C)

1. Store samples requiring medium-term frozen storage (archived soil samples, tissue samples, reagents requiring –20 C or colder)
2. Label all containers with: sample ID, date, researcher name, and project
3. Organize samples by project; do not mix projects within the same shelf area
4. *TBD – define shelf assignment and inventory system for the –30 C freezer*

7.3. Ultra-Low Freezer (–80 C)

The –80 C freezer is organized using a hierarchical location system. Every sample must be documented in the Freezer Inventory Google Sheet and in your laboratory notebook.

7.3.1. Loading Protocol

1. When loading the freezer for the first time or after defrosting, install a few drawers at a time from top to bottom, allowing the freezer to stabilize between additions
2. Only open the freezer when you are ready to place or remove samples. Minimize the time the door is open.
3. Wear insulated cryogenic mittens and a lab coat when accessing the freezer

7.3.2. Location Hierarchy

The freezer inventory uses the following hierarchy:

- **Shelf:** Each shelf is contained behind an inner door, numbered from top to bottom. (Note: Shelf 1 is currently empty.)
- **Divider:** Each divider is a column of drawers within the shelf, labeled A through E from left to right
- **Drawer:** Drawers are numbered sequentially from top to bottom within each divider
- **Box:** Each drawer contains five boxes, labeled 1 through 5 from front to back
- **Box position:** Each box is divided into 100 positions. Numbering runs from front-left (position 1) to back-right (position 100), counting along rows from left to right

7.3.3. Required Inventory Fields

Record the following in the Google Sheet and in your lab notebook each time you store or remove a sample:

Field	Description
Shelf	Shelf number (top to bottom)
Divider	Column letter (A–E, left to right)
Drawer	Drawer number (top to bottom)
Box	Box number (1–5, front to back)
Box position	Position number (1–100)
Project	Project name or code
Date collected	Date sample was collected in the field
Sample ID	Unique sample identifier
Sample description	Description including any relevant analytical results (e.g., NanoDrop concentration)

> **Tip**
>
> Before opening the freezer, identify the exact shelf, divider, drawer, box, and position you need. Have all your samples labeled and ready to go. This minimizes the time the door is open and protects the integrity of all stored samples.

8. Quality Control and Acceptance Criteria

8.1. Temperature Monitoring

- Check and record the temperature of each unit daily (or use a continuous temperature logger)
- Acceptable ranges: refrigerator 2–6 C; freezer –25 to –35 C; ultra-low freezer –75 to –85 C
- If temperature is outside the acceptable range, investigate immediately (door left ajar, power outage, compressor failure)
- *TBD – establish an alarm system or temperature logging protocol with automatic notifications*

8.2. Power Outage Protocol

! Power Outage Response

If the power goes out, it is imperative that valuable samples in either freezer be moved to a location where power is currently available to maintain freezing conditions. The laboratory generators do not provide sufficient power to keep any of the refrigerators or freezers at their appropriate temperatures.

1. Contact the lab manager and PI immediately
2. Do not open freezer doors unless moving samples to a backup location
3. A closed –80 C freezer can maintain temperature for approximately 24 hours if the door remains shut
4. If power is not expected to be restored within 4 hours, arrange to transfer critical samples to a backup freezer on campus
5. *TBD – identify backup freezer locations on campus and establish agreements for emergency sample transfer*

8.3. Inventory Maintenance

- Conduct a full inventory audit of the –80 C freezer at least once per semester
- Remove samples from completed or abandoned projects promptly
- Ensure the Google Sheet is current; discrepancies between the sheet and actual contents must be resolved immediately

9. Waste Disposal

Waste Type	Disposal Method
Expired or abandoned samples	Dispose per the relevant SOP for the sample type (e.g., biohazard, chemical waste, standard trash)
Broken cryovials	Sweep up carefully; treat as sharps if broken glass
Used cryoboxes	Standard trash (if uncontaminated)

10. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

11. Training Record

Before using the laboratory cold storage independently, personnel must:

1. Read this SOP and SOP-L002 Laboratory Safety in full
2. Demonstrate proper use of cryogenic PPE
3. Demonstrate the ability to locate a sample using the freezer hierarchy and Google Sheet
4. Successfully store and retrieve a sample under supervision, updating the inventory in both the Google Sheet and lab notebook
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L107

Hot Plates and Dry Bath

Standard Operating Procedure

SOP Number	SOP-L107
Revision	0.2
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the safe operation, maintenance, and hazard mitigation for laboratory hot plates, stirring hot plates, and digital dry bath heaters used within the environmental analysis laboratories.

2. Scope

- **Applies to:** Hot plates, stirring hot plates, and digital dry bath (heat block) heaters used for sample heating, digestion, reagent preparation, and enzymatic or thermal incubations
- **Does not apply to:** Muffle furnaces (see SOP-L111), water baths, autoclaves, or microwave digestion systems
- **Location:** Environmental analysis laboratories

3. Definitions

- **Dry bath (heating block):** An aluminum block with drilled wells that fits standard tubes (1.5 mL, 15 mL, 50 mL); heated by a resistive element to provide uniform, conductive temperature control without the hazard of a liquid bath.
- **Flash point:** The lowest temperature at which a liquid's vapor can ignite in air; flammable solvents must never be heated above their flash point on an open hot plate.
- **Hot plate:** An electrically heated flat surface used to heat beakers, flasks, or evaporating dishes; may or may not include an integrated magnetic stirrer.
- **Hot plate / stirrer:** A combination unit providing both heating and magnetic stirring; the most common configuration in the EA Program laboratory.
- **Magnetic stir bar:** A PTFE-coated magnetic rod placed in a vessel; rotated by the stirrer magnet beneath the hot plate surface to provide mixing.
- **Set temperature vs. actual temperature:** The temperature displayed on the hot plate controller may differ substantially from the actual liquid temperature; always verify with a calibrated thermometer.
- **Surface temperature limit:** The maximum safe surface temperature of the hot plate; organic solvents should never be heated on an open hot plate above their boiling point or flash point.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Thermal:** Heating surfaces and metal blocks can exceed 300 °C and remain dangerously hot long after power is disconnected. Contact causes severe thermal burns.
- **Fire and Explosion:** Heating volatile, flammable solvents on an open-bench hot plate can ignite vapors. Standard hot plates are not explosion-proof and may generate sparks from mechanical switches or internal cycling.
- **Electrical:** High current draw can overload circuits. Liquid boil-overs or spills create immediate electrical short risks.

5.1. Required PPE

☐ Safety glasses or chemical splash goggles
☐ Laboratory coat (fully buttoned)
☐ Heat-resistant thermal gloves (when handling active or cooling vessels, blocks, or glassware)
☐ Nitrile gloves (underneath thermal gloves if chemical handling is simultaneous)

5.2. Emergency Procedures

In case of burn, spill, or equipment failure, refer to SOP-L002 Laboratory Safety or call campus safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Hot plate / stirring hot plate	Lab bench	Ceramic, aluminum, or silicone-coated top plate
Digital dry bath (heat block)	Lab bench	Interchangeable aluminum blocks for various tube sizes
PTFE-coated stir bars	Supply drawer	Various sizes
External thermocouple probe	Instrument drawer	For independent temperature verification

6.2. Consumables

Item	Notes
Borosilicate glassware	Pyrex or Kimax; certified for thermal stress

Item	Notes
Interchangeable aluminum blocks	Sized for 1.5 mL, 15 mL, or other tube formats

7. Procedure

7.1. Pre-Operational Inspection

1. Inspect the power cord and plug for fraying, exposed wires, or chemical degradation. Do not use if damaged.
2. Ensure the cord does not drape across or contact the heating surface.
3. Verify that the heating surface and aluminum blocks are free of residual chemical films, crusts, or signs of pitting. Clean with a damp cloth if necessary (unit must be unplugged and cool).

7.2. Sample and Vessel Preparation

1. **Glassware inspection:** For hot plates, use only borosilicate glassware (e.g., Pyrex, Kimax) certified for high thermal stress. Inspect for star cracks, hairline fractures, or deep scratches. Discard compromised glassware.
2. **Dry bath alignment:** Ensure the interchangeable aluminum block profile matches your specific sample tubes (e.g., 1.5 mL microcentrifuge tubes vs. 15 mL conical tubes). Incorrect sizing limits thermal contact and degrades temperature control accuracy.

! Sealed Containers

Never heat completely sealed containers on a hot plate. Pressure buildup can cause catastrophic vessel failure.

7.3. Operation and Parameter Adjustment

1. Plug the unit directly into a grounded wall outlet. Avoid extension cords or daisy-chained power strips due to high wattage requirements.
2. Toggle the main power switch to the `ON` position.
3. **Temperature control:** Set the target temperature via the digital interface or analog dial.

External Temperature Probes

If using an external temperature probe (thermocouple), ensure it is securely immersed in the solution and not resting directly against the bottom or side walls of the glassware.

4. **Stirring control (if applicable):** Place the stir bar flat inside the container. Turn the RPM dial slowly from zero to the target velocity. Sudden adjustments can cause the stir bar to decouple (spin erratically), requiring the dial to be reset to zero to re-engage.

7.4. Shutdown and Post-Run Monitoring

1. Turn off the heating and stirring functions by switching controls to `0` or pressing `Stop`.
2. Turn the main power switch to `OFF`.
3. **Hot Top warning:** If the unit features a "Hot Top" indicator light, leave the unit plugged in until the surface drops below 50 °C and the warning light turns off.
4. Once completely cool, wipe down any condensed moisture or accidental spills.

8. Quality Control and Acceptance Criteria

- Verify the set temperature against an independent calibrated thermometer or thermocouple before use with critical samples.
- If the measured temperature deviates by more than ±5 °C from the set point, remove the unit from service and arrange calibration or repair.

9. Waste Disposal

Waste Type	Container	Disposal Method
Chemical residues	Appropriate waste jug	Per chemical-specific SDS
Damaged glassware	Sharps container	Standard glass waste

10. Maintenance

- **Monthly:** Wipe aluminum dry blocks with a mild laboratory detergent to remove oxidation or mineral buildup. Never use steel wool or abrasive pads.
- **Annually:** Calibrate temperature readouts using a calibrated surface thermometer or reference thermocouple.

11. References

Consult the manufacturer's manual for model-specific temperature calibration procedures and replacement parts. No external peer-reviewed references apply to this general equipment SOP.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Flammable solvent handling
L111	Muffle Furnace	Higher-temperature heating

SOP	Title	Relationship
L409	Microbial Biomass Carbon	Uses dry bath at 56 °C
L315	eDNA Processing	Uses dry bath for extraction

13. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L109

Thermo Scientific Sorvall ST8R Centrifuge

Standard Operating Procedure

SOP Number	SOP-L109
Revision	0.2
Author(s)	Micah Maglasang
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe setup, operation, and maintenance of the Thermo Scientific Sorvall ST8R centrifuge. The centrifuge is used to separate substance mixtures of different densities and is integral to sample preparation workflows across multiple laboratory SOPs (Thermo Fisher Scientific, 2016).

2. Scope

- **Applies to:** Thermo Scientific Sorvall ST8/8R centrifuge in the EA Program laboratory
- **Does not apply to:** Clinical centrifuges (see *SOP-L108*), microcentrifuges (see *SOP-L26*), or field-portable centrifuges
- **Location:** EA Program laboratory, Pomona College

3. Definitions

- **Balancing:** The process of pairing tubes of equal mass (within 0.1 g) in opposite rotor positions; required to prevent rotor imbalance, vibration, and bearing damage; failure to balance is the leading cause of centrifuge damage and injury (Thermo Fisher Scientific, 2016).
- **Fixed-angle rotor:** A rotor that holds tubes at a fixed angle (typically 20–45° from vertical); particles pellet against the side and bottom of the tube.
- **g-force (relative centrifugal force, RCF):** The centrifugal acceleration expressed as a multiple of gravitational acceleration (9.81 m s^{-2}); calculated as $\text{RCF} = 1.118 \times 10^{-5} \times r \times N^2$ where r is the rotor radius in cm and N is speed in rpm (Thermo Fisher Scientific, 2016).
- **Pellet:** The solid material sedimented to the bottom (or side-bottom) of a centrifuge tube after centrifugation.
- **Supernatant:** The liquid layer remaining above the pellet after centrifugation; the fraction of interest in many extraction procedures.
- **Swinging-bucket rotor:** A rotor whose buckets pivot to a horizontal position during spinning; particles pellet at the base of the tube, producing a well-defined pellet and cleaner supernatant.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Schedule maintenance, verify rotor condition
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Rotating rotor stores significant kinetic energy; rotor or bucket failure can eject debris. Maintain a 30 cm clear zone around the centrifuge during operation. Never open the door until the rotor has completely stopped and the display confirms it (Thermo Fisher Scientific, 2016).
- **Chemical:** Corrosive samples can damage rotors and accessories if not cleaned promptly

after use.
- **Biological:** Pathogenic or biohazardous samples require sealed tubes or biosafety rotor lids. The centrifuge is not aerosol-tight in the event of mechanical failure (World Health Organization, 2020).

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling samples)

5.2. Emergency Procedures

In the event of a hazardous spill or rotor failure:

1. Turn off the power supply immediately; pull the power plug if necessary.
2. Leave the area and do not attempt to open the centrifuge.
3. Contact the lab manager and refer to SOP-L003 Hazardous Materials Handling.
4. The emergency door release may be used only to recover samples during a power failure, not during mechanical failure (Thermo Fisher Scientific, 2016).

6. Equipment and Materials

Item	Notes
Thermo Scientific Sorvall ST8/8R centrifuge	(Thermo Fisher Scientific, 2016)
Power supply cable	Grounded outlet required
Rotors and buckets	Must be approved for this centrifuge
Instruction manual	Keep accessible near the instrument
Balance tubes	For balancing samples before runs

7. Procedure

7.1. Site Selection and Setup

1. Operate the centrifuge indoors only.
2. Place on a horizontally leveled, rigid surface with adequate load-bearing capacity.
3. Maintain a safety zone of at least 30 cm around the centrifuge; do not place any hazardous substances within this zone (Thermo Fisher Scientific, 2016).
4. Ensure the setup location is well-ventilated and not exposed to heat or direct sunlight.
5. Plug only into properly grounded sockets.
6. Keep the main plug freely accessible at all times.

7.2. Preparation

1. Inspect the rotor, buckets, and accessories for signs of corrosion, cracks, or removed protective coating; do not use damaged components (Thermo Fisher Scientific, 2016).
2. Do not modify the mechanical components of the rotor or alter any electronic components of the centrifuge.
3. Load the rotor properly: always balance samples symmetrically.
4. Never overload the rotor beyond its rated capacity.
5. Verify that the rotor is locked securely into place before closing the door.
6. When working with corrosive samples, ensure tubes are fully sealed.

! Hazardous Substances

- Do not centrifuge explosive or flammable materials.
- Do not centrifuge toxic, radioactive, or pathogenic materials without appropriate safety precautions and sealed rotor lids (World Health Organization, 2020).
- The centrifuge is neither inert nor explosion-protected; never operate in an explosion-prone environment.
- Use fully sealed tubes for highly corrosive substances that could impair rotor mechanical stability.

7.3. Operation

1. Close the centrifuge door before starting a run.
2. Set the desired speed, temperature (8R model), and time using the control panel.
3. Press START to begin the run.
4. Do not move, lean on, or place anything on top of the centrifuge while it is running.
5. Minimize time spent near the centrifuge during operation (Thermo Fisher Scientific, 2016).
6. Wait until the rotor has come to a complete stop and the display confirms it before opening the door.
7. Never open the centrifuge while the rotor is spinning.

Emergency Stop

Press the STOP key to shut down the centrifuge at any time. In an emergency, pull the power supply plug to disconnect power immediately (Thermo Fisher Scientific, 2016).

7.4. Shutdown and Cleanup

1. Remove samples promptly after the run is complete.
2. Inspect the rotor and chamber for spills or condensation.
3. If corrosive samples were centrifuged, clean the rotor, buckets, and chamber thoroughly with deionized water and dry before storage (Thermo Fisher Scientific, 2016).
4. If toxins or pathogenic substances have contaminated the centrifuge, carry out appropriate disinfection before further use (World Health Organization, 2020).

5. Wipe down the exterior of the centrifuge.
6. Leave the door slightly ajar to allow the chamber to dry (if no hazardous residues are present).

7.5. Cover Panel and Door Safety

1. Never operate the centrifuge if cover panels are damaged or missing.
2. Do not attempt to bypass the door interlock mechanism.
3. If mechanical failure occurs (rotor or bucket crash), the centrifuge is not aerosol-tight; leave the room and contact the lab manager (World Health Organization, 2020).

8. Quality Control and Acceptance Criteria

- Visually inspect the rotor and accessories before each use for cracks, corrosion, or wear (Thermo Fisher Scientific, 2016).
- Verify that samples are properly balanced before every run.
- Confirm the door closes and latches securely.
- Report any unusual vibrations, noises, or error messages to the lab manager immediately.
- Follow the manufacturer's maintenance schedule in the instruction manual (Thermo Fisher Scientific, 2016).

9. Waste Disposal

Waste Type	Disposal Method
Contaminated cleaning materials	Dispose per SOP-L003 if hazardous chemicals were involved
Damaged rotors or accessories	Contact lab manager for proper disposal
Biohazardous spill materials	Autoclave before disposal

10. References

- Thermo Fisher Scientific (2016)
- World Health Organization (2020)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Thermo Fisher Scientific. (2016). *Sorvall ST8 and ST8R centrifuge: Instruction manual*. https://www.thermofisher.com/us/en/home/life-science/lab-equipment/centrifuges/benchtop-centrifuges/sorvall-st8.html

World Health Organization. (2020). *Laboratory biosafety manual* (4th ed.). World Health Organization. https://www.who.int/publications/i/item/9789240011311

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials	Chemical and biological spills
L403	KCl Extraction	Uses centrifuge for soil extraction
L409	Microbial Biomass Carbon	Uses centrifuge for extract clarification

12. Training Record

Before operating the Sorvall ST8R centrifuge independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the manufacturer's instruction manual (Thermo Fisher Scientific, 2016)
4. Observe a trained operator perform rotor loading, a centrifuge run, and post-run cleanup
5. Demonstrate proper sample balancing, rotor installation, and emergency shutdown under supervision
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L111

Muffle Furnace Operation

Standard Operating Procedure

SOP Number	SOP-L111
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the safe operation, temperature programming, and maintenance of the muffle furnace used in the EA Program laboratory. The muffle furnace provides controlled high-temperature combustion (up to 1,100 C) for loss-on-ignition analysis (SOP-L406), pre-combustion of crucibles and glass fiber filters, ashing of organic materials, and flux fusion preparations.

2. Scope

- **Applies to:** All use of muffle furnaces in EA Program laboratories, including routine ignition (550 C for SOM), high- temperature ashing (up to 1,000 C), and pre-combustion of labware
- **Does not apply to:** Drying ovens operating below 300 C (see SOP-L105); tube furnaces for elemental analysis (integral to analytical instruments); kilns
- **Location:** EA Program laboratory, SGM 134

3. Definitions

- **Ashing:** High-temperature combustion of organic matter in an open crucible in the muffle furnace, leaving only the inorganic (mineral) residue; the basis of loss-on-ignition analysis.
- **Crucible:** A heat-resistant container (ceramic, porcelain, or platinum) used to hold samples during furnace ignition; must be pre-ignited to constant mass before use.
- **Loss on ignition (LOI):** The mass loss when a dried sample is ignited at 550 °C for 4 hours, expressed as a percentage of dry mass; used as a proxy for soil organic matter content.
- **Muffle:** The interior chamber of the furnace, thermally isolated from the heating elements; provides a controlled oxidizing atmosphere for combustion.
- **Pre-combustion:** Heating glassware, crucibles, or glass fiber filters to 450–550 °C to destroy any organic contamination before use in carbon or organic matter analyses.
- **Ramping:** Programming the furnace to increase temperature at a controlled rate (e.g., 5 °C min^{-1}) to prevent thermal shock to crucibles and to avoid rapid oxidation that can cause sample loss.
- **Set point temperature:** The target temperature programmed into the furnace controller; verify with a calibrated thermocouple if precise temperature is critical.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize furnace use
Lab Manager	Schedule maintenance, verify thermocouple calibration
Trained Personnel	Operate the furnace per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

Hazards

- **Thermal:** The furnace exterior, door, and chamber reach extremely high temperatures. Crucibles removed from the furnace at 550 C or above can cause severe burns on contact. The furnace remains hot for hours after shutdown.
- **Fire:** Combustible materials (paper, wood, plastic) placed on or near the furnace can ignite. Keep a 30 cm clear zone around the furnace on all sides.
- **Fumes:** Combustion of organic matter produces CO_2, water vapor, and potentially odorous or irritating fumes. Ensure adequate room ventilation or connect the furnace exhaust to a fume hood or duct if available.
- **Electrical:** The furnace draws high current. Do not use extension cords. Inspect the power cord for damage before each use.

5.1. Required PPE

- ☐ Heat-resistant gloves (rated to at least 500 C)
- ☐ Safety glasses or face shield
- ☐ Lab coat (cotton preferred; synthetic fabrics melt)
- ☐ Closed-toed shoes

5.2. Emergency Procedures

For burns, apply cool running water immediately and seek first aid. If the furnace produces unusual smoke, odors, or sparks, shut it off at the circuit breaker and evacuate the room. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

Item	Notes
Muffle furnace (programmable)	SGM 134; confirm maximum temperature rating
Crucible tongs (long-handled, heat-rated)	For placing and retrieving crucibles
Ceramic crucibles or porcelain boats	Pre-combusted before first use
Heat-resistant pad or ceramic tile	For resting hot crucibles during transfer to desiccator
Desiccator with active desiccant	For cooling crucibles before weighing
Thermocouple or secondary thermometer	For periodic temperature verification

7. Procedure

7.1. Pre-Use Inspection

1. Inspect the furnace chamber for debris, cracked insulation, or damage to the heating elements. Do not operate if damage is visible; report to the lab manager.
2. Verify that the area around the furnace is clear of combustible materials (minimum 30 cm clearance on all sides and top)
3. Confirm that the furnace is plugged directly into a dedicated wall outlet (no extension cords or power strips)

7.2. Loading

1. Arrange crucibles or boats inside the chamber so they do not touch each other or the heating elements. Leave space for air circulation.
2. Close the furnace door gently; do not slam (the refractory lining is fragile)

7.3. Temperature Programming

1. Set the target temperature and ramp rate per the analytical SOP:
   - Loss on ignition (SOP-L406): ramp to 550 C, hold 4 hours
   - Pre-combustion of glass fiber filters: ramp to 450 C, hold 4 hours
   - Pre-combustion of crucibles: ramp to 550 C, hold 1 hour
   - Ceramic boat preparation (SSM, SOP-L407): ramp to 900 C, hold 30 minutes
2. If the furnace is programmable, set the ramp rate to approximately 10 C per minute unless the analytical SOP specifies otherwise
3. Start the program and verify that the displayed temperature begins to rise

> **Tip**
>
> Avoid placing samples into a furnace that is already at full temperature unless the analytical SOP explicitly requires it. Rapid thermal shock can crack crucibles and cause spattering.

7.4. During Operation

1. Do not leave the furnace unattended during the initial ramp-up (first 30 minutes). Check that the temperature is tracking the set point.
2. Post a “Furnace in Use” sign on the furnace and the lab door, noting the expected completion time
3. Do not open the furnace door during the hold period unless troubleshooting

7.5. Unloading

1. After the hold period, turn off the furnace or allow the programmed cooldown to proceed
2. Allow the furnace to cool to below 200 C before opening the door. Opening at higher temperatures causes rapid thermal shock to samples and exposes the operator to radiant heat.
3. Using crucible tongs and heat-resistant gloves, transfer crucibles to a heat-resistant pad, then immediately to the desiccator
4. Allow crucibles to cool to room temperature in the desiccator (approximately 30–45 minutes) before weighing

7.6. Shutdown

1. Verify that the furnace is off and the temperature is decreasing
2. Remove the "Furnace in Use" sign
3. Leave the furnace door slightly ajar (1–2 cm) after it has cooled below 100 C to prevent moisture buildup inside the chamber

8. Maintenance

Task	Frequency
Visual inspection of chamber and elements	Before each use
Clean furnace floor of spilled material	After each use
Thermocouple verification (compare to NIST-traceable thermometer)	Annually or after suspected malfunction
Replace heating elements	As needed (contact manufacturer or lab manager)
Check door seal and gasket	Every 6 months

> **!** Important
>
> Never use water or wet cloths to clean the furnace interior. Allow the chamber to cool completely, then brush out ash and debris with a dry, soft brush.

9. Quality Control

- Verify furnace temperature with an independent thermocouple or NIST-traceable thermometer at least annually. The furnace set point and the verified temperature should agree within +/- 10 C at 550 C.
- Record the verification date and result in the instrument logbook.
- If temperature uniformity is critical (e.g., for large batches), place thermocouples at the front, center, and back of the chamber and record any gradient.

10. Waste Disposal

Waste Type	Disposal Method
Combusted soil ash	Standard trash (inert mineral residue)
Crucibles	Clean and reuse
Failed heating elements	E-waste; coordinate with campus facilities

11. References

- Furnace manufacturer's operating manual (consult for model-specific instructions and maximum temperature rating)
- Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments. *Journal of Paleolimnology*, 25, 101–110.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L105	Drying Ovens	Low-temperature companion
L200	Balance Calibration	Pre- and post-ignition weighing
L406	Loss on Ignition	Primary application SOP
L407	Total Carbon	Ceramic boat preparation

13. Training Record

Before operating the muffle furnace independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate proper use of heat-resistant gloves and crucible tongs
4. Load, program, and unload the furnace under supervision for one complete cycle
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L112

Autoclave and Steam Sterilization

Standard Operating Procedure

SOP Number	SOP-L112
Revision	0.1
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1. Purpose

This procedure describes the safe operation and maintenance of the laboratory autoclave for steam sterilization of glassware, media, reagent water, and biohazardous waste. Autoclaving uses saturated steam under pressure (typically 121 C at 15 psi for 15–30 minutes) to achieve sterilization

by denaturing proteins and nucleic acids in microorganisms. In the EA Program, the autoclave is used to sterilize equipment for microbiological analyses (e.g., SOP-L305 Colilert), to decontaminate foreign soil samples (SOP-L015), and to sterilize waste before disposal.

2. Scope

- **Applies to:** All use of the laboratory autoclave in EA Program laboratories for sterilization of liquids, solids, glassware, and biohazardous waste
- **Does not apply to:** Dry heat sterilization (use muffle furnace or drying oven); chemical sterilization (e.g., bleach, ethylene oxide); filter sterilization (see SOP-L114)
- **Location:** *TBD* (confirm autoclave location; shared facility or SGM)

3. Definitions

- **Autoclave:** A pressurized vessel that uses saturated steam to achieve sterilization by heating materials to 121 °C at 15 psi (103 kPa) for a defined exposure time.
- **Biological indicator:** A standardized preparation of resistant bacterial spores (typically *Geobacillus stearothermophilus*) used to verify that the autoclave cycle achieved sterilization; must be run periodically to validate cycle effectiveness.
- **Chemical indicator (autoclave tape):** Tape printed with a dye that changes color when exposed to steam and heat; indicates that the item was exposed to the cycle but does not confirm sterilization.
- **Gravity displacement cycle:** A standard autoclave cycle in which steam enters from the top, displaces cooler air downward and out through a drain; used for wrapped instruments, glassware, and liquid media.
- **Liquid cycle:** An autoclave cycle with a slow exhaust phase to prevent superheated liquids from boiling violently when pressure is released; required for all liquid loads.
- **Sterilization:** The complete elimination of all viable microorganisms, including bacterial endospores; achieved by saturated steam at 121 °C for at least 15 minutes at 15 psi.
- **Wrapped load:** Items wrapped in autoclave paper or placed in autoclave pouches before sterilization; wrapping maintains sterility after the cycle.

4. Responsibilities

Role	Responsibility
Lab PI	Authorize autoclave use, approve waste protocols
Lab Manager	Schedule maintenance, verify biological indicators
Trained Personnel	Operate the autoclave per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Thermal/Pressure:** The autoclave operates at 121 C and 15 psi. Pressurized steam causes severe burns. Never attempt to open the door while the chamber is pressurized.
- **Scalding liquids:** Autoclaved liquids are superheated and can boil violently (bumping) when disturbed. Allow liquids to cool inside the chamber for at least 10 minutes after the cycle completes, and open the door slowly.
- **Glassware failure:** Cracked or starred glassware may shatter under pressure. Inspect all glassware before loading.
- **Biological:** Biohazardous waste must be contained in autoclave bags. Assume the exterior of bags is contaminated until the cycle is verified.

5.1. Required PPE

☐ Heat-resistant gloves (autoclave gloves, rated for steam)
☐ Safety glasses or face shield
☐ Lab coat
☐ Closed-toed shoes

5.2. Emergency Procedures

For steam burns, flush with cool running water for at least 20 minutes and seek medical attention. If the autoclave fails to depressurize, do not force the door; shut off power and contact the lab manager. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

Item	Notes
Laboratory autoclave (gravity or pre-vacuum)	Confirm model and location with lab manager
Autoclave-safe trays (stainless steel or polypropylene)	For containing loads
Autoclave bags (clear or orange, printed with biohazard symbol)	For waste sterilization
Autoclave tape (indicator tape)	External indicator; changes color at 121 C
Biological indicator ampoules (e.g., *Geobacillus stearothermophilus* spore strips)	For periodic verification
Chemical integrator strips (Class 5)	Internal indicator in each load
Aluminum foil	For covering flask openings
Heat-resistant secondary containers	For liquids (to contain boil-over)

7. Procedure

7.1. Pre-Use Inspection

1. Check the water level in the autoclave reservoir; refill with distilled or deionized water if below the minimum mark
2. Inspect the door gasket for cracks, brittleness, or debris; clean if needed
3. Verify that the drain screen/strainer is clear of debris
4. Ensure the pressure gauge reads zero and the chamber is at ambient temperature

7.2. Loading

1. Select the appropriate cycle for the load type:

Load Type	Cycle	Temperature	Time	Notes
Wrapped instruments, glassware	Gravity	121 C	30 min	Loosely pack for steam penetration
Liquids (media, water)	Liquid / Slow exhaust	121 C	15–30 min	Leave caps loose or cover with foil; use slow exhaust to prevent boil-over
Biohazardous waste	Gravity	121 C	60 min	Double-bag in autoclave bags; do not seal bags tightly
Foreign soil (SOP-L015)	Gravity	121 C	30 min	Per SOP-L015 requirements

2. Place items in autoclave-safe trays. Do not overload; leave space for steam circulation.
3. For liquids: loosen caps or cover with foil. Place containers in a secondary tray to catch boil-over.
4. For waste bags: leave bags slightly open or use bags with steam-permeable patches. Place bags upright in a tray.
5. Place a chemical integrator strip inside the center of the load (the coldest point)
6. Apply autoclave indicator tape to the outside of each package or bag

7.3. Running the Cycle

1. Close and latch the autoclave door securely
2. Select the appropriate cycle (see table above)
3. Start the cycle and record the start time in the autoclave logbook
4. Monitor the temperature and pressure gauges during the initial pressurization to confirm normal operation
5. Do not leave the area until the autoclave has reached operating temperature and pressure

7.4. Unloading

1. When the cycle is complete, verify that the chamber pressure has returned to zero before opening the door
2. Crack the door open 1–2 cm and wait 2–5 minutes to allow residual steam to vent (prevents steam flash and liquid bumping)
3. Wearing autoclave gloves and face shield, open the door fully
4. Allow liquids to sit in the chamber for an additional 10 minutes after opening the door before removing
5. Remove trays using autoclave gloves; place on a heat-resistant surface away from foot traffic
6. Check the chemical integrator strip: it should show a complete color change indicating adequate steam exposure. If incomplete, the load must be re-processed.
7. Record the cycle completion time, temperature, pressure, and integrator result in the autoclave logbook

7.5. Waste Handling After Sterilization

1. After a successful waste cycle, seal the autoclave bags
2. Sterilized biohazardous waste can be disposed in the regular trash **only if** the institution's waste policy permits this and the biological indicator program confirms efficacy
3. Label the sealed bag with "Autoclaved" and the date
4. If the chemical integrator did not pass, do not dispose; re-run the cycle or contact the lab manager

7.6. Shutdown

1. After the final cycle of the day, drain the chamber if the manufacturer recommends it
2. Leave the door slightly ajar to allow the chamber to dry and prevent odor buildup
3. Wipe the door gasket with a damp cloth to remove debris

8. Verification and Quality Control

- **Chemical indicators:** Include a Class 5 integrator strip in every load. This confirms that the load reached the target temperature for the required duration.
- **Biological indicators:** Run a *Geobacillus stearothermophilus* spore test at least monthly (or per campus EH&S policy). Incubate the exposed ampoule per the manufacturer's instructions. A positive (growth) result means the autoclave failed; quarantine all loads processed since the last successful test and contact the lab manager.
- **Autoclave logbook:** Record every cycle: date, operator, load description, cycle type, temperature, pressure, duration, and indicator results.
- **Annual maintenance:** Schedule a professional inspection and calibration of the pressure gauge, safety valve, and temperature sensor at least annually.

9. Maintenance

Task	Frequency
Check water level in reservoir	Before each use
Clean door gasket	Weekly
Clean drain screen/strainer	Weekly
Run biological indicator test	Monthly
Professional inspection and calibration	Annually
Replace door gasket	As needed (when cracked or leaking)

10. Waste Disposal

Waste Type	Disposal Method
Successfully autoclaved biohazardous waste	Standard trash (if permitted by campus policy)
Failed autoclave loads (indicator did not pass)	Re-process or contact EH&S
Used chemical/biological indicator strips	Standard trash
Autoclave water (drain)	Drain (non-hazardous unless contaminated)

11. References

- CDC/HICPAC. (2008). *Guideline for Disinfection and Sterilization in Healthcare Facilities.* Centers for Disease Control and Prevention.
- Autoclave manufacturer's operating manual (consult for model- specific instructions, cycle parameters, and maintenance schedules)

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L006	Waste Disposal	Waste classification and pickup
L015	Foreign Soil Handling	Autoclave requirement for imported soil
L101	Glassware Cleaning	Pre-sterilization cleaning
L305	Colilert Quanti-Tray	Sterilized glassware requirement

13. Training Record

Before operating the autoclave independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Observe a trained operator run a complete cycle (loading through unloading and indicator verification)
4. Demonstrate proper loading for liquids and for waste (bag preparation, indicator placement, cycle selection)
5. Run one complete cycle under supervision with a passing chemical integrator result
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L113

Laminar Flow Cabinet Operation

Standard Operating Procedure

SOP Number	SOP-L113
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
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1. Purpose

This procedure describes the operation, decontamination, and maintenance of the ESCO laminar flow cabinet (clean bench) used in the EA Program laboratory. The cabinet provides an ISO Class 5 (Class 100) particle-free work environment by passing HEPA-filtered air across the work surface in a unidirectional (laminar) flow pattern. It is used for aseptic work that requires protection of the *sample or product* from airborne contamination, including eDNA extraction setup, PCR master mix preparation, sterile media preparation, and filter handling for microplastics analysis.

> **!** Important
>
> A laminar flow cabinet (clean bench) protects the **work** from contamination. It does **not** protect the **operator** from hazardous aerosols, pathogens, or chemical vapors. For work involving biohazardous materials (BSL-2 organisms, human pathogens), a biological safety cabinet (BSC, Class II) is required. The EA Program does not currently work with BSL-2 materials, but if the scope changes, consult the PI and campus biosafety officer.

2. Scope

- **Applies to:** All use of the ESCO laminar flow cabinet in SGM 134, including eDNA and PCR work, sterile reagent preparation, and microplastics filter handling
- **Does not apply to:** Fume hoods (for chemical vapor protection); biological safety cabinets (BSC, Class II); field work
- **Location:** SGM 134

3. Definitions

- **Biosafety cabinet (BSC):** A ventilated enclosure that protects both the user and the environment from aerosols; classified as Class I, II, or III depending on the level of protection. Not the same as a laminar flow cabinet.
- **HEPA filter (High-Efficiency Particulate Air):** A filter rated to remove at least 99.97% of particles 0.3 µm or larger; used in laminar flow cabinets to produce particle-free air over the work surface.
- **Horizontal laminar flow cabinet:** A clean bench in which HEPA- filtered air flows horizontally from the back of the cabinet toward the user; protects samples from particles but does not protect the user from aerosols (not suitable for hazardous biological agents).
- **Laminar flow:** Smooth, parallel flow of air in one direction at uniform velocity; prevents turbulence that would re-introduce particles to the work surface.
- **UV germicidal lamp:** A short-wave ultraviolet lamp installed in some cabinets to reduce surface contamination between work sessions; effective only on exposed surfaces; never operate with personnel present.
- **Vertical laminar flow cabinet:** A clean bench in which HEPA- filtered air flows downward from the top onto the work surface; the standard configuration in the EA Program lab.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, schedule HEPA filter replacement
Lab Manager	Coordinate annual certification, maintain UV lamp log
Trained Personnel	Operate the cabinet per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **UV radiation:** The cabinet may include a UV germicidal lamp for surface decontamination. UV-C (254 nm) causes skin burns and eye damage. Never operate the UV lamp while hands or face are inside the cabinet. The UV lamp must be off and the sash open before working.
- **Electrical:** The cabinet uses high-voltage blower motors. Do not modify electrical components.
- **Chemical:** If using ethanol or bleach for surface decontamination, follow standard chemical safety precautions.

5.1. Required PPE

☐ Nitrile gloves
☐ Lab coat (clean; lint-free preferred for molecular work)
☐ Safety glasses (if UV lamp has been recently active, as a precaution during the transition)

6. Equipment and Materials

Item	Notes
ESCO laminar flow cabinet	SGM 134; horizontal or vertical flow
70% ethanol spray bottle	For surface decontamination
10% bleach solution (for eDNA work)	Destroys residual DNA on surfaces
Lint-free wipes (Kimtech or equivalent)	For wiping surfaces
UV germicidal lamp (if installed)	Built into cabinet; timer-controlled or manual

7. Procedure

7.1. Startup

1. Ensure the UV lamp is off
2. Raise or open the sash to the working position
3. Turn on the blower fan and allow it to run for at least 15 minutes before beginning work. This purges the cabinet interior and establishes stable laminar flow.
4. While the cabinet purges, wipe down the entire work surface, side walls, and interior of the sash with 70% ethanol using lint-free wipes. For eDNA work, follow the ethanol wipe with a 10% bleach wipe, then a final ethanol wipe to remove bleach residue.
5. Place only the materials needed for the immediate task inside the cabinet. Minimize clutter; objects block airflow and create turbulence.

> **Tip**
>
> Arrange items so that clean (sterile) materials are upstream (closer to the HEPA filter) and used/contaminated items are downstream (closer to the operator). This prevents contaminated air from passing over clean surfaces.

7.2. Working in the Cabinet

1. Wear clean nitrile gloves; spray gloves with 70% ethanol before reaching into the cabinet
2. Work at least 10 cm from the front edge to stay within the laminar flow zone
3. Move slowly and deliberately; rapid arm movements disrupt laminar flow and pull room air into the work zone
4. Do not block the air intake grilles (front and/or rear) with equipment, paper, or notebooks
5. Do not use open flames (Bunsen burners) inside a laminar flow cabinet; they disrupt airflow and can damage the HEPA filter
6. Keep the cabinet running continuously while working; do not turn the blower off between tasks during a session

7.3. Shutdown

1. Remove all materials from the cabinet
2. Wipe the work surface with 70% ethanol (and bleach for eDNA work)
3. Lower the sash if applicable
4. Optionally, turn on the UV lamp for 15–30 minutes for surface decontamination. Ensure no personnel are exposed to UV light during this period. Post a "UV Lamp Active" sign.
5. After UV exposure, turn off the UV lamp
6. Turn off the blower (or leave it running on low if lab policy requires continuous operation to maintain cabinet cleanliness)

7.4. UV Lamp Use

1. The UV lamp is a supplemental decontamination tool, not a substitute for chemical wipe-down
2. Run the UV lamp for 15–30 minutes after each use session
3. Record UV lamp use in the UV lamp log (posted on the cabinet). UV lamps lose efficacy over time; replace after the manufacturer's recommended hours (typically 5,000–8,000 hours).
4. Do not rely solely on UV decontamination for eDNA work; chemical wipe-down with bleach is more effective at destroying DNA.

8. Maintenance

Task	Frequency
Wipe interior with 70% ethanol	Before and after each use

Task	Frequency
Bleach wipe (eDNA/PCR work)	Before and after each use
Check blower operation and airflow	Monthly (hold a tissue at the intake to confirm airflow direction)
Replace pre-filter (if present)	Every 6 months or when visibly dirty
HEPA filter certification (by qualified technician)	Annually
Replace UV lamp	Per manufacturer hours or when annual certification shows reduced germicidal output

9. Quality Control

- Annual HEPA filter certification must confirm that the cabinet meets ISO Class 5 (less than 100 particles per ft^3 at 0.5 um). If it fails, the cabinet must not be used for aseptic work until the filter is replaced and the cabinet re-certified.
- For eDNA/PCR work: periodically swab the work surface and run a PCR negative control to verify that the decontamination procedure is effective
- Log all UV lamp hours to track lamp aging

10. Waste Disposal

Waste Type	Disposal Method
Used wipes (ethanol/bleach)	Standard trash
Used gloves	Standard trash
Spent HEPA or pre-filters	Standard trash (non-hazardous unless contaminated with biohazardous material)
Spent UV lamp	Mercury-containing waste; coordinate with campus EH&S for recycling

11. References

- ESCO Corporation. *Laminar Flow Cabinet User Manual.* (Consult for model-specific instructions.)
- NSF/ANSI 49. *Biosafety Cabinetry: Design, Construction, Performance, and Field Certification.* (Reference standard for BSCs; laminar flow cabinets are distinct but share some maintenance practices.)

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L700	DNA Extraction	eDNA work requiring clean bench
L703	PCR and Gel Electrophoresis	PCR master mix preparation
L308	Microplastics in Water	Filter handling under clean air

13. Training Record

Before operating the laminar flow cabinet independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper startup, surface decontamination, and shutdown
3. Demonstrate correct working technique (arm position, airflow awareness, material arrangement)
4. For eDNA/PCR users: demonstrate the bleach decontamination protocol
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L114

Vacuum Filtration Apparatus

Standard Operating Procedure

SOP Number	SOP-L114
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Author(s)	Marc Los Huertos
Date	2026-06-26
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1. Purpose

This procedure describes the assembly, operation, and maintenance of vacuum filtration systems used in the EA Program laboratory. Vacuum filtration separates suspended solids from liquids by drawing the liquid through a filter membrane or glass fiber filter under reduced pressure. It is used across many analytical methods, including soil and water extract filtration (SOPs L403, L409, L418), microplastics analysis (SOP-L308), chlorophyll extraction (SOP-L310), and eDNA concentration (SOP-L700).

2. Scope

- **Applies to:** All vacuum filtration in EA Program laboratories using filter flasks, filter funnels (Buchner, glass frit, analytical, or Nalgene disposable units), and Fisher Scientific Maxidry vacuum pumps or building vacuum lines
- **Does not apply to:** Syringe filtration (push-through, no vacuum); centrifugal ultrafiltration; field filtration of eDNA samples (covered in SOP-F700)
- **Location:** SGM 133 and SGM 134

3. Definitions

- **Filter funnel:** The upper component of a vacuum filtration assembly that holds the filter membrane and directs liquid onto it; typically borosilicate glass or polysulfone.
- **Filtrate:** The liquid that passes through the filter membrane and collects in the side-arm flask; the fraction of interest in most dissolved-analyte analyses.
- **Glass fiber filter (GFF):** A depth filter composed of borosilicate glass microfibers; commonly 0.7 µm nominal pore size (GF/F); used for chlorophyll, particulate carbon, and TSS analyses.
- **Membrane filter:** A thin polymeric film with a defined pore size (typically 0.45 µm); used to separate dissolved from particulate fractions in water samples.
- **Nominal pore size:** The manufacturer-stated pore size of a filter; actual retention varies by manufacturer and depends on the particle size distribution of the material being filtered.
- **Retentate:** The material retained on the filter membrane; the fraction of interest for TSS, chlorophyll, and particulate analyses.
- **Side-arm (vacuum) flask:** A thick-walled borosilicate flask with a side arm for connection to the vacuum line; designed to withstand the pressure differential during filtration.
- **Vacuum trap:** A secondary flask or bottle interposed between the filtration funnel and the vacuum pump to catch filtrate overflow and protect the pump.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain pumps, replace tubing

Role	Responsibility
Trained Personnel	Operate filtration systems per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Glassware under vacuum:** Filter flasks (Erlenmeyer with sidearm) are under reduced pressure during operation. Cracked or starred flasks can implode. Inspect all glassware before each use; reject any with visible damage.
- **Chemical:** Filtrates may contain hazardous chemicals (acids, solvents). Handle filtrate according to the analytical SOP.
- **Biological:** Samples may contain pathogens. Wear gloves.
- **Sharp edges:** Broken glass fiber filters and fritted glass funnels can cut skin. Handle with forceps.

5.1. Required PPE

☐ Safety glasses (mandatory; protects against glass implosion)
☐ Nitrile gloves
☐ Lab coat

5.2. Emergency Procedures

If a filter flask implodes, do not touch the broken glass. Sweep up fragments with a brush and dustpan (not hands) and dispose in a sharps container or labeled "Broken Glass" box. If contaminated with hazardous chemicals, follow SOP-L004 Chemical Spill Response.

6. Equipment and Materials

6.1. Filtration Hardware

Item	Qty	Location	Notes
Fisher Scientific Maxidry vacuum pump	4	SGM 134	Oil-free diaphragm pump
Filter flask (sidearm Erlenmeyer, 500 mL or 1 L)	Multiple	SGM 134	Borosilicate glass; inspect for cracks
Rubber stopper with hole (matched to funnel stem)	Multiple	SGM 134	Connects funnel to flask
Vacuum tubing (thick-wall rubber or silicone)	–	SGM 134	Check for cracks and kinks

Item	Qty	Location	Notes
In-line vacuum trap (flask or bottle between the filter flask and pump)	1 per pump	SGM 134	Prevents liquid from reaching the pump

6.2. Funnel Types

Funnel Type	Application
Buchner funnel (porcelain or polypropylene)	General filtration with flat filter paper or GF filters
Glass frit funnel (sintered glass, various porosities)	Fine filtration; reusable; requires careful cleaning
Nalgene analytical filter unit (disposable, sterile, 0.45 um PES)	Sterile filtration for microbiological and eDNA work
Millipore glass filter holder (47 mm)	Membrane filtration for water quality (TSS, chlorophyll, microplastics)

6.3. Filter Media

Filter Type	Pore Size	Application
Whatman GF/A (glass fiber)	1.6 um	General particulate filtration
Whatman GF/F (glass fiber)	0.7 um	Chlorophyll, DOC
Whatman 934-AH (glass fiber)	1.5 um	TSS, microplastics (SOP-L308)
Cellulose nitrate membrane	0.45 um	Bacterial enumeration, general
PES membrane (polyethersulfone)	0.45 um	Sterile filtration, eDNA
PVDF membrane	0.45 um	DOC filtration (low carbon blank)
PTFE membrane	0.45 um	Organic solvent filtration

> **Note**
>
> Select the filter type and pore size specified in the relevant analytical SOP. Using the wrong filter can bias results (e.g., cellulose acetate filters leach DOC and are unsuitable for carbon analysis).

7. Procedure

7.1. Assembly

1. Inspect the filter flask for cracks or stars. Do not use damaged flasks.
2. Connect vacuum tubing from the flask sidearm to the vacuum trap inlet, and from the vacuum trap outlet to the pump
3. Place the rubber stopper into the neck of the flask

4. Seat the appropriate funnel into the stopper
5. If using a 47 mm filter holder: assemble the base, place the filter on the fritted support using forceps (avoid touching the filter with fingers), and clamp the funnel top in place

7.2. Pre-Wetting or Pre-Combustion (If Required)

- For gravimetric analyses (TSS): pre-dry and pre-weigh the filter per the analytical SOP
- For DOC filtration: pre-rinse the filter with 50–100 mL of sample or DI water and discard the first rinse (removes filter-leached carbon)
- For pre-combusted GF/F filters (chlorophyll, DOC): filters should have been fired at 450 C for 4 hours per SOP-L111 and stored in a clean container

7.3. Filtration

1. Turn on the vacuum pump. Verify suction by briefly covering the funnel opening with a gloved hand (you should feel resistance).
2. Pour the sample into the funnel slowly and steadily. For particulate-laden samples, add in small aliquots to avoid overloading the filter.
3. Allow the liquid to pass through completely
4. Rinse the funnel walls with a small volume of DI water (or the matrix specified in the analytical SOP) to transfer any adhering particles to the filter
5. Continue vacuum for 10–30 seconds after the last visible liquid has passed through to remove residual moisture (do not over-dry if the filter will be extracted for chlorophyll or DOC)
6. Turn off the vacuum pump
7. Carefully disconnect the funnel from the stopper

7.4. Filter Handling

1. Remove the filter from the funnel using clean forceps
2. Transfer the filter to the appropriate container per the analytical SOP:
    - Aluminum tin (for drying/weighing: TSS)
    - Amber vial with extraction solvent (chlorophyll)
    - Extraction tube (eDNA, microplastics)
    - Petri dish (if storing before analysis)
3. Label the container immediately

7.5. Filtrate Handling

1. If the filtrate is the analyte of interest (e.g., dissolved nutrients, DOC), transfer it from the filter flask to a labeled sample vial using a clean graduated cylinder or by pouring
2. Acidify or preserve the filtrate per the analytical SOP
3. If the filtrate is waste, dispose per SOP-L006

7.6. Cleanup

1. Discard used disposable funnels and filters per the analytical SOP
2. Rinse reusable funnels, stoppers, and filter flasks with DI water immediately after use
3. For glass frit funnels: back-flush with DI water, then soak in an appropriate cleaning solution (acid bath for inorganic work, detergent for general use) per SOP-L101
4. Coil vacuum tubing loosely (do not kink) and store

8. Vacuum Pump Maintenance

Task	Frequency
Check vacuum tubing for cracks/kinks	Before each use
Empty and clean the vacuum trap	After each use session
Verify pump suction (should reach at least 20 in Hg / 500 mmHg)	Monthly
Replace vacuum tubing	Annually or when degraded
Service pump (diaphragm replacement)	Per manufacturer schedule or when suction declines

> **!** Important
>
> Always use a vacuum trap (secondary flask or bottle) between the filter flask and the pump. If liquid reaches the pump, it can damage the diaphragm and contaminate the pump internals.

9. Quality Control

- For analytical methods requiring pre-weighed filters (TSS), follow the weighing QC in the analytical SOP
- For DOC filtration, verify filter blanks by filtering DI water and analyzing the filtrate; blank DOC should be less than 0.5 mg/L
- Inspect all glassware for cracks before every use

10. Waste Disposal

Waste Type	Disposal Method
Used filters (non-hazardous)	Standard trash
Used filters (hazardous matrix)	Chemical waste per SOP-L006
Filtrate (hazardous)	Appropriate waste stream per SOP-L006
Filtrate (non-hazardous)	Drain with water if approved by EH&S
Broken glassware	Sharps container or “Broken Glass” box

11. References

- Fisher Scientific. *Maxidry Vacuum Pump User Manual.*
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater.* 22nd ed. Section 2540: Solids.

12. Related SOPs

SOP	Title	Relationship
L101	Glassware Cleaning	Cleaning funnels and flasks
L111	Muffle Furnace	Pre-combustion of GF filters
L308	Microplastics in Water	Filtration application
L310	Chlorophyll Extraction	Filtration application
L403	KCl Extraction	Extract filtration
L409	Microbial Biomass C	Extract filtration
L700	DNA Extraction	eDNA filter processing

13. Training Record

Before operating vacuum filtration systems independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper assembly of the filtration system (flask, stopper, funnel, trap, pump, tubing)
3. Demonstrate correct filter handling with forceps
4. Perform one filtration under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L115

Sonicator – Ultrasonic Bath and Probe

Standard Operating Procedure

SOP Number	SOP-L115
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation and maintenance of the ultrasonic bath and probe sonicator used in the EA Program laboratory. Sonication uses high-frequency sound waves (typically 20–40 kHz) to generate cavitation bubbles in a liquid medium. The collapse of these bubbles produces localized shear forces that disaggregate particles, disperse samples, degas solutions, enhance extraction, and clean labware. In the EA Program, sonication is used for soil particle dispersion, glassware cleaning, sample homogenization, and cell lysis in DNA extraction workflows.

2. Scope

- **Applies to:** The Fisher Scientific ultrasonic bath (SGM 134) and any probe-tip sonicators available in EA Program laboratories
- **Does not apply to:** Vortex mixing (see future SOP-L104); bead beating for DNA extraction (integral to the extraction protocol in SOP-L700); industrial-scale ultrasonics
- **Location:** SGM 134

3. Definitions

- **Amplitude:** The peak displacement of the sonicator probe tip, expressed as a percentage of maximum; higher amplitude delivers more energy per unit time to the sample.
- **Cavitation:** The formation and violent collapse of microscopic bubbles in liquid exposed to ultrasound; the primary mechanism by which ultrasound disrupts cells and disperses particles.
- **Duty cycle:** For pulsed sonication, the fraction of each cycle during which the probe is active (e.g., 0.5 s on / 0.5 s off = 50% duty cycle); used to limit heat input to sensitive samples.
- **Probe sonicator (ultrasonic processor):** A device that converts electrical energy to ultrasonic vibrations (typically 20 kHz) via a piezoelectric transducer; the probe tip vibrates in direct contact with the sample.
- **Sonication bath:** An ultrasonic cleaning bath in which the sample container is immersed in water through which ultrasound is transmitted; less intense than probe sonication; used for dispersing particles or aiding extraction.
- **Specific energy ($J\ mL^{-1}$):** Total ultrasonic energy delivered per unit volume; the parameter most predictive of cell disruption yield for a given sample type; increases with amplitude, time, and volume.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain sonicator, replace bath water
Trained Personnel	Operate the sonicator per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Hearing:** Ultrasonic baths produce audible noise (buzzing/ humming) during operation. Probe sonicators at high power can produce high-intensity noise. Wear hearing protection if exposure exceeds a few minutes at high power settings.
- **Aerosol generation:** Probe sonication of open samples generates aerosols. If the sample contains hazardous biological or chemical material, sonicate inside a fume hood or biological safety cabinet.
- **Heat:** Extended sonication heats the bath water and the sample. Monitor temperature if heat-sensitive analytes are involved.
- **Skin contact:** Do not immerse hands in the bath while it is operating. Cavitation can damage skin tissue.
- **Glassware:** Thin-walled or cracked glassware may fail under sonication. Use containers rated for ultrasonic use.

5.1. Required PPE

☐ Safety glasses
☐ Nitrile gloves
☐ Lab coat
☐ Hearing protection (for probe sonication at high power or extended bath sonication)

5.2. Emergency Procedures

For chemical spills from broken containers, follow SOP-L004 Chemical Spill Response. If the sonicator sparks, smokes, or produces unusual odors, unplug immediately and report to the lab manager.

6. Equipment and Materials

Item	Location	Notes
Fisher Scientific ultrasonic bath	SGM 134	With lid, timer, and heater (if equipped)
Probe sonicator (if available)	SGM 134	With microtip and standard tip
Rack or beaker holder for bath	SGM 134	Keeps sample containers off the bath floor for uniform sonication
Deionized water	–	For filling the bath
Detergent solution (for cleaning applications)	–	Per SOP-L101

7. Procedure

7.1. Ultrasonic Bath

7.1.1. Setup

1. Fill the bath with deionized water to the manufacturer's recommended level (typically 2–3 cm above the transducer surface or to the fill line). Do not operate the bath without water; this damages the transducer.
2. If using the bath for cleaning, add the appropriate detergent concentration per SOP-L101
3. Degas the bath water by running the sonicator for 5 minutes without a load (degassed water transmits ultrasonic energy more effectively)

7.1.2. Operation

1. Place sample containers (tubes, beakers, flasks) in the bath using a rack or holder. Containers should be partially submerged but not resting on the bath floor (direct contact with the floor can cause hot spots and uneven sonication).
2. Set the timer for the duration specified in the analytical SOP (typical ranges: 1–15 minutes for sample dispersion, 15–30 minutes for glassware cleaning)
3. If the bath has a heater, set the temperature as required (common: room temperature for samples, 40–50 C for cleaning)
4. Start the sonicator. Verify cavitation by observing fine bubbles rising from the bath surface.
5. Do not open the lid or reach into the bath during operation
6. When the timer stops, remove containers using tongs or gloves

7.1.3. Post-Use

1. Turn off the sonicator
2. If the bath water is visibly contaminated, drain, rinse, and refill with fresh DI water
3. Replace the bath water at least weekly even if it appears clean
4. Wipe the exterior with a damp cloth

7.2. Probe Sonicator

7.2.1. Setup

1. Select the appropriate tip (microtip for volumes less than 10 mL; standard tip for 10–50 mL)
2. Attach the tip securely; a loose tip reduces energy transfer and can damage the probe
3. Clamp the probe in a ring stand so the tip is immersed in the sample to approximately one-third of the liquid depth. Do not touch the container walls or bottom with the tip.

7.2.2. Operation

1. Set the power level and pulse settings per the analytical SOP (common: 20–40% amplitude, 5 seconds on / 5 seconds off pulsing to minimize heating)

2. If the sample is hazardous, work inside a fume hood or biological safety cabinet with the sash lowered
3. Start sonication. A faint hissing and visible agitation confirm cavitation.
4. Monitor sample temperature; if overheating is a concern, place the sample container in an ice bath during sonication
5. After the prescribed duration, stop the sonicator

7.2.3. Post-Use

1. Immediately wipe the probe tip with a lint-free tissue dampened with 70% ethanol or DI water
2. For molecular biology work (eDNA, PCR), decontaminate the tip with 10% bleach, rinse with DI water, then wipe with 70% ethanol
3. Inspect the tip for pitting or erosion; a heavily pitted tip reduces performance and should be replaced

8. Maintenance

Task	Frequency
Replace bath water	Weekly
Clean bath interior (drain, wipe, refill)	Weekly
Inspect probe tip for pitting	Before each use
Replace probe tip	When pitted or eroded
Check bath transducer (listen for changes in tone)	Monthly

9. Quality Control

- For soil particle dispersion, verify that the sonication time and power are sufficient by checking particle size distribution results against the analytical SOP's acceptance criteria
- For glassware cleaning, verify cleanliness by rinsing with DI water and checking for water sheeting (clean glass sheets evenly; dirty glass beads)
- If the bath fails to produce visible cavitation, contact the lab manager (the transducer may be failing)

10. Waste Disposal

Waste Type	Disposal Method
Used bath water (no hazardous chemicals)	Drain
Used bath water (with detergent or chemicals)	Dispose per SOP-L006

Waste Type	Disposal Method
Spent probe tips	Standard trash (stainless steel)

11. References

- Fisher Scientific. *Ultrasonic Bath User Manual*. (Consult for model-specific instructions.)
- Mason, T. J. & Lorimer, J. P. (2002). *Applied Sonochemistry: Uses of Power Ultrasound in Chemistry and Processing*. Wiley-VCH.

12. Related SOPs

SOP	Title	Relationship
L101	Glassware Cleaning	Ultrasonic cleaning application
L401	Soil Texture (Hydrometer)	Particle dispersion
L700	DNA Extraction	Cell lysis application

13. Training Record

Before operating the sonicator independently, personnel must:

1. Read this SOP in full
2. Demonstrate proper bath fill level and degassing
3. For probe sonicator users: demonstrate tip attachment, immersion depth, and power/pulse settings under supervision
4. Perform one sonication task under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L116

Sample Grinding and Homogenization – Ball Mill, Mortar and Pestle

Standard Operating Procedure

SOP Number	SOP-L116
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the grinding and homogenization of dried soil, sediment, plant tissue, and other solid environmental samples to produce a fine, uniform powder suitable for chemical, elemental, and isotopic analysis. Many analytical methods require a homogeneous subsample of 0.5–20 mg (e.g., EA-IRMS capsules, SOP-L414) or 0.5–1.0 g (e.g., acid digestion, SOP-L410, SOP-L411). Without thorough grinding and homogenization, small subsamples drawn from an incompletely mixed bulk sample may not represent the true composition of that sample, introducing analytical error unrelated to the instrument or method (Bremner, 1996; Nelson & Sommers, 1996). Particle-size reduction to ≤ 150 μm also improves combustion completeness in high-temperature elemental analyzers and reduces between-capsule mass variance in EA-IRMS work (Sollins et al., 1996; Thermo Fisher Scientific, 2010).

2. Scope

- **Applies to:** Dry grinding and homogenization of air-dried or oven-dried (60–105 °C) solid samples, including mineral soils, organic soils, sediments, dried plant tissue, compost, and biochar, using agate or steel mortar and pestle, ball mill, or mechanical grinder.
- **Does not apply to:** Wet grinding or homogenization of fresh tissue (cryogenic grinding for molecular biology applications); sieving-only sample preparation (see SOP-F400 for 2 mm sieving); bead beating for DNA extraction (integral to SOP-L700); ball milling specifically for TC and EA-IRMS (see SOP-L117).
- **Location:** EA Program laboratory, SGM 134, Edmunds 017 - Rock Grinding

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP
Lab Manager	Maintain grinding equipment, replace worn components
Trained Personnel	Grind and homogenize samples per this SOP
New Students	Complete training before independent use

4. Definitions

Term	Definition
Grinding	Mechanical size reduction of solid particles by impact, compression, or abrasion (Bernhardt, 1994).

Term	Definition
Homogenization	Mixing and size reduction to produce a sample in which any subsample of a given mass has the same composition as any other subsample of that mass (Nelson & Sommers, 1996).
Particle size (d_{50})	Median particle diameter by mass; the most common target for soil analysis is 150 μm (U.S. No. 100 mesh sieve) (Nelson & Sommers, 1996).
Cross-contamination	Transfer of material from a previously processed sample to a subsequent one via residue retained on grinding equipment surfaces (Boutton, 1991).
Sacrificial subsample	A small amount of the upcoming sample ground and discarded before collecting the analytical aliquot, to purge residual material from equipment surfaces (Boutton, 1991).
Respirable crystalline silica (RCS)	Quartz and cristobalite particles $\leq$ 10 μm aerodynamic diameter released during grinding of mineral soils; an IARC Group 1 carcinogen (International Agency for Research on Cancer, 1997).
Air-dried	Dried at ambient temperature ($\leq$ 40 °C) until mass is constant; the standard pre-treatment for 2 mm sieving (Nelson & Sommers, 1996).

5. Safety and Hazards

Hazards

- **Respirable dust and silica:** Grinding dry soil and plant tissue generates fine respirable dust including crystalline silica (quartz), which is an IARC Group 1 carcinogen at particle sizes $\leq$ 10 μm (International Agency for Research on Cancer, 1997). Always perform grinding inside a fume hood or wear a NIOSH-approved N95 respirator.
- **Contaminated soils:** Soils from legacy-contaminated sites (Pb, As, Hg, pesticides, PAHs) pose additional inhalation and dermal hazards during grinding. Consult the Lab PI before processing (U.S. Environmental Protection Agency, 2007).
- **Noise:** Ball mills and mechanical grinders can exceed 85 dB. Wear hearing protection for runs $>$ 1 min (National Institute for Occupational Safety and Health, 1998).
- **Pinch/crush hazards:** Milling cups and clamps are heavy. Secure milling cups before starting the mill and keep hands clear during operation.
- **Heat**: Milling cups may get hot if samples are run for extended periods of time. Use protective gloves to handle hot items, or wait until they have cooled down enough to avoid burns.
- **Cross-contamination:** Residual sample in grinding equipment is the primary quality

risk in sequential grinding. Thorough cleaning between samples is essential (Section 6.5).

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ N95 dust mask or work in fume hood
- ☐ Hearing protection (ball mill or mechanical grinder)

5.2. Emergency Procedures

For dust inhalation, move to fresh air immediately. For cuts from broken equipment, apply first aid per SOP-L002. Report all incidents to the Lab Manager.

6. Equipment and Materials

6.1. Grinding Equipment

Item	Location	Best For
Agate mortar and pestle (various sizes)	SGM 134	Small batches (< 50 g); trace metal work (low contamination)
Ceramic (porcelain) mortar and pestle	SGM 134	General purpose; not for trace metals (contributes Al, Si)
Ball mill (planetary or mixer mill)	Edmunds 017 - Rock Grinding	Small batches (< 5g); estimated particle size
Mechanical grinder (Wiley mill, Thomas mill)	TBD	Plant tissue; coarse initial grinding

6.2. Accessories

Item	Notes
Stainless steel spatula	Transferring sample
Brush (soft bristle, nylon)	Cleaning mortar and milling cups
Lint-free wipes (Kimtech)	Wiping surfaces
70% ethanol spray bottle	Decontamination
Compressed air (can or line)	Blowing dust from crevices
Test sieves (No. 60 = 250 μm; No. 100 = 150 μm; No. 200 = 75 μm)	Verifying particle size

Item	Notes
Labeled sample vials or coin envelopes	Storing ground samples
Analytical balance	Per SOP-L200
Weighing boat/paper	Used for weighing samples

7. Procedure

7.1. Sample Selection and Drying

Samples must be thoroughly dry before grinding. Residual moisture causes particles to clump, impedes size reduction, and can promote microbial activity that alters sample chemistry during storage (Nelson & Sommers, 1996).

1. Use air-dried samples sieved to 2 mm (SOP-F400), or oven-dry per the drying requirements in the table below:

Analytical Pathway	Drying Temperature	Rationale
TC, LOI, metals, or general chemistry	105 °C to constant mass	Standard gravimetric drying; isotope fractionation not a concern (Nelson & Sommers, 1996)
δ^{15}N, δ^{13}C (EA-IRMS)	40–60 °C to constant mass	Temperatures > 60 °C volatilize ^{14}N-enriched NH_4^+, artificially elevating δ^{15}N (Kool et al., 2011; **Guntiñas2012?**)
Plant tissue (any analysis)	60 °C to constant mass	Higher temperatures degrade labile N compounds and alter tissue δ^{15}N (Kalra, 1998)

2. Cool samples to room temperature in a desiccator before grinding.
3. Record drying temperature, start and end times, and initial and final mass in the lab notebook.

7.2. Mortar and Pestle (Manual Grinding)

Manual grinding with mortar and pestle is appropriate for small batches (< 50 g), hard materials requiring careful size control, and laboratories where a ball mill is unavailable (Nelson & Sommers, 1996).

1. Place a clean, dry mortar on a stable bench surface. If working with contaminated soil or generating visible dust, work inside a fume hood.

2. Add a small amount of dried sample (5–20 g) to the mortar.
3. Grind with a circular and pressing motion, periodically scraping material from the sides back to the center.
4. Continue grinding until the sample passes through the target sieve (No. 60 [250 μm] for general chemistry; No. 100 [150 μm] for EA-IRMS (Thermo Fisher Scientific, 2010)). For plant tissue, grind to a uniform fine powder.
5. Transfer the ground sample to a labeled vial or coin envelope with a clean spatula.
6. Record the sample ID and particle size achieved.

> **Tip**
>
> For hard or fibrous samples (e.g., woody roots, calcium carbonate nodules), pre-crush coarsely with a hammer inside a thick plastic bag before transferring to the mortar. This reduces grinding time and wear on the mortar.

7.3. Ball Mill (Mechanical Grinding)

Ball milling provides more reproducible particle size reduction and higher throughput than manual grinding, and is preferred for batches > 20 samples or where particle size uniformity is critical (Bernhardt, 1994).

1. Select the appropriate milling cup and ball material (*confirm which materials we have for balls/jars*):

Material	Best Use Case	Notes
Stainless steel	General chemistry (non-metal)	Faster; may contribute Fe, Cr, Ni, Mn
Ceramic	TBD	TBD

2. Load the sample and milling balls into the milling cup. The milling cup's volume should be occupied roughly by 1/3 sample, 1/3 grinding ball(s) and 1/3 air.

> **Safe Running**
>
> - **Filling:** Sample quantity should not be below 25% of the milling cup's holding capacity.
> - **Material** Milling ball and milling cup material must always be the same.
> - **Single Running** Do not run machine with only one milling cup loaded. If you only need to use one milling cup, still load the second one completely empty (no sample nor milling balls)

3. Tightly seal milling cup and load it into the milling cup holder. Make sure to insert cups in the correct orientation, as shown below:



Figure 1: *Orientation of milling cup for left hand side holder. Cup should be mirrored for the right hand side holder.*

4. Turn locking pin into the locked position as shown in the image below and turn the handwheel until maximum clamping range is reached. Close hood.



Figure 2: *Locked and unlocked pin positions*

5. Set speed and duration per the table below or per the analytical SOP:

Sample Type	Frequency (Hz)	Duration	Target Size
Organic soil	30	2 min	≤ 75 μm
Quartz Sand	30	1 min	N/A

6. Run the mill. If the milling cup becomes warm to the touch, alternate 2-minute grinding intervals with 1-minute rest periods to prevent thermal alteration of labile organic matter and N compounds (Kool et al., 2011).
7. After run ends, open the hood and unlock the locking pin before removing milling cups. Wait 30 seconds after stopping before opening the milling cup (dust settles). Open inside the fume hood or with N95 protection.
8. Transfer the ground sample to a labeled container.
9. If required, verify particle size by passing a subsample through the target sieve.
10. Perform cleaning run with quartz sand in between sample runs. Add the same volume of quartz sand as the samples you are running, and the milling balls you are using. The quartz run will help clean the cups and remove any sample that might have gotten stuck to the cup. After the quartz run, clean cup thoroughly according to the "Cleaning Between Samples" section.

7.4. Mechanical Grinder (Plant Tissue)

A Wiley-type or Thomas-type cutting mill provides rapid coarse grinding of dried plant tissue and is generally faster than mortar and pestle for batch processing (Kalra, 1998).

1. Set the screen mesh to the target particle size (1 mm [No. 18] or 0.5 mm [No. 35]).
2. Feed dried tissue into the hopper slowly; do not overload.
3. Collect ground material from the output chamber.
4. For analyses requiring finer particle size (e.g., EA-IRMS), follow with mortar and pestle or ball mill to reduce further (Thermo Fisher Scientific, 2010).

7.5. Cleaning Between Samples

Cross-contamination is the primary quality risk in sequential grinding. For isotope work, residues as small as 0.05 mg C from a high-carbon sample can produce measurable δ^{13}C bias in a subsequent low-carbon sample (Boutton, 1991). Clean thoroughly between every sample:

1. Disassemble the mortar/pestle or milling cup and balls.
2. Brush out all visible residue with a soft-bristle brush.
3. Wipe all surfaces with a lint-free tissue dampened with 70% ethanol.
4. **Trace metal work:** rinse with 10% HNO_3 (trace-metal grade), then triple-rinse with Type I deionized water (18.2 MΩ), and air-dry (U.S. Environmental Protection Agency, 2007).
5. **Isotope work:** rinse with Type I deionized water, wipe with ethanol, and blow dry with clean compressed air.

6. **Wiley mill:** run a small amount of clean quartz sand through the mill between samples to abrade residues from the cutting chamber, then brush and wipe.
7. Allow all equipment to dry completely before grinding the next sample.

> **!** Important
>
> When transitioning between samples with very different compositions (e.g., high-carbon compost followed by low-carbon mineral soil), grind a sacrificial subsample of the next sample and discard it before collecting the analytical aliquot. This purges micro-residues that ethanol-wiping alone may not remove (Boutton, 1991). This step is mandatory for EA-IRMS work.

7.6. Storage

1. Transfer ground powder to a labeled vial (glass or polypropylene with a polyethylene-lined cap). Avoid foam-gasketed caps, which can off-gas organic vapors.
2. Label with: sample ID, date ground, equipment and milling cup material, and target particle size.
3. Store in a desiccator at room temperature. Ground samples have large surface area and are hygroscopic; even brief exposure to humid air can add 0.5–2% mass as adsorbed water (Nelson & Sommers, 1996).
4. For long-term storage (> 2 weeks), seal with Parafilm and store at $-$20 °C.
5. Record grinding date, equipment, milling cup material, speed, and duration in the lab notebook and sample tracking spreadsheet.

8. Quality Control

QC Check	Frequency	Acceptance Criterion
Particle size verification (sieve)	$\geq$ 10% of samples per batch	$\geq$ 90% by mass passes target sieve; re-grind failures
Equipment blank (clean milling cup 2 min)	Once per batch and after new milling cup	No visible powder; TC blank $<$ 0.05 mg C if applicable
Certified reference material (CRM) – trace metals	1 per batch (trace metal work)	Recovered elemental concentration within 10% of certified value (U.S. Environmental Protection Agency, 2007)
Reference standard – isotopes (USGS40, USGS41a)	1 per batch (isotope work)	δ^{13}C within $\pm$ 0.5 ‰; δ^{15}N within $\pm$ 0.5 ‰ of certified value (Brand et al., 2014)
Sample replicate (duplicate grind)	1 per 10 samples	%C or %N: CV $\leq$ 5%; isotopes: SD $\leq$ 0.3 ‰
Sacrificial subsample log	Every inter-sample cleaning (isotope work)	Recorded in lab notebook

Document grinding equipment and milling cup material for every batch; different materials introduce different trace elements and the record is essential for troubleshooting contamination (Bernhardt, 1994).

9. Waste Disposal

Waste Type	Disposal Method
Grinding residue (non-hazardous soil/plant)	Standard solid waste or return to sample stock
Grinding residue (contaminated soil)	Hazardous solid waste per SOP-L006
Quartz sand (cleaning aid)	Standard solid waste
Used ethanol wipes	Standard solid waste (non-halogenated)
Worn grinding balls or milling cups (agate, ceramic)	Standard solid waste
Worn grinding components (stainless steel)	Metal recycling
HNO_3 rinse waste	Acid waste per SOP-L006

10. References

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11. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	2 mm sieving before grinding
F608	Plant Tissue Sampling	Dried tissue requiring grinding
L105	Drying Ovens	Pre-grinding drying
L117	Ball Mill Prep for TC and EA-IRMS	Instrument-specific ball mill protocol
L200	Balance Calibration	Weighing ground subsamples
L406	Loss on Ignition	Downstream analysis
L410	Hot-Plate Acid Digestion	Requires ground sample
L411	MARS Microwave Digestion	Requires ground sample
L414	EA-IRMS Combustion	Requires finely ground sample
L415	Carbonate GasBench-IRMS	Requires ground carbonate sample

12. Training Record

Before performing carbonate isotope analysis independently, personnel must:

1. Read this SOP in full and sign the SOP acknowledgment form.
2. Complete SOP-L002 Laboratory Safety training.
3. Demonstrate proper mortar-and-pestle technique including grinding motion and inter-sample cleaning.
4. If using the ball mill, demonstrate proper milling cup loading, speed/time selection, and milling cup cleaning per Section 7.5.
5. Grind at least 5 samples under supervision with acceptable particle size verification (all material passes the target sieve).
6. Be signed off by the PI or Lab Manager.

Trainee	Trainer	Date	Initials

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L200

Analytical Balance Calibration and Operation – Mettler Toledo

Standard Operating Procedure

SOP Number	SOP-L200
Revision	1.0
Author(s)	Marc Los Huertos
Date	2026-06-26
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1. Purpose

This procedure describes the routine calibration, verification, and proper operation of Mettler Toledo analytical balances used in the EA Program laboratory. Accurate mass measurements are fundamental to nearly every quantitative laboratory procedure, including soil moisture determination (SOP-L400), loss on ignition (SOP-L406), DNA extraction (SOP-L700), and chemical reagent preparation. Regular calibration ensures that balance readings are traceable to national standards and within acceptable tolerance for analytical work (Harris & Lucy, 2020; Mettler Toledo, 2023).

2. Scope

- **Applies to:** All Mettler Toledo analytical balances (0.1 mg readability) and precision balances (0.01 g or 0.001 g readability) in the EA Program laboratory
- **Does not apply to:** Portable field balances, top-loading balances used only for rough weighing (greater than 1 g tolerance), or microbalances (0.001 mg readability) requiring specialized calibration procedures
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Accuracy:** The closeness of a measurement to the true (reference) value; assessed by weighing a certified reference weight and comparing the result to the certified mass.
- **Calibration:** Comparison of a balance's readings against reference weights of known mass to establish a correction curve or verify performance within tolerance.
- **Certified reference weight:** A weight whose mass is traceable to national or international measurement standards (NIST or equivalent); classified by tolerance class (OIML E1 through M3 or ASTM 0 through 7).
- **Drift:** Slow, systematic change in balance readings over time, caused by temperature fluctuation, vibration, or electronic aging.
- **External calibration:** Calibration using calibrated weights placed manually on the pan; required on balances without internal weights.
- **Internal calibration (AutoCal):** Automatic calibration using a built-in reference weight that is mechanically engaged by the balance; the Mettler Toledo XPE205 performs internal calibration automatically in response to temperature changes.
- **Linearity:** The degree to which balance readings are proportional to applied mass across the full weighing range; assessed by weighing multiple reference weights spanning the range.
- **Repeatability:** The standard deviation of repeated measurements of the same weight under identical conditions; a key indicator of short-term balance precision.
- **Tare:** The function that zeroes the displayed mass with a container on the pan, so that subsequent readings show only the mass of material added.

4. Responsibilities

Role	Responsibility
Lab PI	Ensure annual professional calibration is scheduled
Lab Manager	Perform monthly verification checks, maintain calibration log
Trained Personnel	Perform daily checks before use, report malfunctions
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Physical:** Calibration weights are dense metal objects; dropping them can cause injury to feet or damage to the balance pan. Handle with forceps only.
- **Chemical:** Some samples weighed on the balance may be hazardous. Refer to the relevant SOP for the material being weighed.

5.1. Required PPE

☐ Lab coat
☐ Nitrile gloves (when handling calibration weights or chemical samples)
☐ Safety glasses (when weighing chemical reagents)

5.2. Emergency Procedures

If a chemical spill occurs on or near the balance, clean the spill per SOP-L003 Hazardous Materials Handling before resuming use. If the balance pan or draft shield is damaged, remove the balance from service and contact the lab manager.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Mettler Toledo analytical balance	Lab bench, SGM 133	*TBD – confirm model number (e.g., ME204, MS205DU, or XPE205)*
Anti-vibration table or stone slab	Lab bench	Balance must sit on a stable, vibration-free surface
Spirit level (built into balance)	On balance base	For leveling verification

6.2. Calibration Weights and Supplies

Item	Notes
ASTM Class 1 or OIML Class E2 calibration weight set	Traceable to NIST; includes 1 mg, 10 mg, 100 mg, 1 g, 10 g, 50 g, 100 g, 200 g (or as appropriate for balance capacity)
Weight handling forceps	Never touch weights with bare hands
Lint-free wipes (Kimwipes)	For cleaning the pan
Compressed air canister (filtered)	For removing dust from the chamber
Calibration log binder or digital log	For recording all checks

7. Procedure

7.1. Balance Placement and Environment

Proper placement is essential for accurate weighing. The balance should remain in the same location and should not be moved unless absolutely necessary (Mettler Toledo, 2023).

1. Place the balance on a dedicated anti-vibration table or stone slab, away from doors, windows, air vents, and foot traffic
2. Keep the balance away from direct sunlight and heat sources (ovens, hot plates), which cause thermal drift
3. Verify the balance is level using the built-in spirit level. Adjust the leveling feet until the bubble is centered.
4. Allow the balance to warm up for at least 30 minutes after being powered on before performing any measurements or calibration (Harris & Lucy, 2020)

7.2. Internal Calibration (FACT/iSOCAL)

Most Mettler Toledo analytical balances are equipped with an internal calibration function (Fully Automatic Calibration Technology, FACT, or iSOCAL) that uses a built-in reference weight (Mettler Toledo, 2023).

1. Ensure the balance is unloaded and the draft shield doors are closed
2. Press the internal calibration button or navigate to **Menu > Calibration > Internal Calibration** (exact path varies by model)
3. The balance will automatically perform the calibration sequence. Do not touch the balance, lean on the bench, or create air currents during this process.
4. When the display reads “Calibration Done” or returns to the weighing screen, record the date and result in the calibration log
5. If the internal calibration fails, repeat the process. If it fails again, remove the balance from service and contact the lab manager.

> **Note**
>
> If the balance is set to automatic FACT mode, it will self-calibrate whenever it detects a temperature change of 1.5 C or more. However, manual internal calibration should still be performed monthly as part of the verification schedule.

7.3. External Calibration Verification (Monthly)

External verification uses traceable reference weights to confirm that the balance is reading accurately across its range. This check does not replace the annual professional calibration but provides ongoing confidence in measurement quality (**aac_guide2019?**).

1. Perform an internal calibration first (Section 6.2)
2. Allow the balance to stabilize with an empty pan and closed draft shield. Tare to read 0.0000 g (for a 0.1 mg balance).
3. Using forceps, place the reference weights on the center of the pan one at a time, starting with the smallest. Record the displayed mass for each weight.
4. Test at a minimum of three points spanning the expected working range of the balance (e.g., low, mid, and high):

Check Point	Reference Weight	Acceptance Criterion (analytical, 0.1 mg)
Low	1 g	Displayed value within +/- 0.5 mg of nominal
Mid	50 g	Displayed value within +/- 0.5 mg of nominal
High	200 g	Displayed value within +/- 1.0 mg of nominal

5. If any reading falls outside the acceptance criterion, re-zero, repeat the measurement, and clean the pan and weight with a lint-free wipe. If the reading is still out of tolerance, perform an external calibration (Section 6.4) or remove the balance from service.
6. Record all results, the date, and the operator initials in the calibration log.

> **Tip**
>
> Always place weights in the center of the pan. Off-center loading (corner loading) can introduce errors, especially on high-resolution balances. Periodically test corner loading by placing a single weight at each corner and comparing readings.

7.4. External Calibration (As Needed)

If the internal calibration or monthly verification indicates the balance is out of tolerance, perform a full external calibration using a traceable reference weight.

1. Navigate to **Menu > Calibration > External Calibration**

2. When prompted, place the specified calibration weight (the balance will display the required mass, typically its full capacity or a specific test weight)
3. Place the weight on the center of the pan using forceps
4. Wait for the calibration to complete and record the result
5. Repeat the verification checks in Section 6.3 to confirm the balance is now within tolerance
6. *TBD – confirm external calibration weight value for the specific Mettler model in the lab*

7.5. Annual Professional Calibration

1. Schedule an annual on-site calibration by a Mettler Toledo service technician or an ISO 17025-accredited calibration laboratory
2. The technician will perform a comprehensive calibration including linearity, eccentricity (corner loading), repeatability, and hysteresis tests
3. Retain the calibration certificate in the lab's quality records binder
4. *TBD – record the current service contract details and contact information for scheduling*

7.6. Daily Use and Best Practices

Follow these practices every time you use the balance to ensure accurate and reproducible results (Harris & Lucy, 2020; Mettler Toledo, 2023):

1. Check that the balance is level before use
2. Close all draft shield doors before taring or reading a mass
3. Allow the reading to stabilize fully (stability indicator appears) before recording a value
4. Use appropriate weighing vessels (aluminum tins, ceramic crucibles, weighing paper, or glass vials depending on the application)
5. Tare the vessel before adding sample material
6. Handle objects with forceps, spatulas, or gloves; fingerprints add mass and oils that cause drift
7. Never place chemicals directly on the pan
8. Clean up any spills immediately with a lint-free wipe or brush
9. Leave the draft shield closed and the balance powered on between uses to maintain thermal equilibrium
10. Record the balance ID, date, and any anomalies in the lab notebook

7.7. Cleaning and Maintenance

1. Wipe the pan and interior of the draft shield with a lint-free wipe after each use
2. Use filtered compressed air to remove dust from hard-to-reach areas inside the weighing chamber
3. If the pan becomes stained or corroded, remove it and clean with mild soap and water; dry thoroughly before replacing
4. Do not use solvents to clean the draft shield (they may damage acrylic or glass)
5. Inspect the draft shield gaskets periodically for wear; replace if they no longer seal properly

8. Quality Control and Acceptance Criteria

- Internal calibration (FACT): at minimum monthly, or after any balance relocation or significant temperature change
- External verification with traceable weights: monthly, recorded in the calibration log
- Annual professional calibration: required; certificate on file
- Daily pre-use level check: required
- Acceptance tolerance for routine analytical work (0.1 mg readability): displayed value within +/- 0.5 mg of the nominal weight for loads up to 100 g, and within +/- 1.0 mg for loads above 100 g
- If tolerance is exceeded after re-calibration, tag the balance "OUT OF SERVICE" and notify the lab manager

8.1. Calibration Log

Maintain a calibration log (paper binder or spreadsheet) with the following columns:

Date	Operator	Check Type	Weight Used (g)	Displayed Value (g)	Pass/Fail	Notes

9. Waste Disposal

No waste is generated by this procedure. Cleaning materials (Kimwipes) can be disposed of in standard trash unless they contacted hazardous chemicals, in which case follow SOP-L003.

10. References

Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.

Mettler Toledo. (2023). *Good weighing practice: The SOP for balances and scales*. https://www.mt.com/us/en/home/library/guides/laboratory-weighing/good-weighing-practice.html

11. Training Record

Before using the analytical balance independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform internal calibration and external verification
3. Demonstrate proper leveling, taring, weighing, and draft shield use
4. Successfully complete a verification check with reference weights under supervision
5. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Reading drifts continuously	Air currents or vibration	Close draft shield doors; check placement away from vents and traffic
Reading drifts slowly in one direction	Temperature change or warm sample	Allow balance to equilibrate; cool samples to room temperature before weighing
Balance does not tare to zero	Residue on pan or overloaded tare	Clean pan; remove vessel and re-tare
Internal calibration fails	Significant temperature change or mechanical issue	Allow 30 min warm-up; retry. If persistent, contact lab manager.
Displayed value differs from reference weight by more than tolerance	Calibration drift	Perform external calibration (Section 6.4). If still out, remove from service.
"Overload" message	Sample exceeds balance capacity	Use a balance with higher capacity
Balance will not power on	Power supply disconnected or fuse blown	Check power connection; contact lab manager
Unstable reading with small masses (less than 10 mg)	Static charge on sample or vessel	Use anti-static device or ionizer; ground yourself before weighing

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L203

Trilogy Laboratory Fluorometer

Standard Operating Procedure

SOP Number	SOP-L203
Revision	0.5
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration, and maintenance of the Turner Designs Trilogy Laboratory Fluorometer, a compact, multifunctional instrument used for fluorescence, absorbance, and turbidity measurements with interchangeable snap-in optical modules. The Trilogy is the primary instrument in the EA Program laboratory for chlorophyll *a* determination and turbidity analysis of water samples (Arar & Collins, 1997; Turner Designs, 2014).

2. Scope

- **Applies to:** Turner Designs Trilogy Laboratory Fluorometer equipped with fluorescence, absorbance, and turbidity optical modules
- **Does not apply to:** Field fluorometers, in-vivo chlorophyll measurements with multiprobes (see SOP-F200), or benchtop spectrophotometers (Hach DR3900; see SOP-L303)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure calibration standards are current
Lab Manager	Maintain optical modules, replace light sources as needed
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Blank correction:** Subtraction of the fluorescence signal from a blank (solvent only, no sample) from sample readings to remove background fluorescence from the solvent and optical components.
- **Chlorophyll *a*:** The primary photosynthetic pigment in phytoplankton and algae; quantified by the Trilogy using a chlorophyll *a* module (excitation ~430 nm, emission ~685 nm); expressed in µg L^{-1} (water) or µg g^{-1} (sediment) (Arar & Collins, 1997).

- **Emission wavelength:** The wavelength at which the fluorophore emits light after excitation; detected by the fluorometer's photodetector.
- **Excitation wavelength:** The wavelength of light used to illuminate the sample; chosen to match the absorption maximum of the target fluorophore.
- **Fluorometer:** An instrument that measures fluorescence intensity emitted by a sample after excitation; more sensitive than spectrophotometry for low-concentration analytes such as chlorophyll *a* and CDOM (Turner Designs, 2014).
- **Linear dynamic range (LDR):** The concentration range over which instrument response is linear; for the Trilogy chlorophyll module, approximately 0–1000 µg/L (Turner Designs, 2014).
- **Module (Trilogy):** An interchangeable optical block that sets the excitation and emission wavelengths for a specific analyte; available modules include chlorophyll *a* (acidification and non-acidification), CDOM, rhodamine WT, and turbidity.
- **Pheophytin *a*:** A degradation product of chlorophyll *a* formed by loss of the central magnesium ion; contributes to total fluorescence and is distinguished from chlorophyll *a* by the acidification step of EPA Method 445.0 (Arar & Collins, 1997).
- **Quinine sulfate unit (QSU):** A fluorescence unit based on the fluorescence of a standard quinine sulfate solution; used for CDOM and some other modules.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Chlorophyll extraction uses 90% acetone, which is flammable and an irritant. Handle in a fume hood. Acidification with 0.1 N HCl is required for EPA 445.0. See SOP-L003.
- **Physical:** Glass test tubes used for extracted chlorophyll measurements can break. Handle with care.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (especially when handling acetone or standards)

5.2. Emergency Procedures

For acetone spills, extinguish all ignition sources, ventilate the area, and clean up per SOP-L003 Hazardous Materials Handling. For skin or eye contact with acetone or acid, flush with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Turner Designs Trilogy Fluorometer	With color touch screen (Turner Designs, 2014)
Fluorescence optical module(s)	Application-specific (e.g., chlorophyll *a*, rhodamine WT)
Absorbance optical module	With interchangeable filter paddles (560/10, 600/10, 750/10 nm)
Turbidity optical module	Infrared LED at 850 nm (ISO 7027)
Solid secondary standard	For verifying instrument stability
Printer or PC connection	For data export (USB or serial)

6.2. Consumables

Item	Notes
Methacrylate cuvettes (10 × 10 mm)	Minimum 2 mL; for ammonium and general fluorescence
Polystyrene cuvettes (10 × 10 mm)	For turbidity measurements
Glass cuvettes (10 × 10 mm)	Minimum 1.5 mL; for absorbance
Glass test tubes (12 × 35 mm)	For extracted chlorophyll measurements

6.3. Reagents and Standards

Item	Notes
Chlorophyll *a* standard	Pure chlorophyll *a* in 90% acetone; Turner Designs or equivalent
90% acetone (reagent grade)	For chlorophyll extraction
0.1 N HCl	For acidification step (EPA 445.0) (Arar & Collins, 1997)
Formazine turbidity standards	4000 NTU primary standard; valid 4 weeks after preparation
Ultrapure water	For blanks

7. Procedure

7.1. Startup

1. Turn on the Trilogy using the power switch on the back of the instrument.

2. Allow the instrument to warm up for approximately 10 minutes before taking measurements (Turner Designs, 2014).
3. Connect the Trilogy to a printer or PC if data export is needed.

7.2. Installing an Optical Module

1. Select the appropriate optical module for the analysis:
   - **Fluorescence module** for chlorophyll *a*, rhodamine WT, or other fluorescent analytes
   - **Absorbance module** for colorimetric assays
   - **Turbidity module** for nephelometric turbidity measurements
2. Open the sample compartment lid.
3. Align the module with the slot and press firmly until it clicks into place.
4. The Trilogy will automatically detect the installed module and display the corresponding menu on the touch screen.
5. If using the absorbance module, install the appropriate filter paddle for the target wavelength.

7.3. Selecting the Measurement Mode

Mode	When to Use
Raw mode	Qualitative measurements; no calibration required; results in RFU, raw absorbance, or NTU
Direct Concentration mode	Quantitative measurements in concentration units; calibration required

7.4. Fluorescence Module: Chlorophyll *a* (EPA Method 445.0)

EPA Method 445.0 is the standard fluorometric method for measuring extracted chlorophyll *a* and pheophytin *a* in marine and freshwater algae (Arar & Collins, 1997).

1. Calibrate the fluorescence module in Direct Concentration mode using a known concentration of pure chlorophyll *a* in 90% acetone (see §7.3).
2. Insert the glass test tube containing the extracted sample.
3. Touch **Measure Fluorescence** to obtain the pre-acidification reading.
4. Add the prescribed volume of 0.1 N HCl, mix gently, and wait 90 seconds.
5. Measure again to obtain the post-acidification reading.
6. Calculate chlorophyll *a* and pheophytin *a* per EPA 445.0 (Arar & Collins, 1997).

> **Standard Stability**
>
> Recalibrate with a fresh chlorophyll *a* standard every few months. The standard degrades over time, especially if exposed to light or warmth (Turner Designs, 2014).

7.5. Single-Sample Measurements

1. Open the lid and insert the appropriate cuvette or test tube.
2. Close the lid.
3. Optionally, touch **Sample ID** to name the sample; touch **Save**.
4. Touch the **Measure** button corresponding to the installed module.
5. The Trilogy measures the sample for 6 seconds and reports the average reading.
6. Results for the most recent 20 measurements are displayed on the home screen.

> **!** Important
>
> The Trilogy stores a maximum of 20 measurements at a time. Older readings are overwritten. Measurements are not retained between power cycles. Always export data to a printer or PC before turning off the instrument or exceeding 20 readings.

7.6. Shutdown

1. Export all data to a printer or PC.
2. Remove the cuvette or test tube from the sample compartment.
3. Clean the cuvette holder if any liquid was spilled (wipe with 70% ethanol and a lint-free tissue).
4. Close the sample compartment lid.
5. Turn off the instrument using the rear power switch.

8. Quality Control and Acceptance Criteria

8.1. When to Calibrate

1. Before running a new batch of samples
2. After a temperature change of ±5 °C in the lab
3. After changing to a different optical module or analyte
4. When verification readings deviate unacceptably from the expected value

8.2. Calibration Overview

Calibration converts the raw instrument signal to concentration units. The Trilogy supports single-point and multi-point calibrations (up to five standards plus a blank). Multi-point calibrations provide superior accuracy across a wide concentration range (Turner Designs, 2014).

8.3. Direct Concentration Calibration Procedure

1. Turn on the Trilogy and confirm the correct module is installed.
2. Touch **Calibrate** on the home screen.
3. Select **Run New Calibration**.

4. Select the desired unit of measurement.
5. Insert the blank (ultrapure water or 90% acetone, as appropriate) and touch **OK**.
6. Enter the concentration of the first standard. For multi-point calibrations, begin with the lowest concentration.
7. Insert the standard and touch **OK**.
8. After the reading, either select **Proceed with Current Calibration** or **Enter More Standards**.
9. Optionally, save the calibration for future use.
10. Verify calibration by measuring the same standard used in step 6; the displayed concentration should match the known value within the acceptable tolerance (Turner Designs, 2014).

8.4. Verification

- After calibration, re-read a mid-range standard to confirm accuracy.
- Re-read the standard every few hours during extended sessions to detect drift.
- Optionally, read the solid secondary standard and record its value for instrument stability tracking.
- If verification readings drift beyond acceptable limits, recalibrate before continuing.

8.5. QC Acceptance Criteria

- Laboratory reagent blank (LRB) every 10–15 samples; must read below the lower detection limit.
- QC standards must recover within ±20% of the known value (Arar & Collins, 1997).
- Relative standard deviation (RSD) of replicate measurements must be within ±20%.
- Field duplicates must agree within ±20% RPD.
- Samples, standards, LRBs, and QCs must all be at ambient room temperature; fluorescence is temperature-dependent (APHA et al., 2012).

9. Waste Disposal

Waste Type	Disposal Method
Acetone extracts	Collect in organic waste carboy in fume hood; EH&S pickup
Acidified samples (HCl)	Neutralize and dispose per SOP-L003
Used cuvettes (non-hazardous)	Standard trash (polystyrene, methacrylate) or recycle (glass)
Formazine standards	Collect in aqueous waste; EH&S pickup

10. References

- Turner Designs (2014)
- Arar & Collins (1997)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll textita and pheophytin textita in marine and freshwater algae by fluorescence*. U.S. Environmental Protection Agency, Office of Research; Development, National Exposure Research Laboratory. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309417

Turner Designs. (2014). *Trilogy laboratory fluorometer: User's manual*. https://www.turnerdesigns.com/trilogy-laboratory-fluorometer

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Acetone and HCl handling
L310	Chlorophyll Extraction	Primary application of this instrument

12. Training Record

Before operating the Trilogy Fluorometer independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the Turner Designs Trilogy User's Manual (Turner Designs, 2014)
4. Observe a trained operator perform module installation, calibration, and sample measurement
5. Perform a calibration and measure a set of standards with acceptable results under supervision
6. Be signed off by the PI or lab manager

13. Appendix A: Troubleshooting – Fluorescence

Symptom	Probable Cause	Corrective Action
"Bad Calibration" error	Blank brighter than standard	Compare blank and standard in Raw mode; ensure standard is more concentrated than blank
Erratic readings	Incorrect standard value entered	Review the standard value entered; must match actual concentration
Negative values	Sample near zero	Normal if near blank; blank value is automatically subtracted

Symptom	Probable Cause	Corrective Action
Low readings	Wrong optical module	Verify that module excitation/emission wavelengths match the analyte
High background signal	Contaminated cuvette holder	Clean holder with 70% ethanol and a lint-free tissue

14. Appendix B: Troubleshooting – Turbidity

Symptom	Probable Cause	Corrective Action
Readings disagree with other meters	Different light sources (visible vs IR)	Calibrate with the same standard; color interference affects visible-light meters but not the Trilogy
Readings vary between replicates	Particle movement in sample	Normal; particles shift and scatter light differently each time
Readings change after recalibration	Formazine standard has aged	Use fresh formazine (valid for 4 weeks per ISO 7027)



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L204

Shimadzu TOC-L Analyzer with TNM-L and SSM-5000A

Standard Operating Procedure

SOP Number	SOP-L204
Revision	2.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the general operation, calibration, maintenance, and shutdown of the Shimadzu TOC-L total organic carbon analyzer and its companion modules: the TNM-L Total Nitrogen Module and the SSM-5000A Solid Sample Module. The TOC-L uses a 680 C combustion catalytic oxidation method, which is one of two principal approaches to TOC analysis (the other being wet chemical oxidation with persulfate); the high-temperature combustion method is preferred for environmental samples because it more completely oxidizes refractory organic matter such as humic substances (Sharp, 1993; Sugimura & Suzuki, 1988).

All instrument control is performed through the LabSolutions TOC software on the dedicated computer.

This SOP covers the instrument itself. For application-specific sample preparation, measurement modes, and calculations, refer to the relevant analytical SOPs:

- SOP-L311 Dissolved Organic Carbon in Water (NPOC)
- SOP-L407 Total Carbon (Soils) (SSM)
- SOP-L409 Microbial Biomass Carbon (CFE extracts)
- SOP-L418 Water-Extractable DOC (soil extracts)

2. Scope

- **Applies to:** All use of the Shimadzu TOC-L analyzer, TNM-L module, SSM-5000A module, and ASI-L autosampler in the EA Program laboratory, operated through LabSolutions TOC software
- **Does not apply to:** Manual or standalone operation without computer control; instruments at other institutions; application- specific sample preparation or data interpretation (see analytical method SOPs)
- **Location:** EA Program analytical laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Authorize instrument access, approve maintenance
Instrument Manager	Perform preventive maintenance, replace consumables, manage software updates
Trained Personnel	Operate the instrument per this SOP, log all runs
New Students	Complete training before independent use

4. Definitions

- **Catalytic combustion:** Oxidation of organic carbon in the sample at 680 °C over a platinum catalyst in the presence of oxygen; the standard method used by the Shimadzu TOC-L for liquid samples.
- **Dissolved organic carbon (DOC):** Organic carbon in a water or soil extract sample that passes through a 0.45 µm filter; the NPOC method is the standard approach for DOC measurement.
- **IC (inorganic carbon):** Carbon present as carbonate, bicarbonate, and dissolved CO_2; removed by acidification and sparging before NPOC measurement.
- **NDIR detector (non-dispersive infrared detector):** A detector that measures CO_2 concentration by its absorption of infrared light at ~4.3 µm; the detection element in the Shimadzu TOC-L.
- **NPOC (non-purgeable organic carbon):** The organic carbon fraction remaining in a sample after acidification and sparging to remove inorganic carbon and purgeable volatile organic carbon; the operationally defined DOC measured by the Shimadzu TOC-L.
- **SSM-5000A:** The Shimadzu Solid Sample Module that combusts dried, ground solid materials (soils, sediments, plant tissue) directly in ceramic boats at up to 900 °C for total carbon analysis.
- **TC (total carbon):** The sum of all carbon forms (organic and inorganic) in a sample; measured by direct combustion without acidification.
- **TOC (total organic carbon):** TC minus IC; in practice, usually determined as NPOC for environmental samples.

- **TON (total organic nitrogen):** Total nitrogen minus inorganic nitrogen; measured simultaneously by the TNM-L module using chemiluminescence detection of NO produced during combustion.

5. Safety and Hazards

⚠ Hazards

- **Thermal:** The combustion furnace operates at 680 C in liquid analysis mode and up to 900 C in SSM mode. Ceramic boats, combustion tubes, and furnace surfaces can cause severe burns.
- **Chemical:** Hydrochloric acid (2 M HCl) is used for acidification and inorganic carbon sparging. Phosphoric acid (25%) is used as the IC reagent. Avoid skin and eye contact with both.
- **Compressed gas:** The instrument requires high-purity carrier gas (air or oxygen) supplied from a pressurized cylinder at approximately 200 kPa. Follow all compressed gas safety procedures (National Research Council, 2011).
- **Electrical:** The instrument operates on high-voltage mains power. Do not open instrument panels or attempt internal repairs.
- **Ozone:** The TNM-L chemiluminescence detector uses an ozone generator. The instrument vents ozone through an internal decomposer, but ensure that the exhaust line is connected and unobstructed. If an ozone odor is detected, shut down the TNM-L and ventilate the area.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling acid, standards, or samples)
☐ Heat-resistant gloves (when handling ceramic boats from the SSM)

5.2. Emergency Procedures

For acid splashes, flush the affected area with water for at least 15 minutes. For burns from hot surfaces, apply cool running water and seek first aid. If ozone is detected, shut down the TNM-L, open windows, and leave the room until the odor dissipates. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Instrument Description

6.1. System Overview

The Shimadzu TOC-L is a combustion catalytic oxidation analyzer that quantifies carbon (and, with the TNM-L, nitrogen) in liquid samples (Shimadzu Corporation, 2019b). The system in the

EA Program laboratory consists of four main components:

- **TOC-L analyzer:** Combusts liquid samples at 680 C over a platinum catalyst. The resulting CO_2 is measured by a non-dispersive infrared (NDIR) detector. The analyzer supports multiple measurement modes (TC, IC, TOC, NPOC, POC).
- **TNM-L Total Nitrogen Module:** Measures total nitrogen (TN) simultaneously with carbon analysis. Nitrogen compounds in the combustion gas are converted to nitric oxide (NO), which is detected by a chemiluminescence detector after reaction with ozone (Shimadzu Corporation, 2019a). The TNM-L operates in parallel with the NDIR carbon detector, so carbon and nitrogen data are acquired from the same injection.
- **SSM-5000A Solid Sample Module:** Accepts solid samples in ceramic boats for direct combustion at up to 900 C. Connects to the TOC-L for CO_2 detection. The TNM-L is not used during SSM operation.
- **ASI-L Autosampler:** Holds up to 93 sample vials (8 mL or 40 mL) for unattended sequential analysis of liquid samples. Includes an automated rinse function between injections.

6.2. Measurement Modes (Liquid Samples)

Mode	Full Name	What It Measures
TC	Total Carbon	All carbon (organic + inorganic) by direct combustion
IC	Inorganic Carbon	Carbonate/bicarbonate carbon by acidification and sparging only
TOC	Total Organic Carbon (by difference)	TOC = TC - IC; requires two sequential measurements
NPOC	Non-Purgeable Organic Carbon	Sample is acidified and sparged to remove IC, then combusted; the most common mode for routine analysis (APHA et al., 2012, Section 5310B)
POC	Purgeable Organic Carbon	Volatile organic carbon recovered during the sparging step; POC = TC - (NPOC + IC)
TN	Total Nitrogen (TNM-L)	All nitrogen species measured simultaneously with any carbon mode

6.3. Detection Principles

The TOC-L and TNM-L rely on two detectors operating in series on the same combustion gas stream:

- **NDIR detector (carbon):** Infrared absorption by CO_2 is proportional to the mass of carbon injected. The detector is selective for CO_2 and insensitive to other combustion gases.
- **Chemiluminescence detector (nitrogen, TNM-L only):** NO in the combustion gas reacts with ozone (O_3) to produce excited NO_2*, which emits light as it returns to the ground state. The emitted light intensity is proportional to the mass of nitrogen injected (Walsh, 1989).

7. Required Materials

7.1. Instrument Components

Component	Notes
Shimadzu TOC-L analyzer	With combustion furnace and NDIR detector
TNM-L Total Nitrogen Module	Chemiluminescence detector for TN
SSM-5000A Solid Sample Module	For direct combustion of solid samples
ASI-L Autosampler	93-position; 8 mL or 40 mL vials
Dedicated computer	Running LabSolutions TOC software
Carrier gas (high-purity air)	Regulated to 200 kPa (or per manual)
Exhaust tubing	Connected to fume hood or building exhaust

7.2. Consumables

Item	Notes
TC catalyst (Pt on alumina beads)	Replace when blanks rise or sensitivity drops; typical life 3,000–5,000 injections
IC reagent (25% phosphoric acid)	For IC/NPOC acidification; check level weekly
Combustion tubes (quartz)	Inspect monthly; replace if cracked or discolored
Ceramic sample boats	For SSM; pre-combust at 900 C before first use
Halogen scrubber	Replace every 6–12 months or per manufacturer schedule
Dehumidifier / moisture trap	Drain weekly; more often in humid weather
Autosampler vials and septa	Acid-washed for trace-level work; use pre-combusted glass vials for NPOC
Syringe needle (instrument syringe)	Inspect for bends or clogs; replace as needed

7.3. Calibration Standards

Standard	Use	Preparation
Potassium hydrogen phthalate (KHP)	Primary TC/NPOC calibration	2.125 g/L = 1,000 mg C/L stock (APHA et al., 2012)
Sodium bicarbonate / sodium carbonate	Primary IC calibration	See Shimadzu manual for concentrations
Potassium nitrate (KNO_3)	Primary TN calibration (TNM-L)	7.218 g/L = 1,000 mg N/L stock
Deionized water (18.2 MOhm)	Blanks, dilutions, and standard preparation	From Milli-Q; verify TOC less than 0.05 mg/L

8. Procedure

> **!** Computer Control Only
>
> All operations described below are performed through LabSolutions TOC. Do not adjust furnace temperature, gas flow, or other parameters using the instrument front panel unless directed by the instrument manager during troubleshooting.

8.1. Instrument Startup (Cold Start)

A cold start applies when the instrument has been fully powered down (furnace off, gas closed). Allow approximately 60 minutes from power-on to running samples.

1. Open the carrier gas cylinder valve. Verify the regulator output pressure is set to 200 kPa at the instrument inlet. Check for leaks at all fittings (listen for hissing; apply leak-detection solution if uncertain).
2. Verify that the exhaust line from the TNM-L ozone decomposer is connected and routed to the fume hood or building exhaust
3. Check consumable levels:
   - IC reagent bottle (25% phosphoric acid): refill if below the minimum line
   - Dehumidifier / moisture trap: drain if water is visible
   - Rinse water reservoir on the ASI-L: fill with fresh deionized water
4. Power on the TOC-L main unit using the switch on the right side panel
5. If the TNM-L is needed, power on the TNM-L using the switch on its rear panel
6. Power on the ASI-L autosampler
7. Turn on the dedicated computer and launch LabSolutions TOC
8. In LabSolutions TOC, verify that communication with all modules is established. The status bar should show “Warming Up”
9. **Wait for the furnace** to reach 680 C (approximately 20–30 minutes). The software status changes from “Warming Up” to “Ready”
10. **Wait for the TNM-L** ozone generator and chemiluminescence detector to stabilize (approximately 30–45 minutes after power-on; the TNM-L status indicator changes to “Ready”)
11. Run 3–5 injections of deionized water as system conditioning blanks to flush the injection port, syringe, and combustion tube. Discard these results.
12. Run 3 additional deionized water blanks and verify baseline stability: TC blank values should be below 0.1 mg/L C; TN blank values should be below 0.1 mg/L N

8.2. Instrument Startup (From Standby/Sleep)

When the instrument is in standby mode (furnace at temperature, gas flowing, software idle), startup is faster:

1. Launch LabSolutions TOC if not already open
2. Verify that the status bar shows “Ready” for the TOC-L and (if applicable) the TNM-L

3. Check the IC reagent level and drain the moisture trap
4. Fill the ASI-L rinse reservoir with fresh deionized water
5. Run 3 deionized water conditioning blanks. If blanks are below 0.1 mg/L C and 0.1 mg/L N, proceed to calibration.

8.3. Standby and Sleep Modes

Choose the appropriate idle mode based on how soon the instrument will be used again:

Mode	Furnace	Carrier Gas	TNM-L	When to Use
Active standby	680 C (on)	Flowing	On	Next run within 24 hours
Reduced standby	680 C (on)	Flowing	Off	Next run within 2–3 days; no TN needed
Sleep	Off (cooling)	Closed	Off	No runs planned for 3+ days
Full shutdown	Off (cold)	Closed	Off	Extended break, maintenance, or relocation

8.3.1. Entering Active Standby

1. After the final sample, run 3–5 deionized water blanks to flush the system
2. Leave the furnace at 680 C and the carrier gas flowing
3. Leave the TNM-L powered on
4. Empty the autosampler rack and cover with a dust cover
5. The instrument manager may set the software to a low carrier gas flow rate overnight to conserve gas (consult the manual for the minimum flow that maintains a positive purge of the combustion tube)

8.3.2. Entering Reduced Standby

1. Flush the system with deionized water blanks as above
2. Power off the TNM-L (rear panel switch). This extends the ozone decomposer lifetime.
3. Leave the TOC-L furnace at 680 C and gas flowing
4. Cover the autosampler

8.3.3. Entering Sleep Mode

1. Flush the system with deionized water blanks
2. Power off the TNM-L
3. In LabSolutions TOC, initiate a furnace cooldown (or simply power off the TOC-L)
4. Once the furnace temperature drops below 200 C (approximately 90 minutes), close the carrier gas cylinder valve
5. Power off the ASI-L
6. Close LabSolutions TOC and shut down the computer

8.3.4. Full Shutdown

Follow the Sleep Mode steps, then:

1. Allow the furnace to cool to room temperature
2. Disconnect the carrier gas line if the instrument will be moved or serviced
3. Empty the IC reagent bottle if the shutdown will exceed 30 days (phosphoric acid can corrode the reagent lines over long idle periods)
4. Empty the ASI-L rinse reservoir to prevent microbial growth
5. Cover the instrument with a dust cover

8.4. Software Navigation

LabSolutions TOC organizes work into the following areas:

- **Instrument Monitor:** Real-time display of furnace temperature, carrier gas flow, detector signal (NDIR and chemiluminescence), and system status
- **Method Editor:** Define measurement mode (TC, IC, NPOC, TN, etc.), injection volume, number of replicate injections, sparge time (for NPOC), acidification volume, and calibration curve parameters
- **Sample Table:** Enter sample IDs, vial positions, dilution factors, and measurement method for each sample
- **Calibration:** Build, view, and edit calibration curves. The software fits curves by least-squares regression and reports r^2 and residuals.
- **Data Browser:** Review completed runs, view peak integrations, export results to CSV, and reprocess data

8.5. Calibration

Calibrate at the start of each analytical session or whenever the method, measurement mode, or concentration range changes. Carbon and nitrogen calibrations are built independently but can be run simultaneously if both detectors are active.

8.5.1. Carbon Calibration

1. Prepare a multi-point calibration series from KHP dissolved in deionized water. Choose concentrations that bracket the expected sample range (e.g., 0, 1, 5, 10, 25, 50, 100 mg/L C). Prepare fresh daily or from a refrigerated stock per SOP-L020 (APHA et al., 2012).
2. For IC calibration, prepare standards from sodium bicarbonate/sodium carbonate in deionized water
3. In LabSolutions TOC, open the Method Editor and select the measurement mode (e.g., NPOC, TC, or TC-IC)
4. Enter calibration standard concentrations in the Calibration window
5. Load standards into the autosampler in order of increasing concentration
6. Run the calibration sequence. The software will perform replicate injections (typically 3) of each standard and fit a regression

7. Verify that $r^2 \geq 0.999$ and that each standard back- calculates to within 10% of its nominal value (15% at the lowest standard is acceptable)
8. Save the calibration curve

8.5.2. Nitrogen Calibration (TNM-L)

1. Prepare a multi-point calibration series from KNO_3 dissolved in deionized water (e.g., 0, 0.5, 1, 5, 10, 25, 50 mg/L N)
2. In the Method Editor, confirm that TN measurement is enabled alongside the carbon mode
3. Enter TN calibration concentrations in the Calibration window
4. The TN calibration runs simultaneously with the carbon calibration if both are loaded in the same sample table; otherwise, run the TN standards as a separate sequence
5. Verify that $r^2 \geq 0.999$ for the nitrogen curve
6. Save the calibration curve

8.6. Setting Up an Analytical Run (Liquid Samples)

1. In the Method Editor, confirm the measurement mode, injection volume, number of replicate injections, and (for NPOC mode) sparge time and acid volume
2. In the Sample Table, enter the run sequence:
    a. Calibration verification standard (mid-range) as the first sample
    b. Instrument blanks (deionized water) at the beginning, every 10–15 samples, and the end of the run
    c. Method blanks appropriate to the application (e.g., 0.5 M K_2SO_4 for soil extracts; consult the analytical method SOP)
    d. Sample vials in the order they are loaded in the autosampler
    e. A final calibration verification standard
3. Set the dilution factor for each sample if applicable
4. Verify that autosampler vial positions in the Sample Table match the physical rack positions
5. Confirm that the rinse reservoir on the autosampler is filled with deionized water
6. Click **Start** to begin the sequence

> **Replicate Injection Settings**
>
> The software default is 3 replicate injections per sample. If the coefficient of variation (CV) among replicates exceeds 2%, the software can be configured to add up to 2 additional injections automatically (set in Method Editor under "Maximum Injections"). The outlier replicate is excluded from the reported mean.

8.7. Setting Up a Solid Sample Run (SSM-5000A)

1. In LabSolutions TOC, switch to SSM mode
2. Pre-combust ceramic boats at 900 C in a muffle furnace (SOP-L111) to remove residual carbon. Allow boats to cool in a desiccator.

3. Weigh the solid sample into a clean ceramic boat. Record the mass to 0.01 mg.
4. In the Sample Table, enter the sample ID, boat number, and sample mass
5. Place the ceramic boat on the SSM sample port and push it into the furnace when prompted by the software
6. The SSM combusts the sample; evolved CO_2 flows to the TOC-L NDIR detector
7. The software reports carbon content in mg C or as a percentage of sample mass

> **TNM-L and SSM**
>
> The TNM-L chemiluminescence detector is not used during SSM solid sample analysis. Total nitrogen in solid samples requires a separate instrument (e.g., the Flash EA; see SOP-L208).

8.8. Monitoring a Run in Progress

1. Use the Instrument Monitor to watch real-time peak shapes and detector baselines
2. If a peak appears saturated (flat top), the sample exceeds the calibration range. Pause the run, dilute the sample, and re-queue.
3. If the CV among replicate injections exceeds 2%, investigate (air bubble, particulates, insufficient sample volume) and consider re-running.
4. If the system blank rises above 0.5 mg/L C or 0.5 mg/L N during a run, the combustion tube or catalyst may need replacement. Complete the current run, note affected samples, and consult Section 9.

8.9. Data Export

1. After the run, open the Data Browser and review all results
2. Check that calibration verification standards recovered within 90–110% of their nominal values
3. Export results to CSV: **File > Export**, including sample ID, mode, mean concentration, SD, CV, and number of valid injections
4. Save the LabSolutions TOC run file (.toc) to the instrument computer and back up to the lab network drive
5. Record the run date, analyst, method, and file name in the instrument logbook

9. Quality Control

QC procedures are tailored to each analytical method and are specified in the relevant method SOP. The following instrument-level QC applies to all runs (US Environmental Protection Agency, 2009):

- System blanks (deionized water) at the start of every session must read below 0.1 mg/L C and 0.1 mg/L N
- A calibration verification standard (mid-range) must be run at the beginning and end of every sample sequence, with recovery within 90–110%
- Replicate injection CV must be below 2% for liquid samples

- All QC results are recorded in the instrument logbook and in the exported data file
- For additional QC requirements (method blanks, duplicates, matrix spikes, continuing calibration verification frequency), refer to SOP-L802 Quality Control

10. Maintenance

10.1. Routine Maintenance Schedule

Task	Frequency	Notes
System blank check	Before each analytical session	
Drain moisture trap / dehumidifier	Weekly (more often in humid conditions)	Prevents water from reaching the NDIR detector
Check IC reagent level	Weekly	Refill with fresh 25% H_3PO_4 when below the minimum line
Inspect injection syringe needle	Weekly	Replace if bent, corroded, or clogged
Check autosampler rinse reservoir	Before each session	Refill with fresh DI water
Inspect combustion tube	Monthly or every 500 injections	Look for discoloration, cracks, or catalyst settling
Replace TC catalyst	Every 3,000–5,000 injections or when blanks rise	Pt on alumina; record replacement date
Replace halogen scrubber	Every 6–12 months	Prevents halide damage to the NDIR detector
Replace TNM-L ozone decomposer	Annually	Degraded decomposer leaks ozone into the room
Replace SSM combustion tube	As needed	Inspect after each batch of solids
Carrier gas cylinder replacement	As needed	Monitor regulator pressure; order when below 500 psi
Software and firmware updates	As released by Shimadzu	Coordinate with instrument manager

10.2. Catalyst Replacement Procedure

1. Allow the furnace to cool to room temperature (power off the TOC-L and wait at least 3 hours, or use the forced-air cooling function if available)
2. Wearing heat-resistant gloves and safety glasses, remove the combustion tube from the furnace per the manufacturer's procedure
3. Dispose of the spent catalyst (Pt on alumina) as hazardous waste (precious metal content; collect for EH&S pickup per SOP-L006)

4. Fill the combustion tube with fresh catalyst to the manufacturer's specified depth (approximately 12 cm)
5. Add a thin layer of quartz wool above and below the catalyst bed
6. Reinstall the combustion tube. Reconnect all tubing and fittings.
7. Restart the furnace and allow it to reach 680 C
8. Run 20–30 deionized water conditioning injections to stabilize the new catalyst. Blanks should decrease progressively to below 0.1 mg/L C.
9. Run a calibration verification standard to confirm sensitivity before resuming sample analysis
10. Record the replacement date, catalyst lot number, and conditioning results in the instrument logbook

10.3. Combustion Tube Inspection and Replacement

1. Remove the combustion tube as described in Section 9.2, step 2
2. Inspect for cracks, discoloration, or accumulation of non-volatile residue on the inner walls
3. If the tube is damaged, replace with a new quartz combustion tube
4. Repack with catalyst and condition as in Section 9.2

10.4. Syringe and Needle Maintenance

1. Remove the syringe from the autosampler or manual injection port
2. Inspect the needle for bends, burrs, or corrosion
3. Flush the syringe barrel with deionized water, then with dilute HCl (0.1 M), then with deionized water again
4. If the syringe plunger binds or the needle is damaged, replace the entire syringe assembly
5. Reinstall and run 3 deionized water blanks to verify proper function

11. Troubleshooting

Symptom	Probable Cause	Corrective Action
Blank values increasing over time	Catalyst degradation; contaminated combustion tube	Replace catalyst; inspect and replace combustion tube
Poor replicate precision (CV > 2%)	Air bubble in syringe; particulates in sample; insufficient volume	Check syringe for air leaks; re-filter sample; verify vial volume
NDIR signal saturated (flat-top peaks)	Sample exceeds calibration range	Dilute sample and re-run
TNM-L "Not Ready" status persists	Ozone generator not warmed up; decomposer exhausted; exhaust blocked	Allow more warm-up; replace decomposer; check exhaust tubing
No communication with instrument	Cable disconnected; software crash; instrument not powered on	Check cables; restart software; verify power

Symptom	Probable Cause	Corrective Action
Autosampler skips vials	Position mismatch; septa not pierced	Verify positions; check that septa are intact
SSM signal noisy or irreproducible	Boat not fully inserted; sample mass too large; residual carbon on boat	Confirm position; reduce mass; pre-combust boats longer
Carrier gas pressure alarm	Cylinder empty or regulator set incorrectly	Replace cylinder; adjust regulator
Moisture trap fills rapidly	High humidity; furnace tube leak	Drain more frequently; inspect tube fittings for leaks
IC reagent not dispensing	Reagent bottle empty; tubing kinked or clogged	Refill bottle; inspect and replace tubing

12. Waste Disposal

Waste Type	Disposal Method
Acidified liquid waste (HCl/H_3PO_4)	Neutralize and dispose per SOP-L006
Spent TC catalyst (Pt on alumina)	Collect for EH&S hazardous waste pickup (precious metal)
Used combustion tubes (quartz)	Standard glass waste (broken-glass box) if not contaminated
Used ceramic boats (SSM)	Clean and reuse, or standard trash
Autosampler rinse water	Drain
Calibration standard waste	Aqueous waste; drain if dilute

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14. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials Handling	Chemical safety for acids
L006	Waste Disposal	Catalyst and chemical waste disposal
L020	Reagent and Standard Preparation	KHP and KNO_3 standard prep
L111	Muffle Furnace	Pre-combustion of ceramic boats
L311	Dissolved Organic Carbon (Water)	NPOC application SOP
L407	Total Carbon (Soils)	SSM application SOP
L409	Microbial Biomass Carbon (CFE)	DOC extract application SOP
L418	Water-Extractable DOC	Soil extract application SOP
L802	Quality Control	QC requirements for all runs

15. Training Record

Before operating this instrument independently, personnel must:

1. Read this SOP in full and the relevant sections of the Shimadzu TOC-L, TNM-L, and SSM-5000A user manuals
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Observe a trained operator perform a complete session: cold start, calibration (carbon and nitrogen), sample analysis, data export, and transition to standby
4. Demonstrate competence preparing calibration standards (KHP and KNO_3) per SOP-L020
5. Perform a supervised analytical run with acceptable QC results (blanks below 0.1 mg/L, calibration verification recovery 90–110%, replicate CV below 2%)
6. Demonstrate entering and exiting standby and sleep modes
7. Be signed off by the instrument manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L205

X-Ray Fluorescence (XRF) Sample Preparation and Analysis

Standard Operating Procedure

SOP Number	SOP-L205
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the complete workflow for preparing geological rock samples and analyzing their major-element oxide composition by wavelength-dispersive X-ray fluorescence (WD-XRF). The procedure covers sample powdering, flux fusion bead preparation, bead polishing, and instrument operation on the PANalytical XRF spectrometer using the SuperQ software with the "Geology Beads" application (Claisse, 2003; Norrish & Hutton, 1969; PANalytical, 2012).

2. Scope

- **Applies to:** Rock, mineral, and geological samples analyzed as lithium tetraborate fusion beads for major-element oxide determination (e.g., SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, K_2O, TiO_2, P_2O_5, MnO)
- **Does not apply to:** Pressed-pellet trace-element analysis, handheld (portable) XRF measurements, or non-geological materials
- **Locations:** Rock preparation lab (powdering and polishing); analytical lab / wet lab (weighing, fusion); XRF lab (instrument operation)

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, ensure training compliance
Lab Technician	Maintain instrument, assist with troubleshooting
Trained Personnel	Follow this SOP, maintain sample tracking, clean up
New Students	Complete training before independent use

Contact the lab technician, Jade Star, or Bob with any questions.

4. Definitions

- **Certified reference material (CRM):** A geological standard with certified major-element oxide concentrations from an accredited producer (e.g., NIST, USGS, BCR); used to verify XRF accuracy during each analytical session (Norrish & Hutton, 1969).

- **Detection limit (MDL):** The lowest concentration of an element reliably detected above background; varies by element, matrix, and instrument settings; typically 1–50 mg/kg for common elements by WD-XRF.
- **Flux (dilithium tetraborate, $Li_2B_4O_7$):** A reagent mixed with the rock powder in a 2:1 (flux:sample) ratio; lowers the melting point and produces a homogeneous glass bead that eliminates mineralogical matrix effects during analysis (Claisse, 2003).
- **Fluorescence (X-ray):** The emission of characteristic X-rays from a sample excited by primary X-rays; each element emits at unique energies that identify and quantify it (Norrish & Hutton, 1969).
- **Fundamental parameters (FP) method:** An XRF calibration approach that uses known physical constants to calculate element concentrations from measured fluorescence intensities without matrix-matched standards for every sample type.
- **Fusion bead:** A glass disc produced by melting the powdered rock sample with lithium tetraborate flux at 1000 °C; the homogeneous glass eliminates mineralogical matrix effects (Claisse, 2003).
- **Geology Beads:** The SuperQ application/archive configured for major-element oxide analysis of fusion beads on the PANalytical XRF spectrometer (PANalytical, 2012).
- **K-alpha and K-beta lines:** The most commonly measured characteristic X-ray emission lines for elements; produced when an electron is ejected from the K shell and replaced from the L or M shell, respectively.
- **Loss on ignition (LOI):** The mass percentage lost when a pre-weighed rock powder is heated to 1000 °C; reflects H_2O, CO_2, and organic matter content; used as a correction in major-element summation (Norrish & Hutton, 1969).
- **Matrix effects:** Absorption and enhancement of X-ray signals by other elements in the sample; minimized by the flux fusion method (Claisse, 2003).
- **Shatterbox:** A vibratory ring-and-puck mill used to grind rock chips to a fine powder suitable for fusion.
- **SuperQ:** The PANalytical software used to control the XRF spectrometer, define measurement sequences, and retrieve results (PANalytical, 2012).
- **WD-XRF (wavelength-dispersive XRF):** An XRF configuration in which characteristic X-rays are separated by a crystal monochromator before detection; provides higher spectral resolution and lower detection limits than energy-dispersive (ED-XRF) systems.
- **X-ray fluorescence (XRF):** A non-destructive elemental analysis technique in which a sample excited by a primary X-ray beam emits characteristic fluorescent X-rays that are measured to determine elemental composition (Norrish & Hutton, 1969).

5. Safety and Hazards

⚠ Hazards

- **High-temperature furnace:** The muffle furnace operates at 1000 °C. Crucibles and tiles removed from the furnace are extremely hot. Do not touch them, even with gloves, until fully cooled.
- **Compressed air:** Loud; wear ear protection when blowing out equipment.
- **Rock chipper:** Flying rock fragments are common. Always wear safety glasses. Never look into the chipper while it is running.

- **Shatterbox:** The unit vibrates intensely and can travel across the floor. Get help repositioning it if needed.
- **Chemical reagents:** Dilithium tetraborate flux is a fine powder; avoid inhalation. Ethanol is flammable.
- **X-ray radiation:** The XRF instrument contains an X-ray source. Follow all posted radiation safety procedures. Do not open the instrument lid while the arm is moving or the flap is up.

5.1. Required PPE

- ☐ Safety glasses (required in the rock prep lab at all times)
- ☐ Laboratory coat
- ☐ Nitrile gloves (powder handling and polishing)
- ☐ Ear protection (when using the shatterbox or compressed air)
- ☐ Heat-resistant gloves (when handling crucibles or the tile from the furnace)

5.2. Emergency Procedures

Do not work in the lab without letting someone know your plans and estimated return time. Phones and safety showers are in all labs. Refer to SOP-L002 Laboratory Safety for all emergency procedures.

6. Prerequisites and Required Reading

- SOP-L002 Laboratory Safety
- Furnace operating instructions (posted above the furnace)
- This SOP in full

! Important

Minimize contamination at every step. Wear gloves, clean equipment thoroughly between samples, and assume any shared equipment is dirty unless you just cleaned it. Leave the lab and equipment cleaner than you found it.

7. Required Materials

7.1. Equipment

Item	Location / Notes
Small rock chipper	Rock prep lab
Rocklabs shatterbox (tungsten carbide head)	Rock prep lab

Item	Location / Notes
Analytical balance	Analytical lab, corner by XRD room
Vortex mixer	Counter left of weigh station
Deionizing bar	Right of the Vortex mixer
Muffle furnace (1000 °C)	Far left corner, wet lab
Grinding/polishing laps (4 stages)	Darkroom / grinding lab
Ultrasonic cleaner (sonicator)	Grinding lab or XRF lab
Diamond-tipped pen or rotary tool	Darkroom
PANalytical XRF spectrometer	XRF lab (PANalytical, 2012)

7.2. Consumables

Item	Notes
Dilithium tetraborate flux	Stored in desiccator, left of weigh station
Graphite crucibles (9 per batch)	Drawer under the furnace
Plastic cups with lids	Cabinet left of weigh station
Brown butcher paper	Shelves, back wall, rock prep lab
White printer paper	For transferring powder
Kimwipes	General use
Ethanol	Bottle on shatterbox table; bulk jug on side counter
Sand (for cleaning shatterbox)	Bowl in center of table, rock prep lab
Green Brillo pads	Drawer, rock prep lab
Sample bags and labels	Drawer under counter, XRF lab
XRF sample sheets	Drawer left of weigh station
SiC sandpaper	For removing saw marks

8. Procedure

8.1. Step 1: Powdering the Sample

A complete fusion batch is 9 samples. If you plan fusions, turn on the furnace first (see §6.3) since it takes about 1 hour to reach 1000 °C.

8.1.1. Chipping

1. Use the rock chipper to reduce each sample to pieces smaller than approximately 1 cm. Wear safety glasses. Clean the chipper jaws thoroughly between samples with compressed air and an ethanol wipe.
2. Weigh out approximately 20–30 g of chipped material into a labeled sample bag.

8.1.2. Shatterbox Milling

3. Place a piece of brown butcher paper under the shatterbox head assembly to catch any spillage.
4. Wipe all shatterbox bowl components (ring, puck, and bowl) with an ethanol-dampened Kimwipe. Allow to dry completely.
5. Load the chipped sample into the tungsten carbide bowl. Do not overfill (maximum ~two-thirds full).
6. Lock the lid and run the shatterbox for 60–90 seconds. Longer milling times reduce particle size further.
7. Unlock the lid and carefully transfer the powder onto white printer paper, keeping all components on the paper edge so exterior metal surfaces do not contact the sample. Funnel the powder into the labeled container. Use a fresh sheet of paper for every sample.

8.1.3. Cleaning the Shatterbox

8. If powder is stuck, scrub with a green Brillo pad. If that is insufficient, run a scoop of sand for 15 seconds and discard.
9. Detail all pieces with compressed air (hold pieces in the airflow; scrub with the Brillo pad). **Do not let the metal hose tip touch any bowl surface.** Use ear protection.
10. Finish with an ethanol wipe on a Kimwipe. After the last sample, clean up, recycle paper, replace the bowl, and turn off the shatterbox.

8.2. Step 2: Weighing and Mixing

1. Bring your powdered samples to the analytical lab. Get an XRF sample sheet from the drawer left of the weigh station, fill out the top portion, and keep it on a wooden tray next to the balance.
2. Retrieve a small cup of flux from the desiccator.
3. For each sample:
    a. Label a plastic cup lid with the sample number.
    b. Place the cup (no lid) in the cardboard holder on the balance, close the sliding door, and zero.
    c. Weigh 3.5000 g (±0.0004 g) of sample powder. Record the exact mass. Zero the balance.
    d. Add 7.0000 g (±0.0004 g) of flux (2:1 flux-to-sample ratio) (Claisse, 2003). Record the mass. If you add too much flux, remove the excess but **do not return it to the reagent container**.
    e. Seal the lid tightly.
4. Mix each cup on the Vortex mixer for about one minute. If powder clings to the sides, pass the deionizing bar over the cup.

8.3. Step 3: First Fusion

8.3.1. Furnace Startup

1. Flip the black switch on the front of the furnace.

2. Press the T1 temperature button and confirm T = 1000 °C.
3. Set a duration (e.g., 24, 48, or 72 hours). Press Enter and Start. It takes roughly 1 hour to reach 1000 °C.

> **Caution**
>
> Do not spill glass in the furnace or introduce liquids. Do not use this furnace for temperatures below 500 °C – use a regular drying oven instead. Do not shut off the furnace mid-cycle with the power button; let it run out its programmed time and shut off automatically.

8.3.2. Loading Crucibles

4. Select 9 graphite crucibles from the drawer under the furnace. Clean each inside by gently wiping with a Kimwipe and blowing out with compressed air.
5. Back at the analytical lab, carefully empty each cup into the corresponding crucible. **Maintaining the correct order is critical – once the powder is in the crucible, there is no way to tell samples apart.**
6. Place the empty cups (lids on) in the box labeled "Dirty Cups for Blowing Out."

8.3.3. Fusing

7. In the wet lab, place a tile on the brick stand (small hole facing toward you) and crank the stand up until the tile is about halfway up the side of the furnace.
8. Put on both long heat-resistant gloves. Using the tongs, slide the tile into the furnace. Close the door as quickly as possible.
9. Wait 20–30 minutes for the furnace to recover to 1000 °C, then leave the samples for 10–15 minutes. Longer fusion times help carbon-rich samples degas fully.

The tile and crucibles are extremely hot when removed from the furnace. Do not touch them, even with gloves, until fully cooled.

10. Wearing gloves, open the furnace and carefully pull the tile out using the tongs and the tile hole. Move the tile to the hot plate and close the furnace.

8.4. Step 4: Regrinding and Second Fusion

1. Once the crucibles are cool enough to handle, pour the first-fusion glass back into the shatterbox bowl. Re-grind for 60 seconds.
2. Return the powder to the crucible and re-fuse at 1000 °C for 15–20 minutes to remove any remaining bubbles and produce a homogeneous bead.
3. Once cooled, inspect each bead visually. Beads should be clear, bubble-free glass covering the full crucible bottom. If a bead has a dark carbon pellet at the bottom, fuse for an additional 15 minutes.

8.5. Step 5: Polishing the Bead

Polish the flat (analytical) surface of each bead on the 4-stage grinding/polishing laps to remove surface scratches and contamination that would scatter X-rays.

1. Use the coarsest lap first (e.g., 600 grit SiC), working through progressively finer stages.
2. After each stage, clean the bead in the ultrasonic cleaner with distilled water (3 minutes).
3. After the final lap, inspect the flat surface under the lab light; it must be mirror-smooth with no visible scratches.
4. Label each bead on the curved (non-analytical) side using a diamond-tipped pen or rotary tool.

8.6. Step 6: XRF Analysis

1. Open a "Measure and Analyze" window from the SuperQ Manager. Wear gloves and avoid touching the flat (analytical) surface of the bead (PANalytical, 2012).
2. Click the multi-cup icon to open the Sample Changer. Double-click position A1. Enter the sample name; set type to "Routine," application to "Geology Beads," priority to "Normal," and verify weights are correct. Click **Add**. Available positions are A1–A8 and B5–B8.
3. Once all samples are entered, click **Measure**.

> **Note**
>
> If the XRF has not been running recently, the instrument needs to warm up until the front-panel readouts display 60 and 50. Run the first bead until those values are reached, then click Abort. Wait for the arm to return the sample, then restart. Running samples before warm-up can compromise the analysis (PANalytical, 2012).

8.7. Step 7: Retrieving Results

1. Open a "Results Evaluation" window. Set the archive to "Geology Beads" and adjust the date range. Use the "Name Mask" field to filter results. Click on a sample and then click "View."
2. To export data: open a "Results Quantitative" window. Adjust date and name mask. Click the Print icon, then "Delimited Values Report" to clipboard. Paste into a spreadsheet.

9. Quality Control

- Verify the 2:1 flux-to-sample ratio for every sample; record exact masses on the XRF sample sheet (Claisse, 2003).
- Visually inspect beads before loading; reject cracked, broken, or bubble-compromised beads.
- Run at least one CRM alongside unknowns to monitor instrument drift and accuracy (Norrish & Hutton, 1969).
- If a bead required a third fusion, note this on the sample sheet.

10. Cleanup Checklist

- ☐ All paper recycled (butcher paper, white paper)
- ☐ Shatterbox bowl cleaned and replaced; shatterbox off
- ☐ Chipper cleaned (ethanol wipe on all contact surfaces)
- ☐ Crucibles returned to the drawer (or discarded if degraded)
- ☐ Plastic cups blown out and restocked (or discarded after 6 uses)
- ☐ Polishing laps spun dry
- ☐ Sonicator drained if not in regular use
- ☐ XRF sample sheet completed and filed
- ☐ All personal items removed from the lab
- ☐ Work surfaces wiped down

11. Troubleshooting

Symptom	Possible Cause / Action
Glass does not cover crucible bottom	Insufficient fusion time; re-fuse longer
Bead cracked or has deep air bubbles	Air trapped during fusion; re-fuse briefly
Carbon pellet at bottom of bead	Carbon not fully degassed; fuse longer next time
Powder clings to plastic cup walls	Pass the deionizing bar over the cup
XRF front-panel numbers not at 60/50	Instrument not warmed up; run a bead until ready
XRF data appear anomalous	Sample run before warm-up; re-run

12. References

- Norrish & Hutton (1969)
- Claisse (2003)
- PANalytical (2012)

Claisse, F. (2003). *Fusion and fluxes for XRF sample preparation.* Corporation Scientifique Claisse.

Norrish, K., & Hutton, J. T. (1969). An accurate X-ray spectrographic method for the analysis of a wide range of geological samples. *Geochimica Et Cosmochimica Acta*, *33*(4), 431–453. https://doi.org/10.1016/0016-7037(69)90126-2

PANalytical. (2012). *SuperQ software: User manual.*

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L111	Muffle Furnace	Furnace operation details

14. Training Record

Before performing XRF sample preparation and analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review furnace safety instructions posted above the furnace
4. Observe a trained operator perform a complete batch (powdering through analysis)
5. Prepare and analyze a batch under supervision with acceptable CRM recovery
6. Be signed off by the lab technician or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L208

ThermoFisher Delta V IRMS – General Instrument Use

Standard Operating Procedure

SOP Number	SOP-L208
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the general operation, care, and policies governing the ThermoFisher Delta V Isotope Ratio Mass Spectrometer (IRMS) and its four front-end peripherals in the Oxtoby Environmental Isotope Lab (Coplen, 2011; Werner & Brand, 2001). It is the single instrument-level reference for all IRMS work. Individual application SOPs (e.g., SOP-L414 for soil CN analysis, SOP-L415 for carbonate isotopes) build on this SOP and should be read in conjunction with it.

2. Scope

- **Applies to:** The Delta V IRMS and all peripherals (ConFlo IV, Flash IRMS EA, TC/EA, GasBench II) in the Oxtoby Isotope Lab, SGM Rm 135, Pomona College.
- **Does not apply to:** Application-specific sample preparation, data reduction, or QC criteria. See the relevant application SOP.
- **Location:** David W. and Claire B. Oxtoby Environmental Isotope Lab (SGM Rm 135). Sample preparation: wet lab (SGM Rm 133).

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP; authorize new users
Lab Manager / Super-User	Certify new users; maintain reactors and gas supply
Certified User	Operate instrument per this SOP; log all sessions
Student Researcher	Operate only under supervision until certified

4. Definitions

- **ConFlo IV:** Interface hub connecting peripherals, reference gases, and the IRMS; handles reference-gas dilution and peak-center switching.
- **Delta V IRMS:** The ThermoFisher Delta V Isotope Ratio Mass Spectrometer housed in the Oxtoby Isotope Lab, SGM Rm 135.
- **Flash IRMS EA:** Elemental analyzer peripheral that combusts samples at 1020 °C to produce N_2, CO_2, and (optionally) SO_2 for simultaneous $\delta^{15}N$ and $\delta^{13}C$ analysis.
- **GasBench II:** Low-flow peripheral for headspace gas sampling from sealed vials; used for carbonate CO_2, DIC, and water $\delta^{18}O$.
- **H_3^+ Factor:** Correction applied to δ^2H data for H_3^+ ions formed in the source that interfere with HD^+ at m/z 3.
- **Isodat:** ThermoFisher software used to design analytical sequences, control the instrument, and export raw isotope data.
- **Linearity:** The dependence of measured δ on signal amplitude; a well-tuned instrument shows minimal linearity offset across the working signal range (Werner & Brand, 2001).
- **Super-User:** Staff or faculty member certified for IRMS operation and minor maintenance (gas replacement, reactor exchange).
- **TC/EA:** Thermal Conversion/Elemental Analyzer peripheral; pyrolyzes samples at ~1450 °C to produce H_2 and CO for δ^2H and $\delta^{18}O$ analysis.
- **User:** Student, staff, or faculty member certified to prepare samples and operate the IRMS without direct supervision.
- **Zero-Enrichment Test:** Series of reference-gas on/off pulses run before analysis to verify the IRMS produces consistent δ values independent of signal amplitude (Coplen, 2011).

5. Safety and Hazards

⚠ Hazards

- **Compressed gases:** He, O_2, CO_2, N_2, SO_2 (where used). Cylinders must be chained; O_2 must be stored away from flammables.
- **High-voltage electronics:** The IRMS source operates at high voltage. Do not open the source housing without authorization.
- **High temperatures:** Flash EA reactor operates at 1020 °C; TC/EA at ~1450 °C. Allow to cool before maintenance.
- **Chemical:** Tin capsules contain sample material; some samples may be hazardous. Handle per the sample-specific SOP.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (during sample loading and reactor maintenance)

5.2. Required Training

- SOP-L002 Laboratory Safety
- This SOP (Section on User Certification) before any independent use

6. Equipment and Materials

Item	Location	Notes
Delta V IRMS	SGM Rm 135	Do not power off without authorization
ConFlo IV interface	SGM Rm 135	Always on when IRMS is on
Flash IRMS EA	SGM Rm 135	Application-specific reactor config
GasBench II	SGM Rm 135	Requires dedicated Isodat method
TC/EA	SGM Rm 135	High-temperature; Super-User only for maintenance
He supply (cylinder)	SGM Rm 135	Check pressure before each session
Reference gas cylinders	SGM Rm 135	CO_2, N_2, H_2, SO_2 as needed
Tin capsules (5 x 9 mm)	SGM Rm 133	Sample prep area
Lab logbook	SGM Rm 135	Sign in/out for every session

7. Procedure

7.1. User Certification

Before operating the IRMS independently, personnel must complete the Training Record (Section 13). Contact the lab manager to schedule certification.

7.2. Pre-Analysis Checklist

1. Sign in to the lab logbook.
2. Verify He carrier gas pressure (minimum 1500 psi on the regulator).
3. Check reference gas pressures (CO_2, N_2 as applicable).
4. Confirm Isodat is running and the instrument status screen shows no error flags.
5. Verify the correct peripheral is selected in Isodat.
6. Run a Zero-Enrichment Test (3 on/off pulses) and confirm δ values are stable within $\pm 0.1‰$.
7. Run an on/off linearity check if not done within the past 48 h.

7.3. Analytical Sequence Design

1. Open the Isodat Sequence Editor.
2. Load the appropriate method file for the peripheral and analyte.
3. Build the sequence: blank(s), conditioning samples, reference materials, unknowns, check standards, blanks.
4. Reference material placement: minimum 1 RM per 10 unknowns; two-point normalization requires at least 2 different RMs.
5. Save the sequence file using the naming convention: `YYYYMMDD_Project_SeqID_User.seq`

7.4. Starting and Monitoring a Run

1. Load samples into the autosampler or carousel per the application SOP.
2. Press **Start** in the Isodat Sequence Editor.
3. Monitor the first 3–5 injections on-screen to confirm peaks are present, within expected amplitude, and baseline-resolved.
4. Do not leave the instrument unattended during the first 30 min.

7.5. Shutdown

1. After the sequence completes, export raw data per the application SOP.
2. Remove all samples and consumables from the autosampler.
3. Log gas usage and any issues in the lab logbook.
4. Leave the instrument in standby per the lab manager's current instructions (the Delta V and ConFlo IV are typically left powered on continuously).

8. Troubleshooting

Symptom	Possible Cause / Action
High m/z 28 background	Air leak; check fittings and reactor seals
High m/z 18 background	Moisture; extend bake-out time
High m/z 40 background	Air leak (Ar); check fittings
Poor standard reproducibility	Capsule sealing; exhausted reactor
No peaks in chromatogram	Gas flow interrupted; check He supply
Sequence stops mid-run	Autosampler jam; check carousel alignment
On/off tests fail repeatedly	Source needs tuning or maintenance
Red/yellow lights on IRMS panel	Check Isodat error log; contact lab manager

If you cannot resolve an issue, **stop the run** and contact the lab manager. Do not attempt reactor replacement or major maintenance unless you are a certified Super-User.

9. Quality Control

- Run at least two certified reference materials per analytical sequence for two-point scale normalization (Paul et al., 2007).
- Reference material δ values must recover within $\pm 0.2‰$ of certified values; flag sequences that exceed this threshold.
- Blanks (empty tin capsules) must produce no detectable N_2 or CO_2 peak above the noise baseline.
- Archive all raw Isodat sequence files per SOP-L801.

10. References

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, *25*(17), 2538–2560. https://doi.org/10.1002/rcm.5129

Paul, D., Skrzypek, G., & F'orizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry*, *21*(18), 3006–3014. https://doi.org/10.1002/rcm.3185

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11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite

SOP	Title	Relationship
L414	Soil CN by Flash EA-IRMS	Application SOP
L415	Carbonate Isotopes by GasBench-IRMS	Application SOP

12. Training Record

Before using the IRMS independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety
3. Read the application SOP for the peripheral(s) they will use
4. Observe at least one complete analysis session by an experienced user
5. Complete supervised practice runs
6. Demonstrate a start-to-finish analysis to the satisfaction of the lab technician or a Super-User
7. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L211

Echo Labs Revolve Microscope

Standard Operating Procedure

SOP Number	SOP-L211
Revision	0.1
Author(s)	Dmaia Curry, Aparna Chintapalli
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, image capture, and maintenance of the Echo Labs Revolve microscope. The Revolve is a hybrid instrument that combines an upright microscope (for prepared glass slides) and an inverted microscope (for live samples in dishes) in a single rotating platform, with an iPad retina display replacing the traditional eyepiece (Echo Labs, 2016; Murphy & Davidson, 2012). The microscope supports bright field, phase contrast, fluorescence, and dark field imaging modes. It represents a substantial investment by the EA Program and requires careful handling.

2. Scope

- **Applies to:** All use of the Echo Labs Revolve microscope in the EA Program laboratory, including bright field, phase contrast, fluorescence, and dark field modes in both upright and inverted configurations
- **Does not apply to:** Electron microscopy, confocal microscopy, dissecting/stereo microscopes, or other optical microscopes not described here
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage software updates, maintain optics
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training (including video modules) before independent use

4. Definitions

- **Brightfield illumination:** Standard transmitted-light microscopy in which the sample is illuminated from below and absorbing or scattering structures appear dark against a bright background; the most common mode for stained biological specimens.

- **Darkfield illumination:** A transmitted-light mode in which the direct beam is blocked; only obliquely scattered light reaches the objective, making unstained transparent structures appear bright against a dark background.
- **Epifluorescence:** A fluorescence microscopy mode in which excitation light is directed through the objective onto the sample, and the emitted fluorescence is collected back through the same objective; enables fluorescent stain visualization.
- **Eyepiece (ocular):** The lens assembly through which the observer views the magnified image; typically 10x; total magnification equals eyepiece magnification times objective magnification.
- **Fluorescence filter cube:** An interchangeable optical block containing an excitation filter, dichroic mirror, and emission filter matched to a specific fluorophore (e.g., DAPI, FITC, TRITC).
- **Numerical aperture (NA):** A dimensionless number characterizing the angular range of light an objective can accept; higher NA provides better resolution and light-gathering; the resolving power is approximately $d = 0.61\lambda/NA$.
- **Objective:** The primary lens closest to the specimen; available in magnifications of 4x, 10x, 20x, 40x, and 100x (oil immersion); higher magnification objectives have shorter working distances.
- **Working distance:** The distance between the front lens of the objective and the coverslip when the specimen is in focus; decreases with increasing magnification; critical for thick samples.

5. Safety and Hazards

⚠ Hazards

- **Optical:** The fluorescence LED produces intense illumination. Avoid looking directly into the excitation light source. The iPad display eliminates the need to view through an eyepiece, reducing direct optical exposure, but reflected excitation light can still cause eye strain.
- **Electrical:** Keep liquids away from the iPad and the microscope base. If immersion oil or sample fluid contacts the electronics, disconnect power immediately and notify the lab manager.
- **Physical:** The microscope is heavy. Use both hands when rotating the platform between upright and inverted modes. Objective lenses and condensers are precision optics; handle with care.

5.1. Required PPE

☐ Safety glasses (especially during fluorescence mode)
☐ Lab coat
☐ Nitrile gloves (when handling biological specimens or immersion oil)

5.2. Emergency Procedures

If a biological spill occurs on or near the microscope, clean and disinfect per SOP-L002 Laboratory Safety. If immersion oil contacts the iPad or electronics, power off immediately and contact the lab manager before attempting cleanup.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Echo Labs Revolve microscope	With iPad retina display
Objective lenses	4x, 10x, 40x, 100x (oil immersion)
Low-magnification condenser	For 4x through 60x objectives
High-magnification condenser	For 100x oil immersion objective
Fluorescence LED module	Excitation wavelength TBD nm
Phase contrast rings	PhL, Ph1, Ph2, Ph3 (matched to objectives)

6.2. Consumables

Item	Notes
Microscope slides	Standard 25 x 75 mm
Cover slips	No. 1.5 thickness (0.17 mm)
Immersion oil (Type A or NF)	For 100x objective only
Lens cleaning solution	Optical-grade; for objectives and condenser
Lens cleaning tissue (lint-free)	Do not use Kimwipes on coated optics
Compressed gas duster	For removing dust from the optical path
Flash drive or cloud account	For exporting captured images

7. Procedure

7.1. Microscopy Modes Overview

The Revolve supports four imaging modes. Select the mode appropriate for the specimen and analysis:

Mode	Best For	Staining Required?
Bright field	Stained specimens, gross morphology	Recommended
Phase contrast	Unstained transparent specimens, live cells	No
Fluorescence	Specimens with fluorescent labels or autofluorescent pigments (e.g., cyanobacteria)	Fluorophore-dependent

Mode	Best For	Staining Required?
Dark field	Fine structures, edges, unstained specimens	No

7.2. Starting the Microscope

1. Press the power button on the top left corner of the iPad (Echo Labs, 2016).
2. Swipe to unlock. The overhead microscope light should turn on and a live image should appear on the screen.
3. If the light does not turn on or no image appears, open the **Echo Labs** app from the home screen
4. If the screen reads "Light Off," tap **BF** at the top of the screen to enable bright field illumination
5. Load the prepared slide into the stage clips (upright mode) or place the culture dish on the stage (inverted mode)

7.3. Rotating Between Upright and Inverted Mode

> **!** Important
>
> This is the most mechanically delicate operation on the Revolve. Follow each step carefully to avoid damaging the objectives or condenser.

1. Rotate the nosepiece to move all objective lenses out of the optical path
2. Unscrew the stage and carefully lift it away from the microscope body without contacting the condenser
3. Push the iPad flat so it is out of the rotation path
4. Press the release button on the upper back of the microscope body
5. Rotate the microscope body 180 degrees counterclockwise until it clicks into place
6. Reinstall the stage and tighten the mounting knob until secure
7. Rotate the desired objective lens back into position and realign the iPad

7.4. Using Bright Field Mode

1. Confirm that **BF** is selected on the screen
2. Place the slide or specimen on the stage
3. Start with the lowest magnification objective (4x) to locate the region of interest
4. Use the outer coarse focus knob (to the right of the iPad) to bring the specimen into approximate focus, then use the inner fine focus knob to sharpen the image
5. Use the stage translation knobs to move the slide: the lower knob moves the stage left/right, the upper knob moves it toward/away from you
6. Increase magnification by rotating the nosepiece to the next objective (10x, 40x)
7. Adjust brightness, contrast, and color balance using the sliding controls on the bottom right of the screen. Adjust the physical light intensity using the flat knob on the bottom right of the microscope body.

7.5. Using Phase Contrast Mode

Phase contrast converts differences in refractive index into differences in brightness, allowing transparent, unstained specimens to be visualized.

1. Set up the specimen in bright field mode first and bring it into focus
2. Select the phase contrast ring that matches the current objective:

Objective	Phase Ring
4x	PhL
10x	Ph1
40x	Ph2
100x	Ph3

3. Tap the **PH** mode selector on the screen (if available) or manually rotate the condenser turret to the matching phase ring
4. Fine-tune focus and centering as needed

> **Note**
>
> Phase contrast images may exhibit a bright "halo" around large objects (positive phase contrast) or a dark edge (negative phase contrast). This is a normal optical artifact caused by diffracted light passing through the phase ring. A "shade-off" effect, in which homogeneous regions appear at the same intensity as the background, is also expected.

7.6. Using Fluorescence Mode

1. Tap **FL** at the top of the screen to switch to fluorescence mode
2. The fluorescence LED will illuminate automatically
3. Place the specimen on the stage and focus using the same procedure as bright field
4. Minimize illumination time to reduce photobleaching. Locate the region of interest in bright field first, then switch to fluorescence only for image capture.

> **Warning**
>
> Fluorophores lose their ability to fluoresce over time when illuminated (photobleaching). Work quickly, minimize unnecessary excitation, and consider using photoprotective mounting media when possible.

7.7. Using Dark Field Mode

Section TBD. Consult the lab manager for dark field setup procedures until this section is completed.

7.8. Capturing and Exporting Images

1. To capture an image, tap the white camera shutter button on the left side of the screen
2. Enable the **anti-shake** feature (hand icon below the shutter button; it should turn orange when active) to reduce vibration blur
3. To review captured images, tap the gallery icon (square) at the top left of the screen. Tap **Done** to return to the live view.
4. Export images via a flash drive connected to the iPad, AirDrop, or the cloud sharing option in the Echo Labs app
5. Use a consistent file-naming convention: `YYYY-MM-DD_SampleID_Objective_Mode.jpg`

7.9. Using Oil Immersion (100x Objective)

1. Focus the specimen at 40x first
2. Rotate the nosepiece to an empty position (no objective in the light path)
3. Place a single small drop of immersion oil directly on the cover slip at the point of illumination
4. Rotate the 100x objective into position. The lens front element should contact the oil without trapping an air bubble.
5. Focus carefully using the fine focus knob only
6. After use, immediately clean the 100x objective with lens cleaning solution and lint-free tissue. Never allow oil to dry on the lens.
7. Clean the slide or dispose of it as appropriate

7.10. Replacing the Condenser

The Revolve has two condensers: one for low magnification (4x through 60x) and one for 100x oil immersion. Condenser swaps require care to avoid contaminating or scratching the optics. Consult the lab manager before performing this procedure for the first time.

Detailed replacement steps TBD; to be documented with the lab manager.

7.11. Shutdown and Daily Maintenance

1. Remove the specimen from the stage
2. Rotate the nosepiece to the lowest-magnification objective (4x) or to an empty position
3. If oil immersion was used, clean the 100x objective immediately
4. Wipe any sample residue or oil from the stage with a Kimwipe dampened with lens cleaning solution
5. Close the Echo Labs app on the iPad
6. Press and hold the power button to turn off the iPad
7. Cover the microscope with the dust cover

8. Quality Control and Acceptance Criteria

8.1. Optics Cleaning

If specks or smudges appear in captured images that do not move when the specimen is translated, the optical path may need cleaning:

1. Do not attempt to clean internal optics without the lab manager present
2. For objective lens surfaces, apply a small amount of lens cleaning solution to lint-free tissue and gently wipe in a circular motion from center to edge
3. For dust on internal surfaces, remove the arm and use short bursts of compressed gas duster. Do not allow the compressed air canister to become cold, as it will deposit a film on the optics.
4. For persistent contamination, contact Echo Labs support or the lab manager

8.2. Image Quality Checklist

Before accepting an image for publication or analysis, verify:

- Focus is sharp across the region of interest
- Illumination is even (no hot spots or vignetting)
- Scale bar or magnification metadata is recorded
- Anti-shake was enabled during capture
- In fluorescence mode, confirm that photobleaching has not significantly degraded the signal

9. Waste Disposal

Waste Type	Disposal Method
Used immersion oil tissues	Standard trash
Used microscope slides (non-hazardous)	Broken-glass box if glass; standard trash if plastic
Biological specimen waste	Biohazard bag if applicable; autoclave before disposal
Spent lens cleaning solution	Standard trash (small volumes on tissues)

10. References

Echo Labs. (2016). *Revolve microscope: User guide*. https://www.echolabs.com

Murphy, D. B., & Davidson, M. W. (2012). *Fundamentals of light microscopy and electronic imaging* (2nd ed.). Wiley. https://doi.org/10.1002/9781118382905

11. Training Record

Before using the Revolve microscope independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Watch the Echo Labs training video at https://www.youtube.com/watch?v=IcubmIsxa7w
4. Observe a trained operator demonstrate upright/inverted rotation, all four imaging modes, and oil immersion setup and cleanup
5. Demonstrate proper startup, focusing, image capture, and shutdown under supervision
6. Be signed off by the PI or lab manager

12. Appendix A: Microscope Anatomy Quick Reference

Component	Function
iPad display (eyepiece replacement)	High-resolution live view and image capture
Arm	Connects optics to base; removable for optical path cleaning
Stage	Flat platform for specimens; translatable via X/Y knobs
Nosepiece (turret)	Holds and rotates objective lenses
Objective lenses	4x, 10x, 40x, 100x (oil); determine magnification
Condenser	Focuses illumination light onto the specimen
Coarse/fine focus knobs	Outer knob = coarse focus; inner knob = fine focus
Light intensity knob	Physical control on the base for illumination brightness
Phase contrast turret	PhL, Ph1, Ph2, Ph3 rings matched to objectives
Fluorescence LED	Excitation source for fluorescence imaging
Platform release button	On the upper back; allows 180-degree rotation between upright and inverted modes

13. Appendix B: Sections Requiring Completion

The following sections from the original SOP were incomplete or marked as placeholders. They should be completed with input from the lab manager:

Section	Status	Notes
Fluorescence LED wavelength	TBD	Record the excitation wavelength from the module specifications
Dark field mode procedure	TBD	Document setup and use
Condenser replacement steps	TBD	Document with the lab manager
Specific catalog numbers for consumables	TBD	Add slide, cover slip, oil, lens cleaner part numbers

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L212

NanoDrop One Spectrophotometer

Standard Operating Procedure

SOP Number	SOP-L212
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration verification, and maintenance of the Thermo Scientific NanoDrop One micro-volume UV-Vis spectrophotometer. The NanoDrop measures nucleic acid and protein concentrations from samples as small as 1–2 uL and provides absorbance ratios (A_{260}/A_{280} and A_{260}/A_{230}) used to assess sample purity. For the role of NanoDrop measurements within the DNA extraction workflow, see SOP-L700 DNA Extraction.

2. Scope

- **Applies to:** All use of the NanoDrop One spectrophotometer in the EA Program laboratory, including nucleic acid quantification, protein quantification (A_{280} method), and custom wavelength measurements
- **Does not apply to:** Cuvette-based spectrophotometers (see SOP-L201), fluorometric quantification (see SOP-L213), or Hach benchtop spectrophotometry (see SOP-L303)
- **Location:** Instrument bench, Seaver ??

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage software updates
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **A_{260}:** Absorbance at 260 nm; the wavelength at which double-stranded DNA and RNA absorb most strongly; used to quantify nucleic acid concentration.
- **A_{280}:** Absorbance at 280 nm; absorbed primarily by aromatic amino acids (tyrosine, tryptophan) and by phenolic contaminants; used to assess protein contamination in nucleic acid preparations.
- **A_{260}/A_{280} ratio:** The ratio of absorbance at 260 nm to 280 nm; pure dsDNA gives ~1.8; pure RNA gives ~2.0; values below 1.7 indicate protein or phenol contamination.
- **A_{260}/A_{230} ratio:** The ratio of absorbance at 260 nm to 230 nm; values of 2.0–2.2 are expected for pure nucleic acids; low values indicate contamination with chaotropic salts (guanidinium), carbohydrates, or humic acids.
- **Micro-volume spectrophotometry:** Absorbance measurement using 1–2 µL of sample held by surface tension between a pedestal and an optical fiber arm; eliminates the need for cuvettes and allows measurement of very small volumes.
- **Pedestal:** The fixed lower optic of the NanoDrop on which the sample droplet is placed; must be cleaned with a lint-free wipe between measurements to prevent carryover.
- **Pathlength normalization:** Automatic adjustment of the measured absorbance to a standardized 10 mm pathlength equivalent; allows direct comparison with traditional cuvette measurements.

5. Safety and Hazards

⚠ Hazards

- **Optical:** The NanoDrop uses a pulsed xenon flash lamp. Do not look directly into the light source when the pedestal arm is open.
- **Biological:** Samples may contain hazardous biological material. Treat all samples as potentially infectious.
- **Chemical:** Cleaning solvents (e.g., 0.5 M HCl for decontamination) require appropriate handling.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For biological spills on or near the instrument, clean and disinfect per SOP-L002 Laboratory Safety. If liquid enters the instrument housing, disconnect power immediately and contact the lab manager.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
NanoDrop One spectrophotometer	Instrument bench, SGM 133	Thermo Scientific
Connected laptop or NanoDrop display	Instrument bench	For data export

6.2. Consumables

Item	Notes
Lint-free wipes (Kimtech)	For pedestal cleaning between samples
Nuclease-free water	For blanking and pedestal cleaning
Elution buffer	Must match the buffer used in extraction

7. Procedure

7.1. Startup

1. Open the NanoDrop One software (or wake the integrated touchscreen)
2. Select the appropriate application (e.g., **Nucleic Acid**, **Protein A280**, or **UV-Vis**)
3. Clean the upper and lower pedestals with 2 uL of nuclease-free water; wipe with a lint-free tissue
4. Perform a blank measurement (see Section 6.2)

7.2. Blanking

1. Pipette 2 uL of the blank solution onto the lower pedestal. The blank must match the sample matrix – use the same elution buffer or solvent that the samples are dissolved in.
2. Lower the sampling arm and tap **Blank**
3. Verify that the blank spectrum is flat and the baseline absorbance at 260 nm is near zero
4. Wipe both pedestals with a lint-free tissue

> **Tip**
>
> Re-blank whenever you switch sample types, change buffers, or if the instrument has been idle for more than 30 minutes.

7.3. Measuring Samples

1. Pipette 2 uL of sample onto the lower pedestal
2. Lower the sampling arm and tap **Measure**
3. Record the following for each sample:
   - Concentration (ng/uL)
   - A_{260}/A_{280} ratio
   - A_{260}/A_{230} ratio
4. Wipe both pedestals with a lint-free tissue between every sample
5. Repeat for all samples

7.4. Interpreting Results

Parameter	Expected Range	Interpretation
A_{260}/A_{280}	1.8–2.0	Values below 1.8 suggest protein or phenol contamination
A_{260}/A_{230}	2.0–2.2	Values below 2.0 suggest humic acid, carbohydrate, salt, or guanidine contamination

> **Note**
>
> For environmental DNA extracts, A_{260}/A_{230} ratios are frequently below 2.0. If the NanoDrop concentration is more than twice the Qubit reading, the UV absorbance is likely inflated by co-extracted contaminants; use the Qubit value for planning downstream reactions.

7.5. Data Export

1. Save the session file (.tsv or .csv) to the designated project folder on the connected laptop
2. Use a consistent file-naming convention: `YYYY-MM-DD_NanoDrop_ProjectName.tsv`
3. Record results in the lab notebook and/or project data sheet

7.6. Shutdown and Daily Maintenance

1. Wipe both pedestals with 2 uL of nuclease-free water, then dry with a lint-free tissue
2. If protein or concentrated DNA residue is visible, clean with 2 uL of 0.5 M HCl, wait 10 seconds, then rinse with nuclease-free water
3. Close the software and lower the sampling arm
4. Cover the instrument with the dust cover if available

8. Quality Control and Acceptance Criteria

- Verify instrument performance at the start of each session by measuring a blank; the 260 nm absorbance should read 0.00 +/- 0.02
- If the NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; trust the Qubit value
- *TBD – establish a schedule for periodic performance verification using Thermo Scientific NanoDrop verification standards*

9. Waste Disposal

Waste Type	Disposal Method
Used lint-free wipes	Standard trash
Pipette tips	Standard trash (or biohazard if samples are hazardous)
Residual sample on pedestal	Wiped and cleaned as part of shutdown

10. References

- Thermo Fisher Scientific. *NanoDrop One/One^C User Guide*. Thermo Fisher Scientific, Waltham, MA.

- Desjardins, P. & Bhatt, T. (2010). Micro-volume quantification of nucleic acids. *Current Protocols*, A.3J.1–A.3J.16.

11. Training Record

Before using the NanoDrop independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Observe a trained operator demonstrate blanking, measurement, and data export
4. Perform a practice session (blank plus at least 5 samples) under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L213

Qubit 4 Fluorometer

Standard Operating Procedure

SOP Number	SOP-L213
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration, and maintenance of the Invitrogen Qubit 4 Fluorometer. The Qubit uses fluorescent dyes that bind selectively to the target molecule (dsDNA, ssDNA, RNA, or protein), providing more accurate quantification than UV absorbance methods, especially at low concentrations or when contaminants are present. For the role of Qubit measurements within the DNA extraction workflow, see SOP-L700 DNA Extraction.

2. Scope

- **Applies to:** All use of the Qubit 4 Fluorometer in the EA Program laboratory, including dsDNA Broad Range (BR), dsDNA High Sensitivity (HS), RNA, and protein assays
- **Does not apply to:** UV-Vis spectrophotometric quantification (see SOP-L212), Turner Designs fluorometry for chlorophyll or other environmental analytes (see SOP-L203)
- **Location:** Instrument bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Maintain reagent inventory, schedule service
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Broad Range (BR) assay:** A Qubit assay kit designed for DNA concentrations of 2–1,000 ng/µL; less sensitive than the HS assay but covers a wider dynamic range; used when high DNA yield is expected.
- **dsDNA:** Double-stranded DNA; the form quantified by most Qubit DNA assay kits; the fluorescent dye binds specifically to dsDNA, not to ssDNA, RNA, or protein contaminants.
- **Fluorophore:** A fluorescent chemical compound that absorbs light at a specific excitation wavelength and emits light at a longer emission wavelength; the Qubit dye is a fluorophore that binds selectively to dsDNA, ssDNA, or RNA depending on the kit.
- **High Sensitivity (HS) assay:** A Qubit assay kit designed for DNA concentrations of 0.2–100 ng/µL; more sensitive than BR; preferred for low-yield environmental DNA extractions from soil or water.
- **Qubit assay tube:** A thin-walled 0.5 mL PCR tube in which the working solution and sample are mixed for measurement; must be free of fluorescent contaminants; do not substitute with other tube types.
- **Working solution:** A freshly prepared mixture of Qubit reagent diluted in buffer at a ratio specified by the kit (usually 1:200); prepared fresh for each session; stable for up to 3 hours at room temperature.

5. Safety and Hazards

Hazards

- **Chemical:** Qubit reagent concentrate contains DMSO. Avoid skin contact; review the SDS before handling.
- **Biological:** Samples may contain hazardous biological material. Treat all samples as potentially infectious.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes and consult the SDS. For biological spills, clean per SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Qubit 4 Fluorometer	Instrument bench, SGM 133	Invitrogen / Thermo Fisher
USB drive or Wi-Fi connection	–	For data export

6.2. Reagents and Consumables

Item	Catalog No.	Storage
Qubit dsDNA BR Assay Kit	Q32850	Room temperature
Qubit dsDNA HS Assay Kit	Q32851	Room temperature
Qubit assay tubes (0.5 mL, thin-wall)	Q32856	Room temperature
Nuclease-free water	Various	Room temperature

Note

Use only the thin-wall 0.5 mL Qubit assay tubes (Cat. Q32856). Standard microcentrifuge tubes are not optically compatible and will produce inaccurate readings.

7. Procedure

7.1. Selecting the Assay

Choose the assay based on expected concentration (Invitrogen, 2019):

Assay	Detection Range	When to Use
dsDNA BR	2–1,000 ng	Most soil/sediment extractions
dsDNA HS	0.2–100 ng	Low-yield samples (e.g., water filters, dilute extracts)
RNA BR / HS	See kit documentation	RNA quantification
Protein	See kit documentation	Protein quantification

7.2. Preparing the Working Solution

1. Calculate the volume of working solution needed: 200 uL per assay tube (all samples plus 2 standards)
2. Dilute the Qubit reagent concentrate 1:200 in Qubit buffer. For example, for 10 tubes: 10 uL reagent + 1,990 uL buffer = 2,000 uL total.
3. Vortex briefly to mix

> **Important**
>
> Prepare the working solution fresh for each session. Do not store diluted working solution.

7.3. Preparing Standards

1. Label two Qubit assay tubes: **Std 1** and **Std 2**
2. Add 190 uL of working solution to each tube
3. Add 10 uL of Standard 1 to the first tube and 10 uL of Standard 2 to the second tube
4. Vortex each tube briefly (2–3 seconds)

7.4. Preparing Samples

1. Label one Qubit assay tube per sample
2. Add 198 uL of working solution to each tube
3. Add 2 uL of sample (DNA extract, RNA, or protein solution) to the corresponding tube
4. Vortex each tube briefly (2–3 seconds)

> **Tip**
>
> The sample volume can range from 1–20 uL as long as the total volume in each tube equals 200 uL (adjust the working solution volume accordingly). Using 2 uL of sample minimizes

consumption of precious extracts.

7.5. Incubation

1. Incubate all tubes (standards and samples) at room temperature for 2 minutes (Invitrogen, 2019)
2. Do not incubate for longer than 30 minutes; fluorescence signal decays over time

7.6. Calibration

1. On the Qubit home screen, select the appropriate assay (e.g., **dsDNA Broad Range**)
2. Tap **Read Standards**
3. Insert the Std 1 tube, close the lid, and tap **Read**
4. When prompted, insert the Std 2 tube, close the lid, and tap **Read**
5. Verify that the calibration is accepted (green checkmark). If it fails, prepare fresh standards and repeat.

7.7. Reading Samples

1. Tap **Run Samples**
2. Set the sample volume to match the volume of sample added (e.g., 2 uL)
3. Set the output units (ng/uL is standard for DNA extracts)
4. Insert the sample tube, close the lid, and tap **Read**
5. Record the concentration (ng/uL, corrected for dilution by the instrument)
6. Repeat for all samples

7.8. Data Export

1. Export results via USB or Wi-Fi to the designated project folder
2. Use a consistent file-naming convention: `YYYY-MM-DD_Qubit_ProjectName.csv`
3. Record results in the lab notebook and/or project data sheet

7.9. Shutdown and Daily Maintenance

1. Remove all tubes from the instrument
2. Wipe the sample chamber with a lint-free tissue if any liquid was spilled
3. Close the lid
4. Dispose of used assay tubes (see Section 8)

8. Quality Control and Acceptance Criteria

- Run fresh standards at the beginning of each session; do not rely on a previous calibration
- Standards must be within the acceptable range indicated by the instrument (green checkmark)

- If a sample reads "Out of Range," re-prepare it using the other assay sensitivity (e.g., switch from BR to HS for low-concentration samples)
- Acceptable DNA yield from 0.25 g of soil: typically 1–20 ng/uL in a 100 uL elution, depending on soil type
- If the NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; trust the Qubit value for downstream planning

TBD – establish a periodic performance check using Qubit DNA standards of known concentration.

9. Waste Disposal

Waste Type	Disposal Method
Used Qubit assay tubes	Standard trash (rinse if biohazardous)
Leftover working solution	Standard drain (dilute DMSO, non-hazardous at this concentration)
Pipette tips	Standard trash (or biohazard if samples are hazardous)

10. References

Invitrogen. (2019). *Qubit 4 fluorometer user guide.* Thermo Fisher Scientific. https://www.thermofisher.com/us/en/home/references/protocols/nucleic-acid-purification-and-analysis/qubit-assay-protocols.html

11. Training Record

Before using the Qubit independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Observe a trained operator demonstrate standard preparation, calibration, sample reading, and data export
4. Perform a practice session (calibration plus at least 5 samples) under supervision
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L214

Bio-Rad QX200 Droplet Digital PCR System

Standard Operating Procedure

SOP Number	SOP-L214
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, maintenance, and troubleshooting of the Bio-Rad QX200 Droplet Digital PCR (ddPCR) system, including the QX200 Droplet Generator, the C1000 Touch Thermal Cycler with the 96-well Deep Well Reaction Module, and the QX200 Droplet Reader. The QX200 partitions each PCR reaction into approximately 20,000 nanoliter-sized droplets, enabling absolute quantification of target DNA sequences without a standard curve. For assay design, primer/probe sequences, and data analysis, see SOP-L701 ddPCR Nitrogen Cycle Primers and SOP-L702 ddPCR Data Analysis.

2. Scope

- **Applies to:** All use of the Bio-Rad QX200 ddPCR system in the EA Program laboratory, including droplet generation, thermal cycling, and droplet reading
- **Does not apply to:** Assay design, primer validation, or data analysis (see SOP-L701 and SOP-L702); conventional qPCR on other instruments
- **Location:** *TBD* (confirm room and bench assignment)

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage consumable inventory, coordinate Bio-Rad service visits
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete Bio-Rad training modules and supervised practice before independent use

4. Definitions

- **Amplitude:** The fluorescence signal intensity of a droplet; positive droplets (containing target DNA) have high amplitude; negative droplets have low amplitude; a clear separation between positive and negative populations is required for accurate quantification.
- **Droplet digital PCR (ddPCR):** A method of absolute quantification of nucleic acids in which the sample is partitioned into ~20,000 nanoliter-sized water-in-oil droplets, each containing zero or one target molecule; after PCR amplification, the fraction of fluorescent positive droplets is used to calculate target concentration using Poisson statistics.
- **FAM:** A green fluorescent dye (fluorescein amidite) used to label one probe or primer in a duplex ddPCR assay; detected in the FAM channel (channel 1) by the QX200.
- **HEX:** A yellow-orange fluorescent dye used to label a second probe in a duplex ddPCR assay, or as an alternative to VIC; detected in channel 2 by the QX200.

- **Poisson correction:** A statistical adjustment applied by the DropletQuant software to account for the probability that some droplets contain more than one target molecule; necessary when target concentrations are high.
- **Copies per microliter (copies/µL):** The absolute concentration of the target DNA in the final ddPCR reaction, as reported by DropletQuant; must be back-calculated to the original sample concentration using the appropriate dilution factor.
- **Threshold:** The fluorescence amplitude boundary separating positive from negative droplets; set manually or automatically in the analysis software; incorrect threshold setting is the most common source of ddPCR quantification error.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Droplet generation oil (Bio-Rad Cat. 1863005) is a fluorinated oil. Avoid skin contact and inhalation. Work in a well-ventilated area or fume hood when handling bulk oil. Review the SDS before first use.
- **Biological:** Samples may contain environmental DNA from unknown organisms. Treat all samples and amplified products as potentially biohazardous.
- **Physical:** The thermal cycler reaches temperatures up to 98 C. Allow the block to cool before removing plates. The Droplet Reader needle is sharp; do not reach into the reader bay.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (change between pre-PCR and post-PCR areas)

5.2. Emergency Procedures

For oil spills, absorb with paper towels and dispose as chemical waste. For thermal burns, apply cool running water and seek first aid. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
QX200 Droplet Generator	*TBD*	Generates water-in-oil emulsion

Item	Location	Notes
C1000 Touch Thermal Cycler	*TBD*	With 96-Deep Well Reaction Module
QX200 Droplet Reader	*TBD*	Reads fluorescence per droplet
Computer with QuantaSoft / QuantaSoft AP	*TBD*	For instrument control and analysis
DG8 Cartridge Holder	*TBD*	Holds cartridges during generation
Plate sealer (PX1 PCR Plate Sealer)	*TBD*	Heat-seals 96-well plates

6.2. Reagents and Consumables

Item	Catalog No.	Storage
DG8 Cartridges for QX200	1864008	Room temperature
DG8 Gaskets	1863009	Room temperature
Droplet Generation Oil for Probes	1863005	Room temperature
Droplet Generation Oil for EvaGreen	1864006	Room temperature
ddPCR 96-Well Plates	12001925	Room temperature
Pierceable Foil Heat Seals	1814040	Room temperature
ddPCR Supermix for Probes (No dUTP)	1863024	–20 C
QX200 ddPCR EvaGreen Supermix	1864034	–20 C

7. Procedure

7.1. Pre-PCR Setup (Clean Area)

> **!** Important
>
> All pre-PCR work (reaction assembly, droplet generation) must be performed in the designated pre-PCR area to prevent amplicon contamination. Use dedicated pipettes and wear clean gloves.

1. Thaw the ddPCR Supermix and DNA templates on ice
2. Prepare the reaction master mix according to the assay protocol (see SOP-L701 for primer/probe concentrations and cycling conditions)
3. Dispense 20 uL of each assembled reaction into the sample wells (middle row) of a DG8 cartridge
4. Add 70 uL of Droplet Generation Oil to the oil wells (bottom row)
5. Attach a DG8 gasket to the cartridge

7.2. Droplet Generation

1. Place the loaded cartridge into the QX200 Droplet Generator

2. Close the lid and press **Start**. Generation takes approximately 2 minutes per cartridge.
3. When complete, carefully open the lid. Do not tilt or shake the cartridge – the droplets are fragile.
4. Using a multichannel pipette set to 40 uL, gently aspirate the droplets from the top row of the cartridge and dispense into the 96-well plate. Pipette slowly (approximately 3 seconds to aspirate, 3 seconds to dispense) to avoid shearing droplets.
5. Repeat for each cartridge

7.3. Plate Sealing

1. Place the pierceable foil heat seal over the loaded 96-well plate
2. Seal using the PX1 PCR Plate Sealer at 180 C for 5 seconds
3. Visually confirm a uniform seal across all wells

7.4. Thermal Cycling

1. Place the sealed plate into the C1000 Touch Thermal Cycler with the 96-Deep Well Reaction Module
2. Program the thermal cycling protocol per SOP-L701 (typical: 95 C activation, 40 cycles of denaturation/annealing, 98 C droplet stabilization, 4 C hold)
3. Set the ramp rate to 2 C/s (critical for ddPCR; do not use the default maximum ramp rate)
4. Start the run. Cycling typically takes 2–2.5 hours.
5. After cycling, plates can be held at 4 C for up to 24 hours before reading, but immediate reading is preferred

7.5. Droplet Reading

1. Open QuantaSoft software on the connected computer
2. Set up the plate layout: assign sample names, target names, experiment type (ABS for absolute quantification), and supermix type
3. Place the sealed plate into the QX200 Droplet Reader
4. Confirm that the reader waste and buffer bottles are at appropriate levels (refill or empty as needed before starting a full plate)
5. Tap **Start** to begin reading. The reader processes each well individually (approximately 1–2 minutes per well).
6. Monitor the first few wells to confirm that droplet counts exceed 10,000 accepted events per well

7.6. Data Review

1. After reading, review the 1-D amplitude plots in QuantaSoft to verify clear separation between positive and negative droplet clusters
2. Set the fluorescence amplitude threshold manually using the NTC and positive control wells as guides
3. Check that all wells have at least 10,000 accepted droplets; flag any wells below this threshold
4. Export results as .csv for downstream analysis (see SOP-L702)

7.7. Shutdown and Daily Maintenance

1. Remove the plate from the Droplet Reader
2. Check the reader waste bottle; empty if more than 75% full
3. Check the reader buffer bottle; refill if less than 25% full
4. Wipe down the Droplet Generator cartridge holder with a lint-free tissue
5. Dispose of used cartridges and gaskets (see Section 8)
6. Close QuantaSoft and shut down instruments if no further runs are scheduled

8. Quality Control and Acceptance Criteria

- Every run must include: no-template controls (NTC, minimum 2 wells), extraction blanks (from SOP-L700), and positive controls (gBlock or plasmid at known concentration)
- Droplet count per well must exceed 10,000 accepted droplets (out of approximately 20,000 generated); wells below this threshold should be excluded
- NTC wells must show 0 or fewer than 3 positive droplets; more than 3 indicates contamination and the plate or affected target should be rerun
- Positive/negative droplet cluster separation must be unambiguous on the 1-D amplitude plot
- *TBD – define acceptable coefficients of variation for technical replicates (typically less than 15–25% for ddPCR)*
- *TBD – establish a maintenance log and schedule for Bio-Rad service visits*

9. Waste Disposal

Waste Type	Container	Disposal Method
Used DG8 cartridges and gaskets	Standard trash	Non-hazardous
Droplet generation oil waste	Chemical waste container	EH&S hazardous waste pickup
Used PCR plates and foil seals	Standard trash	Non-hazardous after thermal cycling
Pipette tips (pre- and post-PCR)	Standard trash	Non-hazardous
Reader waste fluid	Chemical waste container	EH&S hazardous waste pickup

10. References

- Bio-Rad Laboratories. *QX200 Droplet Digital PCR System Instruction Manual.* Bio-Rad Laboratories, Hercules, CA.
- Bio-Rad Laboratories. *Droplet Digital PCR Applications Guide.* Bio-Rad Bulletin 6407.
- Hindson, B. J. et al. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, 83(22), 8604–8610.

11. Training Record

Before using the QX200 system independently, personnel must:

1. Read this SOP, SOP-L701, and SOP-L003 Hazardous Materials Handling
2. Complete the Bio-Rad QX200 online training modules or equivalent manufacturer training
3. Observe a trained operator demonstrate droplet generation, thermal cycling setup, and droplet reading for a full plate
4. Perform a supervised run including reaction assembly, droplet generation, plate sealing, thermal cycling, and reading
5. Demonstrate competence in QuantaSoft plate setup and threshold setting
6. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Low droplet count (below 10,000)	Pipetting error during transfer; clogged cartridge	Pipette more slowly (3 s up, 3 s down); use a fresh cartridge
No separation between positive and negative clusters	Poor primer/probe design; degraded reagents	Review assay (SOP-L701); use fresh Supermix
Rain (droplets between clusters)	Suboptimal annealing temperature; partial inhibition	Run a temperature gradient; dilute template
Reader error or blockage	Air bubble or debris in reader line	Run a cleaning cycle per Bio-Rad instructions; contact lab manager
Positive droplets in NTC wells	Amplicon contamination	Decontaminate pre-PCR area with 10% bleach; use fresh reagents; separate pre- and post-PCR areas strictly

13. Appendix B: Sections Requiring Completion

Section	Status	Notes
Instrument location	TBD	Confirm room and bench assignment
Maintenance schedule	TBD	Establish log and Bio-Rad service intervals
Reader cleaning cycle details	TBD	Document with Bio-Rad field service engineer
Lab-specific LOD/LOQ values	TBD	Establish after primer validation (SOP-L701)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L214

QX200 Droplet Digital PCR System: Instrument Familiarization and Standard Operation

Standard Operating Procedure

SOP Number	SOP-L214
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure orients new personnel to the Bio-Rad QX200 Droplet Digital PCR (ddPCR) system and describes its safe, standard operation: the components of the system, the consumables and reagents, the four-stage workflow (droplet generation, plate sealing, thermal cycling, and droplet reading), basic use of QuantaSoft software, routine maintenance, and common troubleshooting.

It is a familiarization and operation SOP. It does not cover the science of assay or primer design (see SOP-L701) or the analysis and interpretation of results (see SOP-L702 Applications Guide). The workflow described here follows Bio-Rad's published guidance for the platform (Bio-Rad Laboratories, 2018, 2024), which itself implements the droplet-counting principle of absolute quantification (Hindson et al., 2011).

2. Scope

- **Applies to:** Operation of the QX200 ddPCR system in the lab, comprising the QX200 Droplet Generator, the PX1 PCR Plate Sealer, the C1000 Touch Thermal Cycler with 96-deep-well reaction module, and the QX200 Droplet Reader, together with QuantaSoft software.
- **Does not apply to:** Assay/primer design (SOP-L701), data analysis and interpretation (SOP-L702), or DNA extraction (SOP-L700).
- **Prerequisite:** A prepared, validated reaction mix and plate plan from SOP-L701.

> **SOP numbering**
>
> This instrument SOP is numbered L214, placing it ahead of the L700-series analysis SOPs in the lab scheme. Conceptually it precedes analysis: complete this familiarization before running samples for SOP-L702.

3. Responsibilities

Role	Responsibility
Lab PI	Approve instrument access; oversee major maintenance
Lab Manager	Maintain consumable stock, oil, and service records
Trained Personnel	Operate the system per this SOP; log each run
New Students	Complete familiarization and supervised runs before independent use

4. Definitions

- **Amplitude:** The fluorescence signal intensity of a droplet; positive droplets (containing target DNA) have high amplitude; negative droplets have low amplitude; a clear separation between positive and negative populations is required for accurate quantification.
- **Droplet digital PCR (ddPCR):** A method of absolute quantification of nucleic acids in which the sample is partitioned into ~20,000 nanoliter-sized water-in-oil droplets, each containing zero or one target molecule; after PCR amplification, the fraction of fluorescent positive droplets is used to calculate target concentration using Poisson statistics.
- **FAM:** A green fluorescent dye (fluorescein amidite) used to label one probe or primer in a duplex ddPCR assay; detected in the FAM channel (channel 1) by the QX200.
- **HEX:** A yellow-orange fluorescent dye used to label a second probe in a duplex ddPCR assay, or as an alternative to VIC; detected in channel 2 by the QX200.
- **Poisson correction:** A statistical adjustment applied by the DropletQuant software to account for the probability that some droplets contain more than one target molecule; necessary when target concentrations are high.
- **Copies per microliter (copies/µL):** The absolute concentration of the target DNA in the final ddPCR reaction, as reported by DropletQuant; must be back-calculated to the original sample concentration using the appropriate dilution factor.
- **Threshold:** The fluorescence amplitude boundary separating positive from negative droplets; set manually or automatically in the analysis software; incorrect threshold setting is the most common source of ddPCR quantification error.

5. Safety and Hazards

⚠ Hazards and precautions

- **Droplet generation oil and reader oil.** Use the oil specified for the chemistry (probe vs. EvaGreen). Avoid skin and eye contact; wear gloves and eye protection; consult the SDS for each oil. Clean spills promptly.
- **Reagents.** ddPCR supermixes and any sample DNA are handled with gloves. Environmental soil DNA is low biohazard risk, but treat all extracts with standard lab precautions.
- **Moving parts.** The droplet reader autosampler moves during a run. Keep hands clear and the lid closed while running.
- **Electrical and thermal.** Standard bench-instrument electrical safety. The thermal cycler block reaches high temperatures; do not touch the block during or immediately after a run.
- **Sharps / glass.** None in normal operation; cartridges and plates are single-use plastics.

! Consumables are not interchangeable

ddPCR requires Bio-Rad's proprietary droplet-generation reagents, cartridges, and gaskets. Substituting non-specified consumables will produce unusable droplets (Bio-Rad Laboratories, 2018). Match the oil to the supermix chemistry.

6. Equipment, Consumables, and Reagents

6.1. Instruments

Item	Role in workflow
QX200 Droplet Generator	Partitions each 20 uL reaction into ~20,000 droplets
DG8 cartridge holder	Holds the DG8 cartridge during generation
PX1 PCR Plate Sealer	Heat-seals droplets into the 96-well plate with pierceable foil
C1000 Touch Thermal Cycler (96-deep-well module)	Endpoint PCR amplification within droplets
QX200 Droplet Reader	Streams droplets single-file and reads two-color fluorescence
Instrument computer (QuantaSoft)	Plate setup, run control, thresholding, export

6.2. Consumables and reagents

Item	Notes
DG8 cartridges and gaskets	Single-use; one cartridge = 8 samples
Droplet generation oil (probes) **or** QX200 EvaGreen droplet oil	Match to supermix chemistry
Droplet reader oil	Loaded in the reader
ddPCR Supermix for Probes **or** QX200 ddPCR EvaGreen Supermix	Per assay (SOP-L701)
ddPCR 96-well plates and pierceable foil heat seals	For PX1 sealing and reading
Filtered pipette tips, low-bind tubes	Standard molecular-biology consumables

Tip

Keep a stock log for cartridges, gaskets, oils, plates, and foil. The droplet generator and reader each draw oil from onboard bottles; check levels before every session.

Ordering supermixes, oils, and assays

Order supermixes, droplet oils, cartridges, plates, and validated PrimePCR ddPCR assays from the Bio-Rad digital assay catalog: https://www.bio-rad.com/digital-assays. Confirm chemistry (probe vs EvaGreen) before ordering so the supermix and matching oil are consistent.

7. Procedure

7.1. System Overview

The QX200 system performs absolute quantification by partitioning, amplifying, and counting (Bio-Rad Laboratories, 2018; Hindson et al., 2011). A single 20 uL reaction is split into roughly 20,000 uniform water-in-oil droplets. Each droplet is a tiny independent PCR. After endpoint amplification, the reader passes droplets one at a time through a two-color optical detector (channel 1 for FAM, channel 2 for HEX or VIC), scoring each droplet as positive or negative. QuantaSoft fits the positive fraction to a Poisson model to return target concentration in copies per microliter.

The work proceeds through four instruments in sequence:

1. **Droplet Generator** (Section 6.4) makes the droplets.
2. **PX1 Plate Sealer** (Section 6.5) seals them into a plate.
3. **C1000 Touch Thermal Cycler** (Section 6.6) amplifies.
4. **Droplet Reader** (Section 6.7) counts.

7.2. Daily Startup

1. Power on the instrument computer and the instruments to be used.
2. Launch QuantaSoft and confirm it connects to the droplet generator and/or reader.
3. Check oil levels in the droplet generator and the reader; top up or replace bottles per the manual (Bio-Rad Laboratories, 2024).
4. Empty the reader's waste bottle if near full.
5. Confirm consumable stock for the planned run (cartridges, gaskets, oil, plate, foil).

7.3. Consumables Orientation

Before the first run, identify each consumable and where it loads:

- **DG8 cartridge** seats in the cartridge holder. The middle row of wells receives sample; the bottom row receives generation oil; the top row collects the generated droplets.
- **Gasket** snaps over the loaded cartridge to seal it for generation.
- **Generation oil** is chemistry-specific. Using probe oil with EvaGreen supermix (or vice versa) will fail.
- **Pierceable foil** is the only seal type compatible with the reader's autosampler; do not use hard-seal films.

7.4. Droplet Generation

1. Prepare reactions per SOP-L701 and load into the cartridge holder with a DG8 cartridge.
2. Pipette each sample into the sample wells (avoid bubbles; pipette to the first stop).
3. Pipette the matched generation oil into the oil wells.
4. Place the gasket over the cartridge.

5. Load the holder into the QX200 Droplet Generator and close the lid; the run takes about two minutes per cartridge.
6. After generation, slowly transfer the droplets from the top row of the cartridge to the corresponding wells of a ddPCR 96-well plate using a multichannel pipette. Pipette gently and at an angle; droplets are fragile and shearing them lowers the usable count.

> **Warning**
>
> Damaged or sheared droplets reduce accepted-droplet counts and can fail the QC threshold (see SOP-L702, Section 6.2). Pipette droplets slowly and avoid introducing air.

7.5. Plate Sealing (PX1)

1. Place the plate and a sheet of pierceable foil in the PX1 Plate Sealer.
2. Seal at the manufacturer-specified temperature and time (commonly 180 C for 5 s; confirm against the current manual (Bio-Rad Laboratories, 2024)).
3. Inspect the seal: the foil should be uniformly bonded with no lifted edges.

7.6. PCR Amplification

1. Transfer the sealed plate to the C1000 Touch with the 96-deep-well module.
2. Run the validated cycling program for the assay (from SOP-L701).
3. **Set the ramp rate to about 2 C/s.** This slower ramp is important for even amplification across the droplet population on this platform (Bio-Rad Laboratories, 2018).
4. Include a final hold and store at 4 C if not reading immediately; read within the window specified for the chemistry.

A generic template program is given in Appendix B; the assay-specific anneal temperature and cycle number come from SOP-L701.

7.7. Droplet Reading

1. Place the amplified, sealed plate in the QX200 Droplet Reader plate holder with the retaining clamp.
2. In QuantaSoft, set up the plate layout: sample names, assay/target names, fluorophores (FAM, HEX/VIC), experiment type (e.g., absolute quantification), and supermix/chemistry.
3. Start the run. The autosampler aspirates droplets well by well and the detector reads two channels per droplet.
4. Do not open the reader or disturb the plate while running.

7.8. QuantaSoft Orientation (Reading the Output)

This is orientation only; thresholding decisions and full analysis are covered in SOP-L702.

- **1-D plot:** fluorescence amplitude vs. droplet (event) number for one channel. Positive droplets sit above the threshold line; negatives below.
- **2-D plot:** channel 1 (FAM) vs. channel 2 (HEX/VIC) amplitude. In a duplex assay, droplets fall into four clusters: double-negative, FAM-only, HEX-only, and double-positive (Bio-Rad Laboratories, 2018).
- **Thresholding:** the boundary between positive and negative clusters. Review it for every well; adjust manually in QuantaSoft Analysis Pro where the automatic call is poor.
- **Well merging:** replicate wells of the same sample/target may be merged to increase the effective partition count before concentration is calculated.
- **Concentration:** QuantaSoft reports copies per microliter from the Poisson fit. Export as described in SOP-L702, Section 6.1.

7.9. Shutdown

1. Close the experiment and export/back up data (SOP-L702).
2. Remove and discard the plate and used cartridges/gaskets (Section 8).
3. Check and, if needed, empty the reader waste bottle.
4. Power down per the manual; leave the system in the state your lab specifies for the reader oil lines.

7.10. Maintenance and Care

- **Each session:** check oil levels and the waste bottle; inspect for leaks.
- **Routine:** keep the reader's fluidics maintained per the manual; do not let oil bottles run dry mid-run.
- **Do not:** force cartridges or gaskets, reuse single-use consumables, mix oil chemistries, or use non-pierceable seals.
- **Records:** log each run (date, operator, assay, plate, instrument notes) and any maintenance or service in the instrument logbook.

7.11. Troubleshooting

Symptom	Likely cause	Action
Low accepted-droplet count	Sheared droplets; pipetting too fast; bubbles	Pipette droplets slowly at an angle; avoid air; re-generate
Few or no droplets generated	Wrong/insufficient oil; cartridge/gasket seated poorly	Use matched oil; reseat cartridge and gasket
"Rain" (droplets between clusters)	Suboptimal anneal temperature; degraded sample	Optimize anneal (SOP-L701); check DNA quality (SOP-L700)
Reader error / clog	Low oil; debris; fluidics issue	Check oil and waste; run reader maintenance; see manual
High NTC positives	Contamination	Rerun with fresh reagents; review setup (SOP-L702, Section 6.2)

8. Quality Control and Acceptance Criteria

- Each well should yield at least 10,000 accepted droplets (target ~12,000 to 20,000); wells below 10,000 are excluded in analysis (SOP-L702).
- Run the controls defined in SOP-L702 (NTC, extraction blank, positive control) on every plate.
- Confirm uniform plate sealing and correct plate orientation before reading.
- Log instrument run metadata to support digital-PCR reporting standards (The dMIQE Group & Huggett, 2020).

9. Waste Disposal

- Used DG8 cartridges, gaskets, plates, foil, and tips: dispose as standard laboratory plastic/biohazard waste per the lab's waste plan.
- Generation and reader oil and oily waste: collect and dispose per the SDS and the lab's chemical-waste procedures; do not pour oil down the drain.
- Reagent containers: rinse and dispose per local guidance.

10. References

> **i** Finding published methods
>
> For peer-reviewed studies that used ddPCR, including environmental and microbial applications useful when validating an assay or interpreting low target concentrations, search Bio-Rad's ddPCR publication database: https://www.bio-rad.com/ddPCR/publications.

Bio-Rad Laboratories. (2018). *Droplet digital PCR applications guide*. Bio-Rad Laboratories, Inc.

Bio-Rad Laboratories. (2024). *QX200 droplet digital PCR system instruction manual*. https://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system

Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., Bright, I. J., Lucero, M. Y., Hiddessen, A. L., Legler, T. C., Kitano, T. K., Hodel, M. R., Petersen, J. F., Wyatt, P. W., Steenblock, E. R., Shah, P. H., Bousse, L. J., Troup, C. B., Mellen, J. C., … Colston, B. W. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, *83*(22), 8604–8610. https://doi.org/10.1021/ac202028g

The dMIQE Group, & Huggett, J. F. (2020). The digital MIQE guidelines update: Minimum information for publication of quantitative digital PCR experiments for 2020. *Clinical Chemistry*, *66*(8), 1012–1029. https://doi.org/10.1093/clinchem/hvaa125

11. Training Record

Before operating the QX200 system independently, personnel must:

1. Read this SOP and review the current Bio-Rad QX200 manual (Bio-Rad Laboratories, 2024) and Applications Guide (Bio-Rad Laboratories, 2018).
2. Read SOP-L701 Primer Design and SOP-L702 Applications Guide for context on what they are running and why.
3. Observe a full run (generation, sealing, cycling, reading) performed by trained personnel.
4. Complete at least one full run under supervision, including QuantaSoft plate setup and export.
5. Be signed off by the PI or lab manager.

Trainee	Date observed	Date supervised run	Signed off by	Date

12. Appendix A: Pre-Run Checklist

- ☐ Validated reaction mix and plate plan ready (SOP-L701)
- ☐ Instruments powered on; QuantaSoft connected
- ☐ Generation oil matches supermix chemistry; sufficient volume
- ☐ Reader oil level adequate; waste bottle emptied
- ☐ DG8 cartridges, gaskets, plate, and pierceable foil on hand
- ☐ Controls included on the plate (NTC, extraction blank, positive control)
- ☐ PX1 sealer at correct temperature/time
- ☐ Thermal cycler ramp rate set to ~2 C/s
- ☐ Run logged in the instrument logbook

13. Appendix B: Generic Thermal Cycling Template

Anneal/extension temperature and cycle number are assay-specific and come from SOP-L701. The structure below is a starting template for probe-based ddPCR; confirm against the supermix insert and current manual (Bio-Rad Laboratories, 2024).

Step	Temperature	Time	Cycles	Ramp
Enzyme activation	95 C	10 min	1	~2 C/s
Denaturation	94 C	30 s	39–40	~2 C/s
Anneal/extension	(assay)	1 min	39–40	~2 C/s
Enzyme deactivation	98 C	10 min	1	~2 C/s
Hold	4 C	hold	1	~2 C/s

> **Note**
>
> EvaGreen chemistry uses a different supermix, oil, and cycling profile than probe chemistry. If the lab runs EvaGreen assays, add a separate template here once the standard profile is fixed in SOP-L701.

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L215

Hach DR3900 Benchtop Spectrophotometer

Standard Operating Procedure

SOP Number	SOP-L215
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the setup, operation, routine maintenance, and troubleshooting of the Hach DR3900 benchtop spectrophotometer as a laboratory instrument. The DR3900 uses a tungsten lamp (visible range) and a deuterium lamp (UV range) to measure absorbance and transmittance across 320–1100 nm, supporting both factory-stored programs for Hach reagent kits and user-defined wavelength methods (Hach Company, 2024a, 2024b).

For method-specific chemistry, reagent handling, and the analyte Method Registry (nutrients, metals, and general water quality parameters), see SOP-L303 Hach Colorimetric Analysis. For field use of the portable Hach DR900 colorimeter, see SOP-F208 Portable Spectrophotometer / Colorimeter (Field).

2. Scope

- **Applies to:** All use of the Hach DR3900 benchtop spectrophotometer in the EA Program laboratory, including stored-program colorimetric analysis, user-defined single-wavelength measurements, wavelength scans, and time-course (kinetic) measurements
- **Does not apply to:** Micro-volume UV-Vis measurements (see SOP-L212 NanoDrop); fluorometric quantification (see SOP-L203 Trilogy Fluorometer); portable field colorimetry (see SOP-F208); general spectrophotometer theory (see SOP-L201)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule lamp replacement and annual verification, manage firmware updates
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Absorbance (A):** The negative base-10 logarithm of transmittance: $A = -\log_{10}(T)$; directly proportional to analyte concentration (Beer-Lambert law) within the linear range of the instrument.
- **Beer-Lambert law:** The relationship between absorbance, path length, and concentration: $A = \varepsilon \cdot c \cdot l$, where ε is the molar absorptivity, c is concentration, and l is the optical path length (cell width); the basis for all colorimetric quantification.
- **Blank (reagent blank):** A sample containing all reagents except the analyte; used to zero the instrument and correct for absorbance contributed by the reagent matrix.
- **Colorimetric method:** An analytical technique in which a chemical reagent reacts with the analyte to produce a colored complex whose absorbance is proportional to analyte concentration.
- **TNT plus vial:** A Hach proprietary pre-dosed reagent vial for the DR3900; contains all required reagents sealed in a glass vial; provides consistent results without manual reagent preparation.
- **Transmittance (T):** The fraction of incident light that passes through the sample: $T = I/I_0$; related to absorbance by $A = -\log_{10}(T)$.
- **Wavelength scan:** A measurement mode in which the instrument records absorbance across a range of wavelengths to identify the absorption maximum of an unknown compound.

5. Safety and Hazards

⚠ Hazards

- **Optical:** The deuterium lamp emits ultraviolet radiation. Do not look directly into the sample compartment when the lid is open and the lamp is on. The instrument's interlock dims the lamp when the lid is raised, but UV exposure remains possible during diagnostic modes.
- **Chemical:** Sample solutions and reagents placed in the instrument may be hazardous. Refer to the relevant method SOP (e.g., SOP-L303) and the reagent SDS for handling precautions.
- **Electrical:** Do not operate the instrument with wet hands or near standing water. If liquid enters the instrument housing, disconnect power immediately and contact the lab manager.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling reagents or samples)

5.2. Emergency Procedures

For chemical spills on or near the instrument, clean the spill per SOP-L003 Hazardous Materials Handling before resuming use. If liquid enters the instrument, unplug the power cord and do not attempt to power on until the lab manager has inspected the unit.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Hach DR3900 benchtop spectrophotometer	Lab bench, SGM 133	Wavelength range 320–1100 nm
Light shield / adapter	With DR3900	For 13 mm and 16 mm round vials (TNT plus)
USB cable or Ethernet connection	Lab bench	For data export to PC

6.2. Sample Cells

Cell Type	Path Length	Use
10 mm square glass or quartz	10 mm	General absorbance; user-defined methods
1-inch round glass	25.4 mm	Most Hach stored-program methods
16 mm round (TNT plus vials)	16 mm	TNT plus pre-dosed vial methods

6.3. Consumables

Item	Notes
Lint-free wipes (Kimwipes)	For cleaning sample cell exteriors
Silicone oil and applicator cloth	For 1-inch round cells (reduces light scattering at the glass surface)
DI water (Type II or better)	For blanking and rinsing

7. Procedure

7.1. Startup and Self-Check

1. Verify that the sample compartment is empty and the lid is closed
2. Turn on the DR3900 using the power switch on the rear panel
3. The instrument performs an automatic self-check, including lamp tests and wavelength verification. This takes approximately 30 seconds. Do not open the lid or insert samples until the home screen appears.

4. Allow the lamps to warm up for at least 15 minutes before making measurements that require high photometric accuracy (e.g., user-defined methods at low absorbance). For routine stored-program analyses, measurements may begin after the self-check completes.

7.2. Selecting an Operating Mode

From the home screen, select one of the following modes:

Mode	When to Use
Stored Programs	Pre-programmed Hach reagent kit methods; see SOP-L303 for the Method Registry
Single Wavelength	User-defined absorbance or %T at a specific wavelength
Multi Wavelength	Simultaneous measurement at 2–4 wavelengths
Wavelength Scan	Absorbance spectrum over a defined range
Time Course	Kinetic measurement at a fixed wavelength over time

7.3. Preparing and Inserting Sample Cells

1. **Select the correct cell type** for the method (see Section 5). Square cells must be oriented so the clear optical faces align with the light path (arrow on the cell pointing toward the detector).
2. **Fill the cell** to the level required by the method. Do not overfill; liquid on the outside of the cell causes erroneous readings.
3. **Clean the exterior** of the cell with a lint-free wipe. For 1-inch round cells, apply a thin film of silicone oil and wipe with the oiling cloth to eliminate fingerprints and water droplets (Hach Company, 2024a).
4. **Insert the cell** into the cell holder. Close the sample compartment lid fully before reading.

> Cuvette Selection for UV Work
>
> Standard glass and plastic cells absorb UV light below approximately 340 nm. For measurements below 340 nm in user-defined or wavelength scan modes, use quartz cells (Skoog et al., 2018).

7.4. Zeroing (Blanking) the Instrument

1. Fill a clean cell with the blank solution. The blank must match the sample matrix: DI water for simple aqueous samples, or the reagent blank specified by the Hach method.
2. Insert the blank cell and close the lid
3. Press **Zero** (stored-program mode) or **Zero/Blank** (single wavelength mode)
4. Verify that the display reads 0.000 Abs (or 100.0 %T)
5. Remove the blank cell

> **Note**
>
> Re-zero whenever you change wavelengths, switch cell types, or if the instrument has been idle for more than 30 minutes.

7.5. Measuring Samples

1. Insert the sample cell and close the lid
2. Press **Read** (or **Measure** in scan/time-course modes)
3. Record the result (absorbance, concentration, or %T depending on mode)
4. Remove the cell. Rinse and refill for the next sample, or use a fresh cell.

7.6. Wavelength Scan Mode

1. From the home screen, select **Wavelength Scan**
2. Set the start and end wavelengths and the scan increment (typically 1 nm)
3. Zero the instrument with a blank across the full scan range
4. Insert the sample and press **Start Scan**
5. The spectrum is displayed on screen. Use the cursor to read peak wavelengths and absorbance values.
6. Export the scan data via USB (see Section 6.8)

7.7. User-Defined Programs

The DR3900 allows creation of custom calibration curves for analytes not covered by the factory-stored programs:

1. Select **User Programs** from the home screen, then **New**
2. Set the measurement wavelength, units, and cell type
3. Prepare a series of standards spanning the expected concentration range (minimum 5 points plus a blank)
4. Read each standard and enter the known concentration when prompted
5. The instrument fits a calibration curve (linear, polynomial, or segmented). Review the correlation coefficient (r^2); accept only if $r^2 \geq 0.995$ for linear methods (Harris & Lucy, 2020).
6. Save the program with a descriptive name and program number

7.8. Data Export

1. Connect a USB flash drive to the USB-A port on the front panel
2. From the home screen, select **Data Log**
3. Select the readings to export and press **Send to USB**
4. Data are saved as `.csv` files that can be opened in R or Excel
5. Alternatively, connect the DR3900 to a PC via USB cable and use Hach's LINK2SC or Claros software for direct data transfer

7.9. Shutdown

1. Remove all cells from the sample compartment
2. Wipe the cell holder with a dry lint-free tissue to remove any spilled liquid
3. Close the sample compartment lid
4. Turn off the instrument using the rear power switch
5. Cover the instrument with the dust cover if one is available

8. Quality Control and Acceptance Criteria

8.1. Daily Verification

Before each analytical session:

- Run a DI water blank; the reading should be 0.000 ± 0.005 Abs at the working wavelength
- Run a Hach-supplied verification standard (e.g., StablCal formazin standard or NIST-traceable absorbance filter) at the working wavelength. Acceptance: within ± 0.01 Abs of the certified value, or ± 5%, whichever is larger.

8.2. Lamp Monitoring

The DR3900 tracks lamp hours internally. When a lamp approaches its rated lifetime (typically 2000 hours for deuterium, longer for tungsten), the instrument displays a warning.

- Record lamp hours monthly in the instrument log
- Replace lamps per the Hach service manual or contact Hach technical support
- After lamp replacement, run the full wavelength verification procedure

8.3. Annual Verification

At least once per year (or after any service event):

1. Run a multi-wavelength check using a NIST-traceable holmium oxide filter to verify wavelength accuracy (± 1 nm)
2. Run a multi-point photometric accuracy check using neutral density filters or potassium dichromate standards across the range 0.1–1.5 Abs (Skoog et al., 2018)
3. Record results in the instrument log and retain the verification report

9. Troubleshooting

Symptom	Possible Cause	Action
Self-check fails	Lamp failure or firmware error	Note the error code; contact lab manager
Blank does not zero	Dirty cell, wrong cell type, or stale blank	Clean or replace cell; prepare fresh blank
Erratic readings	Air bubbles in cell, condensation, or dirty optics	Degas sample; wipe cell; clean sample compartment
"Lamp intensity low" warning	Lamp nearing end of life	Schedule lamp replacement
Readings drift over time	Lamp not warmed up; temperature change	Allow 15 min warm-up; avoid drafts near instrument
Absorbance exceeds 2.5	Sample too concentrated	Dilute sample and re-read

10. Waste Disposal

Waste Type	Disposal Method
Rinsate from cells (DI water)	Drain
Chemical samples / reagent waste	Per method SOP (see SOP-L303)
Used lint-free wipes	Standard trash unless contaminated
Spent lamps	Hazardous waste (deuterium lamps contain UV-emitting gas); dispose through EH&S

11. References

Hach Company. (2024a). *DR3900 benchtop spectrophotometer: Procedures manual*. https://www.hach.com/dr3900-benchtop-spectrophotometer/product?id=56440352466

Hach Company. (2024b). *Water analysis guide* (7th ed.). https://www.hach.com/wag

Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). *Principles of instrumental analysis* (7th ed.). Cengage Learning.

12. Training Record

Before operating the DR3900 independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Read SOP-L303 if performing colorimetric analyses
4. Observe a trained operator perform a complete session: startup, self-check, zeroing, sample measurement, data export, and shutdown
5. Perform a supervised session with acceptable QC results (blank within specification, verification standard within tolerance)
6. Be signed off by the PI or lab manager

13. Appendix A: Instrument Specifications

Parameter	Specification
Wavelength range	320–1100 nm
Wavelength accuracy	± 1 nm
Wavelength resolution	1 nm
Photometric range	-0.5 to 3.5 Abs
Photometric accuracy	± 0.003 Abs at 0.5 Abs (typical)
Light sources	Tungsten (visible), deuterium (UV)
Cell types	10 mm square, 1-inch round, 16 mm round
Stored programs	200+
Data storage	500+ measurements
Connectivity	USB-A, USB-B, Ethernet
Power	100–240 V AC, 50/60 Hz

Note

Confirm specifications against the serial plate on the unit and the current Hach DR3900 product data sheet. Values above are representative of current production models (Hach Company, 2024a).

14. Appendix B: Relationship to Other SOPs

SOP	Relationship
SOP-L303	Method chemistry, reagent handling, and the analyte Method Registry for the DR3900 and DR900
SOP-F208	Field use of the portable DR900 colorimeter
	
	General spectrophotometer theory and cuvette-based UV-Vis operation
SOP-L212	Micro-volume UV-Vis (NanoDrop)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L216

Dissecting and Compound Microscope

Standard Operating Procedure

SOP Number	SOP-L216
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, image capture, and routine care of the dissecting (stereo) microscopes and compound light microscopes used in the EA Program laboratory. Dissecting microscopes provide low-to-moderate magnification (typically 7–45x) with a wide working distance and are used for sorting biological specimens, gross morphology, and sample preparation. Compound microscopes provide higher magnification (40–1000x) with transmitted illumination and are used for slide-mounted specimens requiring cellular or subcellular resolution (Murphy & Davidson, 2012).

For the Echo Labs Revolve hybrid microscope (which combines upright compound and inverted configurations with an iPad display), see SOP-L211 Revolve Microscope. The Revolve has specialized procedures that differ from the conventional microscopes described here.

2. Scope

- **Applies to:** All conventional dissecting (stereo) microscopes and compound light microscopes in the EA Program laboratory, including units with and without attached cameras
- **Does not apply to:** The Echo Labs Revolve microscope (see SOP-L211); electron microscopy; confocal microscopy; fluorescence microscopy on non-Revolve instruments
- **Location:** EA Program laboratory, SGM 131/133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage optics cleaning supplies, maintain camera software
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Brightfield microscopy:** Transmitted-light microscopy in which the specimen is illuminated from below and appears against a bright background; the standard mode for stained biological specimens.
- **Compound microscope:** A microscope with two or more lens systems (objective and eyepiece) that produce a magnified image; used for cells, microorganisms, and fine structural detail at total magnifications of 40x–1,000x.
- **Condenser:** A lens assembly below the stage that focuses and concentrates the illumination light onto the specimen; its numerical aperture and position strongly affect image contrast and resolution.
- **Dissecting microscope (stereo microscope):** A low-magnification (typically 7x–45x) binocular microscope that provides a three-dimensional image of intact specimens; used for macroinvertebrate sorting, plant tissue examination, and dissection.
- **Immersion oil:** A synthetic or natural oil with a refractive index matching glass (~1.515); applied between the 100x objective and the coverslip to eliminate the air-glass interface and maximize numerical aperture.
- **Iris diaphragm (condenser diaphragm):** An adjustable aperture in the condenser that controls the cone of illumination; reducing the diaphragm increases contrast but reduces resolution.
- **Parfocal:** The property of a set of objectives that allows switching between magnifications with only minor focus adjustment; standard on modern compound microscopes.
- **Working distance:** The distance between the front lens of the objective and the specimen surface when in focus; decreases with increasing magnification; limits the thickness of specimens that can be accommodated.

5. Safety and Hazards

⚠ Hazards

- **Optical:** Prolonged viewing through the eyepieces can cause eye strain. Take breaks during extended sorting or identification sessions. Adjust the diopter rings to match your vision before starting work.
- **Electrical:** Keep liquids away from the illumination base and power supply. If liquid enters the base, disconnect power immediately and notify the lab manager.
- **Physical:** Compound microscope objectives (especially 40x and 100x) can contact and crack the slide or cover slip if the stage is raised too quickly. Always use the coarse focus knob with caution when changing objectives.
- **Chemical/Biological:** Specimens may be preserved in ethanol, formalin, or other fixatives. Wear appropriate PPE and handle preserved material in a well-ventilated area.

5.1. Required PPE

☐ Safety glasses (when not viewing through eyepieces)

☐ Lab coat
☐ Nitrile gloves (when handling preserved specimens, stains, or immersion oil)

5.2. Emergency Procedures

For chemical spills (e.g., ethanol, formalin) on or near a microscope, clean the spill per SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling. If liquid enters the microscope body, unplug power and contact the lab manager before attempting further use.

6. Equipment and Materials

6.1. Dissecting (Stereo) Microscopes

Item	Qty	Location	Notes
Dissecting scope with illuminated base	7	SGM 131/133	7–45x zoom; incident and transmitted light
Dissecting scope with camera	1	SGM 133	Camera-equipped for image capture

6.2. Compound Microscopes

Item	Qty	Location	Notes
Compound light microscope	*TBD*	SGM 133	Objectives: 4x, 10x, 40x, 100x (oil)

6.3. Consumables

Item	Notes
Microscope slides (25 x 75 mm)	Standard glass
Cover slips (No. 1.5, 0.17 mm)	For compound microscope use
Immersion oil (Type A or NF)	For 100x oil immersion objective only
Lens cleaning solution	Optical-grade, for objectives and oculars
Lens cleaning tissue (lint-free)	Use optical-grade tissue, not Kimwipes on coated optics
Compressed gas duster	For removing dust; use short bursts only
Depression slides	For wet mounts of live specimens

7. Procedure

7.1. Choosing the Right Microscope

Task	Microscope Type	Typical Magnification
Sorting macroinvertebrates from debris	Dissecting	7–20x
Gross specimen identification	Dissecting	10–45x
Examining head capsules, chaetae, gill structures	Compound	100–400x
Counting algae or diatoms	Compound	200–400x
Identifying microplastics on filters	Dissecting	10–45x
Viewing stained tissue sections	Compound	40–1000x

7.2. Dissecting Microscope: Setup and Use

1. Plug in the microscope and turn on the illumination using the switch on the base. Most units have both **incident** (top) and **transmitted** (bottom) light sources. Select the mode appropriate for the specimen:
   - Incident light: opaque specimens (insects, soil, rocks)
   - Transmitted light: translucent specimens (small organisms in water, thin sections)
   - Both: general sorting when mixed materials are present
2. Adjust the zoom to the lowest magnification (widest field of view) to locate the area of interest
3. Place the specimen on the stage plate. Use a white plate for dark specimens and a black plate for light or translucent specimens to improve contrast.
4. Adjust the interpupillary distance by sliding the eyepiece tubes until you see a single merged circle of light
5. Focus using the focus knob (single knob on most stereo scopes). With both eyes open, adjust the diopter ring on one eyepiece until the image is sharp in both eyes simultaneously.
6. Increase magnification by turning the zoom dial. Refine focus as needed.
7. When finished, return the zoom to the lowest setting, remove the specimen, turn off the illumination, and unplug the unit if lab policy requires it

7.3. Compound Microscope: Setup and Use

1. Plug in the microscope and turn on the light source using the power switch. Adjust the intensity to a comfortable level.
2. Rotate the nosepiece to the lowest-magnification objective (4x)
3. Place the prepared slide on the stage, securing it with the stage clips. Center the specimen over the condenser aperture.
4. Using the **coarse focus knob**, raise the stage until the specimen comes into approximate focus. Watch from the side to avoid driving the slide into the objective.
5. Sharpen the image with the **fine focus knob**
6. Adjust the condenser height and the iris diaphragm to optimize contrast and resolution. For most work, the condenser should be nearly fully raised (close to the slide) and the diaphragm partially closed to improve contrast without sacrificing too much resolution (Murphy & Davidson, 2012).
7. Increase magnification by rotating the nosepiece to the next objective (10x, then 40x). Refocus with the fine focus knob only.

> **!** Avoid Slide Damage
>
> When switching from the 10x to the 40x objective, watch from the side to confirm that the longer 40x lens does not contact the cover slip. If the working distance is too short, lower the stage slightly before rotating the objective into position.

7.4. Using Oil Immersion (100x Objective)

1. Focus the specimen at 40x first
2. Rotate the nosepiece to an empty position (no objective in the light path)
3. Place a single small drop of immersion oil directly on the cover slip at the point of illumination. Do not use excess oil.
4. Rotate the 100x objective into position. The front element of the lens should contact the oil without trapping an air bubble.
5. Focus carefully using the **fine focus knob only**
6. After use, immediately clean the 100x objective with lens cleaning solution and lint-free tissue. Wipe gently from center to edge. Never allow oil to dry on the lens.
7. Clean the slide or dispose of it as appropriate

7.5. Capturing Images (Camera-Equipped Units)

1. Focus the specimen and center the region of interest in the field of view
2. Open the camera software on the connected computer (software varies by camera model; consult the lab manager for the current setup)
3. Adjust exposure and white balance in the software to match the illumination
4. Capture the image. Record the magnification, scale bar, and sample ID in the file name or metadata.
5. Use a consistent file-naming convention: `YYYY-MM-DD_SampleID_Magnification_Mode.jpg`
6. Export images to the project data folder

7.6. Shutdown and Daily Maintenance

1. Remove the specimen from the stage
2. Rotate the nosepiece to the lowest-magnification objective (4x on compound; lowest zoom on dissecting)
3. If oil immersion was used, clean the 100x objective immediately (see Section 6.4, step 6)
4. Wipe any residue from the stage with a lint-free tissue
5. Turn off the light source and unplug if required by lab policy
6. Cover the microscope with a dust cover

8. Quality Control and Acceptance Criteria

8.1. Optics Cleaning

If specks or smudges appear in the field of view that do not move when the specimen is translated, the optics need cleaning:

1. Start with a few short bursts of compressed gas duster to remove loose dust from the objectives and oculars. Do not invert the can.
2. For objective lens surfaces, apply a small amount of lens cleaning solution to a piece of lint-free optical tissue and gently wipe in a circular motion from center to edge. Do not use dry tissue on coated optics.
3. Clean the oculars (eyepieces) the same way. Fingerprints on the eye lens are the most common source of image degradation.
4. For persistent contamination on internal optics, contact the lab manager. Do not disassemble the microscope.

8.2. Illumination Check

- Replace burned-out bulbs promptly. LED-equipped units should not require bulb replacement during normal use.
- If transmitted light appears uneven, check that the condenser is properly centered and the iris diaphragm is functioning.

8.3. Image Quality Checklist

Before accepting a micrograph for publication or analysis, verify:

- Focus is sharp across the region of interest
- Illumination is even (no hot spots or vignetting)
- A scale bar or magnification is recorded
- The specimen is oriented consistently with project conventions

9. Waste Disposal

Waste Type	Disposal Method
Used immersion oil tissues	Standard trash
Used microscope slides (non-hazardous)	Broken-glass box if glass
Biological specimen waste	Biohazard bag if applicable; autoclave before disposal per SOP-L112
Preserved specimen waste (ethanol)	Flammable waste container
Preserved specimen waste (formalin)	Hazardous waste; dispose through EH&S
Spent lens cleaning solution	Standard trash (small volumes on tissues)

10. References

Murphy, D. B., & Davidson, M. W. (2012). *Fundamentals of light microscopy and electronic imaging* (2nd ed.). Wiley. https://doi.org/10.1002/9781118382905

11. Training Record

Before using dissecting or compound microscopes independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate proper setup, focusing, magnification changes, and shutdown of both dissecting and compound microscopes under supervision
4. If oil immersion work is required, demonstrate proper oil application, focusing, and cleanup
5. If image capture is required, demonstrate camera operation and correct file naming
6. Be signed off by the PI or lab manager

12. Appendix A: Microscope Anatomy Quick Reference

12.1. Dissecting (Stereo) Microscope

Component	Function
Eyepieces (oculars)	Binocular viewing; 10x magnification (typical)
Zoom dial	Continuously adjustable magnification (e.g., 0.7–4.5x objective)
Focus knob	Single knob for coarse/fine focus
Stage plate	Removable; white and black plates for contrast
Incident light	Top illumination for opaque specimens
Transmitted light	Bottom illumination for translucent specimens
Diopter adjustment	On one or both eyepieces; corrects for vision differences between eyes

12.2. Compound Microscope

Component	Function
Eyepieces (oculars)	Binocular viewing; 10x magnification (typical)
Nosepiece (turret)	Holds and rotates objective lenses
Objective lenses	4x, 10x, 40x, 100x (oil); determine magnification
Stage	Holds slide; translatable via X/Y controls
Condenser	Focuses transmitted light onto the specimen
Iris diaphragm	Controls cone angle of illumination; affects contrast and resolution

Component	Function
Coarse focus knob	Rapid focus (large movements)
Fine focus knob	Precise focus (small movements)
Light intensity control	Adjusts brightness of the transmitted light source

13. Appendix B: Total Magnification Reference

Total magnification = eyepiece magnification × objective magnification. For standard 10x eyepieces:

Objective	Total Magnification	Typical Use
4x	40x	Scanning; locating regions of interest
10x	100x	General observation
40x	400x	Detailed cellular structure
100x (oil)	1000x	Fine detail; bacteria, diatom striae, chromosomes

14. Appendix C: Relationship to Other SOPs

SOP	Relationship
SOP-L211	Echo Labs Revolve hybrid microscope (upright/inverted, iPad display, fluorescence)
SOP-L602	Macroinvertebrate sorting and identification (uses dissecting and compound scopes)
SOP-L308	Microplastics enumeration (uses dissecting scope and Revolve)
SOP-L112	Autoclave / sterilization for biohazard waste



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L300

pH and Conductivity – Bench-Top Measurements

Standard Operating Procedure

SOP Number	SOP-L300
Revision	1.0
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes bench-top measurement of pH and specific conductance (conductivity) in water samples, soil extracts, and other aqueous solutions using a laboratory pH/conductivity meter. Accurate pH and conductivity measurements are fundamental to water quality assessment and are referenced by multiple analytical SOPs throughout the EA Program (APHA et al., 2012; Eaton et al., 2005).

2. Scope

- **Applies to:** Bench-top pH and specific conductance measurements on surface water, groundwater, wastewater, soil-water extracts (e.g., 1:1 or 1:5 soil slurries), KCl extracts (see SOP-L403), and reagent solutions
- **Does not apply to:** Field measurements using portable multiprobes (see SOP-F200 Eureka Manta2); continuous monitoring or in-situ deployments
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain meter and electrodes, stock buffer solutions
Trained Personnel	Calibrate meter before each session, measure and record results
New Students	Complete training before independent use

4. Definitions

- **Buffer solution:** A solution that resists changes in pH upon addition of small amounts of acid or base; used to calibrate pH electrodes; standard buffers are pH 4.00, 7.00, and 10.01 at 25 °C.
- **Calibration slope:** The millivolt change per pH unit measured during two-point calibration; theoretical value is -59.16 mV/pH at 25 °C (Nernst equation); acceptable range is 90–102% of theoretical.
- **Conductivity:** A measure of a solution's ability to conduct electrical current, expressed in µS/cm or mS/cm; proportional to the concentration of dissolved ions; temperature-dependent.
- **Junction potential:** A small voltage that develops at the reference electrode junction where two solutions of different composition meet; a source of measurement error that is minimized by proper electrode storage and frequent replacement of the reference filling solution.
- **pH:** The negative base-10 logarithm of the hydrogen ion activity: $pH = -\log_{10}[H^+]$; measured potentiometrically using a glass electrode and a reference electrode.
- **Specific conductance:** Conductivity corrected to a reference temperature of 25 °C; allows comparison of measurements made at different temperatures.

- **Temperature compensation:** Automatic adjustment of conductivity readings to 25 °C using a built-in thermistor; essential for accurate comparisons because conductivity changes approximately 2% per °C.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** pH buffer solutions (pH 4, 7, 10) are mild irritants. Conductivity standards contain dissolved salts. KCl electrode fill solution is a mild irritant. Acidic or alkaline samples may cause skin or eye irritation.
- **Electrical:** Keep the meter and power supply away from water spills on the bench.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Lab coat

5.2. Emergency Procedures

For buffer or sample splashes to eyes, flush with water at the eyewash station for at least 15 minutes. For skin contact, wash with soap and water. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
pH/conductivity meter	Lab bench, SGM 133	*TBD – confirm model (e.g., Accumet AB150, Orion Star, or equivalent)*
pH electrode (combination glass)	Stored in pH 4 or KCl storage solution	Replace annually or when response time degrades
Conductivity cell (2- or 4-electrode)	Stored dry or in DI water	Cell constant typically 1.0 cm^{-1}
Magnetic stir plate and stir bars	Lab bench	Optional, for large-volume samples
Temperature probe (ATC)	Built into meter or separate	For automatic temperature compensation

6.2. Standards and Consumables

Item	Notes
pH buffer 4.01 (red)	NIST-traceable; store at room temperature
pH buffer 7.00 (yellow)	NIST-traceable; store at room temperature
pH buffer 10.01 (blue)	NIST-traceable; store at room temperature
Conductivity standard (1413 uS/cm)	KCl-based; store at room temperature
Conductivity standard (84 uS/cm or 12,880 uS/cm)	For low- or high-range verification
KCl electrode fill solution (3 M or saturated)	For refilling the reference junction
DI water (Type I or II)	For rinsing electrodes between samples
Lint-free wipes (Kimwipes)	For blotting electrodes
Small beakers or sample cups (50–100 mL)	Clean, for holding aliquots

7. Procedure

7.1. Electrode Preparation

1. Remove the pH electrode from its storage solution. Inspect the glass bulb for cracks, chips, or deposits. If the bulb is damaged, replace the electrode.
2. If the electrode has a refillable reference junction, check the KCl fill level. Refill to the indicated line with 3 M KCl or saturated KCl solution if low. Open the fill hole during measurements to allow proper electrolyte flow (Rundle, 2011).
3. Rinse the pH electrode thoroughly with DI water and blot gently with a Kimwipe. Do not rub the glass bulb.
4. Rinse the conductivity cell with DI water and blot dry.

7.2. pH Calibration (Three-Point)

Calibrate before each measurement session and whenever the meter has been turned off (APHA et al., 2012).

1. Pour approximately 30–50 mL of each buffer solution into clean, labeled beakers. Do not pour used buffer back into the stock bottle.
2. Rinse the pH electrode with DI water and blot gently.
3. Place the electrode into pH 7.00 buffer first. Allow the reading to stabilize (stability indicator on the meter display).
4. Accept or set the first calibration point to 7.00.
5. Rinse with DI water, blot, and place into pH 4.01 buffer. Allow to stabilize and accept.
6. Rinse with DI water, blot, and place into pH 10.01 buffer. Allow to stabilize and accept.
7. The meter should display the slope (ideally 95–102% of theoretical Nernstian slope, i.e., approximately 56–60 mV/pH unit at 25 C). If the slope falls below 90%, the electrode may need cleaning or replacement (Illingworth, 1981).
8. Record the calibration date, buffer lot numbers, slope, and any offset in the meter calibration log.

> **Tip**
>
> Always calibrate starting with pH 7.00, then move to pH 4.01, and finish with pH 10.01. The 7.00 buffer sets the zero point (offset), while the acidic and basic buffers define the slope. If you only have two buffers, bracket the expected sample range (e.g., pH 4 and 7 for acidic samples, pH 7 and 10 for alkaline samples).

7.3. Conductivity Calibration (One-Point)

1. Rinse the conductivity cell with DI water and blot dry
2. Pour approximately 30–50 mL of the 1413 uS/cm standard into a clean beaker
3. Immerse the conductivity cell fully in the standard. Tap gently to release air bubbles from the cell opening.
4. Allow the reading to stabilize with temperature compensation active
5. Calibrate (adjust the cell constant) so that the display reads 1413 uS/cm (at 25 C)
6. Record the calibration date and standard lot number in the log
7. *TBD – if your lab uses a different primary conductivity standard (e.g., 84 uS/cm for low-conductivity water or 12,880 uS/cm for brackish water), adjust accordingly*

> **Important**
>
> Never measure conductivity on a water sample that has already been used for pH measurement. The KCl from the pH electrode reference junction leaks into the sample and alters conductivity. Always measure conductivity first, or use a separate aliquot (APHA et al., 2012).

7.4. Measuring pH

1. Rinse the pH electrode with DI water and blot gently
2. Pour approximately 30–50 mL of the sample into a clean beaker. Use a separate aliquot from the one used for conductivity.
3. Immerse the electrode so that the glass bulb and reference junction are fully submerged
4. Allow the reading to stabilize (typically 30–60 seconds for well-buffered samples; may take 2–3 minutes for low-ionic-strength water)
5. Record the pH value, sample temperature, and sample ID in the lab notebook
6. Rinse the electrode with DI water between samples

7.5. Measuring Conductivity

1. Rinse the conductivity cell with DI water and blot dry
2. Pour approximately 30–50 mL of the sample into a clean beaker
3. Immerse the cell fully. Tap gently to dislodge air bubbles.
4. Allow the reading to stabilize
5. Record the specific conductance (uS/cm at 25 C), sample temperature, and sample ID in the lab notebook

6. Rinse the cell with DI water between samples

7.6. Measuring Soil pH and Conductivity (Soil Slurry Method)

For soil pH and electrical conductivity, prepare a soil-water slurry (Rhoades, 1996; Thomas, 1996):

1. Weigh 10 g of air-dried, sieved (2 mm) soil into a clean beaker
2. Add 10 mL of DI water for a 1:1 soil-water ratio (or 50 mL for a 1:5 ratio, depending on the protocol). Record the ratio used.
3. Stir the slurry vigorously for 30 seconds, then allow to stand for 10 minutes
4. Stir again briefly, then insert the pH electrode into the slurry while it is still suspended. Allow to stabilize and record.
5. For conductivity, use a separate slurry aliquot or prepare a fresh slurry. Insert the conductivity cell into the supernatant after the soil has settled. Record.
6. See also *SOP-L57 Soil pH and Salinity* for additional detail.

7.7. Electrode Storage and Maintenance

1. After use, rinse the pH electrode with DI water and return it to its storage solution (pH 4 buffer or KCl storage solution as recommended by the manufacturer). Never store the pH electrode dry or in DI water, as this damages the glass membrane (Illingworth, 1981).
2. Store the conductivity cell dry or in DI water (per manufacturer recommendation).
3. If the pH electrode response becomes sluggish (stabilization takes more than 3 minutes) or the calibration slope drops below 90%, soak the electrode in electrode cleaning solution or dilute HCl (0.1 M) for 15–30 minutes, rinse with DI water, and recalibrate.
4. Replace the pH electrode if the slope remains below 90% after cleaning or if the glass bulb is cracked.

8. Quality Control and Acceptance Criteria

- Calibrate the pH meter before each measurement session using a three-point calibration
- Calibrate the conductivity meter before each session using a traceable standard
- Acceptable pH calibration slope: 90–102% of theoretical Nernstian slope (approximately 54–60 mV/pH at 25 C)
- Check calibration by re-reading pH 7.00 buffer after every 10 samples. If the reading has drifted by more than +/- 0.05 pH units, recalibrate.
- Check conductivity by re-reading the 1413 uS/cm standard after every 10 samples. If the reading has drifted by more than +/- 5%, recalibrate.
- Run duplicate measurements on at least 10% of samples. Acceptable precision: +/- 0.1 pH units and +/- 5% for conductivity.
- Record all calibration data, standard lot numbers, electrode condition, and QC check results in the meter log.

9. Waste Disposal

Waste Type	Disposal Method
Used pH buffer solutions	Collect in waste container; do not pour down the drain
Used conductivity standard	Drain with copious water (KCl solutions are non-hazardous)
Soil slurry waste	Standard trash (if non-hazardous soil)
Spent KCl fill solution	Drain with copious water
DI water rinses	Drain

10. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). *Standard methods for the examination of water and wastewater* (21st ed.). American Public Health Association.

Illingworth, J. A. (1981). A critical review of glass electrode theory and practice. *Biochemical Journal*, *195*(2), 259–262. https://doi.org/10.1042/bj1950259

Rhoades, J. D. (1996). Salinity: Electrical conductivity and total dissolved solids. In D. L. Sparks, A. L. Page, P. A. Helmke, & R. H. Loeppert (Eds.), *Methods of soil analysis, part 3: Chemical methods* (pp. 417–435). Soil Science Society of America.

Rundle, C. C. (2011). *Electrode storage, cleaning, and maintenance*. Technical report, Reagecon Diagnostics. https://www.reagecon.com/technical-papers/

Thomas, G. W. (1996). Soil pH and soil acidity. In D. L. Sparks, A. L. Page, P. A. Helmke, & R. H. Loeppert (Eds.), *Methods of soil analysis, part 3: Chemical methods* (pp. 475–490). Soil Science Society of America.

11. Training Record

Before measuring pH and conductivity independently, personnel must:

1. Read this SOP and SOP-L002 Laboratory Safety in full
2. Demonstrate a three-point pH calibration with acceptable slope
3. Demonstrate a one-point conductivity calibration
4. Perform measurements on at least 5 practice samples under supervision with acceptable duplicate precision
5. Demonstrate proper electrode storage
6. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
pH reading drifts and does not stabilize	Fouled or aging electrode; depleted KCl fill	Clean electrode; refill KCl; replace if slope is below 90%
Calibration slope below 90%	Electrode aging or contamination	Soak in cleaning solution; replace if unresponsive
pH buffer reads correctly but samples do not stabilize	Low ionic strength sample	Allow extra stabilization time (up to 5 min); consider adding ionic strength adjuster
Conductivity reads zero	Air bubble in cell; broken cell	Tap cell to dislodge bubbles; inspect for cracks
Conductivity reading too high on all samples	Contaminated cell or residual standard	Rinse cell thoroughly with DI water; recalibrate
Conductivity standard reads correctly but samples seem wrong	Temperature compensation off or incorrect cell constant	Verify ATC is enabled; recalibrate cell constant

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L301

Alkalinity – Titration Method

Standard Operating Procedure

SOP Number	SOP-L301
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of alkalinity in water samples by potentiometric titration with standardized sulfuric acid, following Standard Methods 2320 B (APHA et al., 2012). Alkalinity is the acid-neutralizing capacity of water and is primarily a function of carbonate, bicarbonate, and hydroxide content. It is a fundamental parameter for characterizing water chemistry, assessing buffering capacity, and interpreting corrosivity, hardness, and suitability for irrigation or aquatic life.

2. Scope

- **Applies to:** Surface water, groundwater, stormwater, and wastewater samples collected per SOP-F300
- **Does not apply to:** Soil alkalinity, industrial process water requiring specialized titration protocols, or samples with alkalinity above 500 mg/L as $CaCO_3$ (which require 0.1 N titrant; see note in Section 6.4)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Prepare and standardize titrant, maintain pH meter
Trained Personnel	Perform titrations, record and report results
New Students	Complete training before independent use

4. Definitions

- **Acid-neutralizing capacity (ANC):** The capacity of a water to neutralize strong acid; for most natural waters, ANC is approximately equal to total alkalinity.
- **Alkalinity:** The capacity of water to resist acidification, expressed in mg/L as $CaCO_3$; in natural waters, primarily contributed by bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and hydroxide (OH^-).

- **Bicarbonate alkalinity:** Alkalinity attributable to HCO_3^-; the dominant form in most fresh waters at pH 6.3–10.3.
- **Endpoint pH:** The pH at which the titration is stopped to correspond to the equivalence point of the reaction between the alkalinity species and the standard acid; 4.5 for routine total alkalinity, 8.3 for phenolphthalein (P) alkalinity.
- **Normality (N):** The number of gram-equivalents of a substance per liter of solution; for H_2SO_4 used in alkalinity titrations, 1 N = 0.5 M because each H_2SO_4 donates 2 protons.
- **Phenolphthalein alkalinity (P alkalinity):** Alkalinity measured to the pH 8.3 endpoint; represents OH^- and half of CO_3^{2-} content; zero if the sample pH is initially below 8.3.
- **Potentiometric titration:** Titration in which the endpoint is determined by monitoring electrode potential (pH) rather than by a color indicator.
- **Standardization:** The process of determining the exact concentration of a titrant by titrating a primary standard of known purity and mass (e.g., anhydrous Na_2CO_3).
- **Total alkalinity (T alkalinity):** Alkalinity measured to the pH 4.3–4.5 endpoint; represents the sum of all alkalinity species.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Sulfuric acid (0.02 N) is a dilute acid but can cause skin and eye irritation. Concentrated H_2SO_4 used to prepare stock solutions is severely corrosive.
- **Physical:** Glassware (burets, beakers) poses a breakage and laceration risk.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For acid splashes to skin or eyes, flush immediately with water at the eyewash station for at least 15 minutes. For concentrated acid spills, neutralize with sodium bicarbonate and refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
pH meter with combination electrode	Calibrated per SOP-L300

Item	Notes
Magnetic stir plate and stir bar	For continuous mixing during titration
Buret, 50 mL (Class A)	For samples with alkalinity above 20 mg/L as $CaCO_3$
Buret, 10 mL (Class A, microburet)	For low-alkalinity samples (below 20 mg/L)
Buret stand and clamp	–
Volumetric flasks (100 mL, 1 L)	For standard preparation
Graduated cylinder (100 mL)	For measuring sample volumes
Beakers (150 – 250 mL)	For titration
Analytical balance	0.1 mg readability; see SOP-L200

6.2. Reagents

Item	Notes
Standard H_2SO_4, 0.1 N	Prepare from concentrated H_2SO_4 or purchase pre-made; standardize against Na_2CO_3 (see Section 6.1)
Standard H_2SO_4, 0.02 N	Dilute from 0.1 N stock (see Section 6.2)
Sodium carbonate (Na_2CO_3), primary standard grade	For standardizing the acid titrant
pH buffer solutions (pH 4.01, 7.00, 10.01)	For pH meter calibration
Deionized water (Type I or II)	For reagent preparation, blanks, and rinsing

6.3. Optional Indicator Solutions

Color indicators may be used for preliminary or routine titrations when the sample is free of interfering color and turbidity. Potentiometric (pH meter) titration is preferred for accuracy.

Indicator	Endpoint pH	Application
Phenolphthalein	8.3	Phenolphthalein (P) alkalinity
Bromcresol green	4.5	Total (T) alkalinity
Mixed bromcresol green – methyl red	4.5	Total (T) alkalinity

7. Procedure

7.1. Standardizing the 0.1 N H_2SO_4

1. Dry 3–5 g of primary standard Na_2CO_3 at 250 C for 4 hours and cool in a desiccator
2. Weigh 2.500 +/- 0.200 g (to the nearest 0.001 g) into a 1 L volumetric flask. Dissolve in DI water and bring to volume. This produces an approximately 0.05 N Na_2CO_3 solution.
3. Pipette 40.00 mL of the Na_2CO_3 solution into a 250 mL beaker. Add approximately 60 mL of DI water.
4. Titrate potentiometrically with the approximately 0.1 N H_2SO_4 to a pH of about 5

5. Remove the electrodes, rinse into the beaker, and gently boil the solution for 3–5 minutes under a watch glass to expel CO_2
6. Cool to room temperature, rinse the watch glass into the beaker, and continue titrating to the pH inflection point
7. Calculate the normality of the acid:

$$N_{\text{acid}} = \frac{A \times B}{53.00 \times C}$$

where A = g Na_2CO_3 weighed into the 1 L flask, B = mL Na_2CO_3 solution taken for titration, and C = mL acid used.

8. Adjust to 0.1000 N or use the measured normality in all subsequent calculations. At 0.1000 N, 1.00 mL = 5.00 mg $CaCO_3$.

7.2. Preparing the 0.02 N H_2SO_4 Working Titrant

1. Dilute 200.00 mL of standardized 0.1000 N H_2SO_4 to 1000 mL with DI water in a volumetric flask
2. Mix thoroughly
3. Verify the normality by titrating 15.00 mL of 0.05 N Na_2CO_3 as described in Section 6.1
4. At 0.02 N, 1.00 mL = 1.00 mg $CaCO_3$
5. Store in a tightly capped borosilicate glass or polyethylene bottle. Do not keep for longer than 1 week without re-standardization.

7.3. Sample Collection and Handling

1. Collect samples in polyethylene or borosilicate glass bottles per SOP-F300
2. Fill sample bottles completely with no headspace; CO_2 exchange with air will alter the alkalinity
3. Analyze samples as soon as possible after collection, ideally within 24 hours. Refrigerate at 4 C if analysis must be delayed.
4. Do not filter, dilute, concentrate, or otherwise alter the sample before titration

7.4. Selecting Sample Size and Titrant

Choose the sample volume and titrant normality to use a reasonable volume of titrant (ideally 20 mL or more from a 50 mL buret) for good volumetric precision:

Expected Alkalinity (mg/L as $CaCO_3$)	Sample Volume	Titrant Normality
Less than 20	200 mL	0.02 N (10 mL microburet)
20 – 100	100 mL	0.02 N
100 – 500	100 mL	0.1 N

If the expected alkalinity is unknown, perform a preliminary titration with indicator to estimate the range before running the potentiometric titration.

7.5. pH Meter Calibration

Calibrate the pH meter with a three-point calibration (pH 4.01, 7.00, 10.01) per SOP-L300 before each titration session. Verify that the calibration slope is between 90% and 102% of theoretical.

7.6. Potentiometric Titration

1. Measure the selected sample volume (e.g., 100 mL) into a clean beaker using a graduated cylinder
2. Place the beaker on the magnetic stir plate and add a clean stir bar. Set the stirrer to a speed that produces a gentle vortex without splashing or entraining air.
3. Immerse the pH electrode in the sample. Record the initial pH.
4. Fill the buret with standardized H_2SO_4 (0.02 N or 0.1 N as selected in Section 6.4). Record the initial buret reading.
5. **Phenolphthalein alkalinity (P):** If the initial pH is above 8.3, titrate to pH 8.3, recording the volume of acid used. This is the P alkalinity endpoint.
6. **Total alkalinity (T):** Continue titrating to the total alkalinity endpoint. Select the endpoint pH from the table below based on the expected alkalinity concentration:

Total Alkalinity (mg/L as $CaCO_3$)	Endpoint pH
Approximately 30	4.9
Approximately 150	4.6
Approximately 500	4.3
Routine or automated analyses	4.5

7. As the endpoint is approached, add acid in smaller increments (0.1–0.2 mL) and allow pH equilibrium to be reached before adding more titrant
8. Record the final buret reading

> **Tip**
>
> For best results, record the pH after each addition of acid and construct a full titration curve. The inflection point (steepest slope) on the curve confirms the endpoint pH. This is especially important for samples with unusual chemistry (e.g., high phosphate or silicate content) where the standard endpoint pH may not correspond to the true equivalence point.

7.7. Low-Alkalinity Method (Below 20 mg/L as $CaCO_3$)

For samples with alkalinity below 20 mg/L, use a 200 mL sample volume and titrate with 0.02 N H_2SO_4 from a 10 mL microburet. Record intermediate pH values to construct a titration curve and determine the endpoint from the inflection point. Report only low-alkalinity values determined by this method.

8. Quality Control and Acceptance Criteria

8.1. Calculations

Total alkalinity (mg/L as $CaCO_3$):

$$\text{Alkalinity} = \frac{A \times N \times 50{,}000}{V}$$

where A = mL of standard acid used, N = normality of the acid, and V = mL of sample.

Phenolphthalein alkalinity uses the same formula but substitutes the volume of acid titrated to pH 8.3.

8.2. Alkalinity Species

Once P and T alkalinity are determined, the individual carbonate species can be calculated:

Condition	Hydroxide (OH^-)	Carbonate (CO_3^{2-})	Bicarbonate (HCO_3^-)
P = 0	0	0	T
P < T/2	0	2P	T – 2P
P = T/2	0	2P	0
P > T/2	2P – T	2(T – P)	0
P = T	T	0	0

All values are expressed as mg/L $CaCO_3$.

8.3. QC Requirements

- Standardize titrant before each batch and at least weekly
- Run a DI water blank at the start of each session; the blank should consume less than 0.2 mL of 0.02 N acid
- Prepare and titrate a laboratory fortified blank (LFB) using a known concentration of Na_2CO_3 at least once per batch of 20 samples. Recovery should be 90–110%.
- Run duplicate titrations on at least 10% of samples. Acceptable precision: +/- 5% relative percent difference for samples above 20 mg/L as $CaCO_3$.
- Record all QC results in the laboratory notebook

9. Waste Disposal

Waste Type	Disposal Method
Titrated samples (no hazardous additions)	Drain with copious water

Waste Type	Disposal Method
Spent acid titrant	Neutralize with sodium bicarbonate to pH 6–8, then drain
Na_2CO_3 standardization waste	Drain with copious water
Used pH buffer solutions	Collect in waste container per SOP-L300

10. References

- APHA, AWWA, WEF. (2012). Standard Methods 2320 B: Alkalinity – Titration Method. In: *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.
- USEPA Method 310.1: Alkalinity (Titrimetric, pH 4.5).

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

11. Training Record

Before performing alkalinity titrations independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L300 pH and Conductivity training
3. Demonstrate pH meter calibration with acceptable slope
4. Prepare and standardize a batch of H_2SO_4 titrant under supervision with normality within 2% of the target value
5. Perform at least three practice titrations on known standards with acceptable recovery (90–110%)
6. Be signed off by the PI or lab manager

12. Appendix A: Titrant Preparation Quick Reference

12.1. N H_2SO_4 from Concentrated H_2SO_4

Concentrated H_2SO_4 is approximately 36 N.

1. Add approximately 900 mL of DI water to a 1 L volumetric flask
2. Slowly add 2.8 mL of concentrated H_2SO_4 to the water (**always add acid to water**)
3. Mix, cool to room temperature, and bring to volume with DI water
4. Standardize against primary standard Na_2CO_3 (Section 6.1)

12.2. N H_2SO_4 from 0.1 N Stock

1. Transfer 200.00 mL of standardized 0.1000 N H_2SO_4 to a 1 L volumetric flask
2. Bring to volume with DI water and mix thoroughly

13. Appendix B: Example Calculation

```
# Alkalinity calculation per Standard Methods 2320 B
acid_mL <- 24.5          # mL of standard acid used
acid_N <- 0.02           # Normality of acid (eq/L)
sample_mL <- 100         # mL of sample titrated

alkalinity_mg_L <- (acid_mL * acid_N * 50000) / sample_mL

cat(sprintf("Total alkalinity: %.1f mg/L as CaCO3\n", alkalinity_mg_L))
```

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Endpoint difficult to identify	Low alkalinity; flat titration curve	Use 200 mL sample, 0.02 N acid, microburet; construct full titration curve
Blank consumes excessive acid	CO_2 absorbed into DI water; contaminated glassware	Use freshly boiled and cooled DI water; acid-wash glassware
Duplicate precision exceeds 5% RPD	Inconsistent titrant delivery; pH meter drift	Check buret for air bubbles; recalibrate pH meter; ensure pH equilibrium at endpoint
Alkalinity result seems too high	Sample exposed to air (CO_2 loss raised pH)	Analyze samples promptly; fill bottles with no headspace
Alkalinity result seems too low	CO_2 absorbed during storage or titration	Minimize exposure to air; do not stir vigorously enough to entrain air



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L303

Hach Colorimetric Analysis Spectrophotometer / Colorimeter

Standard Operating Procedure

SOP Number	SOP-L303
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Author(s)	Marc Los Huertos
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1. Purpose

This procedure describes the general operation, calibration, and quality control framework for colorimetric water analysis using the Hach DR3900 benchtop spectrophotometer and the Hach DR900 portable colorimeter. The SOP is designed as a modular framework: Section 6 covers instrument operation common to all analyses, while the **Method Registry** (Section 7) provides chemistry-specific parameters for each analyte (reagent kit, Hach method number, wavelength, range, reaction time, and interferences). New chemistries can be added to the registry as the lab's needs evolve, without rewriting the core SOP (APHA et al., 2012; Hach Company, 2024a, 2024b).

2. Scope

- **Applies to:** Colorimetric determination of nutrients (phosphorus, nitrate, nitrite, ammonia), metals (iron, manganese, copper, zinc, lead, chromium), and other water quality parameters (silica, sulfate, chloride, hardness) in surface water, groundwater, wastewater, and aqueous extracts using Hach pre-packaged reagent kits and stored programs
- **Does not apply to:** Analyses requiring external standards and user-prepared calibration curves (use the DR3900 in manual wavelength mode with a separate SOP); soil KCl extracts analyzed for NO_3^-/NH_4^+ via ion chromatography or flow injection (see SOP-L403)
- **Location:** EA Program laboratory, SGM 133 (DR3900); field sites (DR900)

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, review QC data
Lab Manager	Maintain instruments, stock reagent kits, track lot numbers and expiration dates
Trained Personnel	Run analyses per this SOP, record results
New Students	Complete training before independent use

4. Definitions

- **Absorbance (A):** The negative base-10 logarithm of transmittance: $A = -\log_{10}(T)$; directly proportional to analyte concentration within the linear range of the instrument (Beer-Lambert law) (Hach Company, 2024a).
- **Beer-Lambert law:** The relationship between absorbance, path length, and concentration: $A = \varepsilon c l$, where ε is the molar absorptivity, c is concentration, and l is the optical path length; the physical basis for all colorimetric quantification (APHA et al., 2012).
- **Blank (reagent blank):** A sample containing all reagents except the analyte; used to zero the instrument and correct for background absorbance contributed by the reagent matrix (APHA et al., 2012).

- **Continuing calibration verification (CCV):** A known-concentration mid-range standard analyzed at regular intervals during a run to confirm that factory-programmed calibration curves remain accurate; acceptance criterion is ±10% of the stated value (Hach Company, 2024a).
- **Detection limit (MDL):** The minimum analyte concentration reliably distinguished from a blank; method-specific and listed in the Method Registry (§7); the blank must read within the MDL of zero at the start of each session (APHA et al., 2012).
- **DR3900:** The Hach benchtop spectrophotometer; wavelength range 320–1100 nm; uses stored Hach programs or user-defined methods; supports 10 mm square, 1-inch round, and 16 mm round cells (Hach Company, 2024a).
- **DR900:** The Hach portable colorimeter; battery-operated; uses the same stored-program approach as the DR3900; limited to a subset of Hach methods compatible with the 1-inch round cell format (Hach Company, 2024b).
- **Hach method number:** A unique numeric identifier for each Hach reagent-kit and instrument-program combination; used to select the correct stored program on the DR3900 or DR900 and to ensure that the correct reagent is paired with the correct calibration curve (Hach Company, 2024c).
- **Method Registry:** The table in §7 of this SOP listing all validated Hach methods in use in the EA Program laboratory, with analyte, chemistry, program number, reagent catalog number, instrument compatibility, range, sample volume, reaction time, key interferences, and validation status.
- **Powder pillow:** A heat-sealed foil packet containing a pre-weighed, factory-prepared reagent mixture; added directly to the sample to initiate the color-forming reaction; eliminates manual reagent preparation and reduces sources of error (Hach Company, 2024c).
- **RPD (relative percent difference):** $|A - B| / ((A + B) / 2) \times 100$; used to assess duplicate precision; acceptable RPD is less than 20% for concentrations above 5 × the detection limit (APHA et al., 2012).
- **TNT plus vial:** A Hach proprietary pre-dosed reagent vial containing all required reagents sealed in glass; used with the DR3900 for total-nutrient digestion methods (e.g., total phosphorus, total nitrogen) (Hach Company, 2024c).
- **Zeroing:** The process of inserting a reagent blank into the sample cell and pressing Zero to set the baseline absorbance to zero for the selected program; required before each analytical batch (Hach Company, 2024a).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Hach reagent powder pillows and liquid reagents contain a variety of chemicals depending on the method. Some contain acids (e.g., sulfuric acid in TNT plus vials), heavy metal indicators (e.g., cadmium in older nitrate methods), or oxidizers. Always review the SDS for each reagent kit before first use.
- **Thermal:** COD and total nutrient digestions use the DRB200 reactor block at 150 °C. Use heat-resistant gloves when handling heated vials.
- **Glassware:** Glass vials can break during handling or if heated unevenly. Handle with care; wear safety glasses.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For reagent splashes to skin or eyes, flush with water for at least 15 minutes. Refer to SOP-L004 Chemical Spill Response for spill cleanup. For burns from the reactor block, apply cool running water and seek first aid.

6. Equipment

Item	Notes
Hach DR3900 benchtop spectrophotometer	SGM 133 (Hach Company, 2024a)
Hach DR900 portable colorimeter	Field use; see SOP-F208
Hach DRB200 reactor block	For COD and total nutrient digestions (150 °C)
Sample cells (1-inch round, 10 mm square, 16 mm round)	Matched to Hach method; keep clean and scratch-free
Lint-free wipes (Kimwipes)	For cleaning cell exterior before reading
Silicone oil and applicator cloth	For 1-inch round cells to minimize light scatter
Timer / stopwatch	For reaction time control
DI water (squeeze bottle)	For blanks and cell rinsing
Waste container (sealable)	For used reagent vials and solutions

7. Procedure

7.1. Startup and Instrument Check

1. Turn on the DR3900 and allow it to complete its self-check (approximately 30 seconds).

2. Verify the lamp status indicator shows "Ready."
3. Select the stored program for your analyte by program number (see §7, Method Registry) (Hach Company, 2024a).

7.2. Zeroing

1. Fill a clean sample cell with DI water to the fill line.
2. Wipe the cell exterior with a lint-free cloth. Apply silicone oil for 1-inch round cells.
3. Insert the cell, close the lid, and press **Zero**. The display must read 0.00 (within the method detection limit). If it does not, clean the cell and re-zero.

7.3. Sample Analysis

1. Fill a clean cell to the fill line with the water sample.
2. Add the reagent (powder pillow or TNT vial) per the method instructions.
3. Cap, invert, and mix as directed. Start the timer for the required reaction time.
4. When the reaction time is complete, wipe the cell exterior (apply silicone oil for 1-inch round cells).
5. Insert the cell, close the lid, and press **Read**.
6. Record the result, analyte, program number, sample ID, units, date, and time in the lab notebook.
7. Rinse the cell three times with DI water before the next sample.

7.4. Shutdown

1. Remove all samples and reagents from the instrument compartment.
2. Wipe the cell compartment with a lint-free tissue if any liquid is present.
3. Log the run in the instrument logbook (date, analyst, analytes run, and any instrument issues).
4. Turn off the DR3900 if the session is complete.

8. Method Registry

The tables below list all Hach methods currently validated in the EA Program laboratory. "Active" methods have been verified with known standards; "TBD" methods are pending validation. Add new methods to the registry per §7.4 before routine use.

8.1. Nutrients

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Range	Sample Vol.	Reaction Time	Key Interferences	Status
Reactive P (ortho-P)	PhosVer 3 (Ascorbic Acid)	490	2106069 (pillows)	DR3900, DR900	0.02–2.50 mg/L PO_4-P	10 mL	2 min	Silica above 200 mg/L; arsenate	Active
Total Phosphorus	Acid persulfate digestion + PhosVer 3	536	TNT843	DR3900 + DRB200	0.06–3.50 mg/L PO_4-P	5 mL	30 min dig. + 2 min	High turbidity	*TBD*
Nitrate (NO_3^-)	Cadmium reduction	355	2106169 (pillows)	DR3900, DR900	0.1–30.0 mg/L NO_3-N	10 mL	5 min	NO_2^- above 2 mg/L; Cl^- above 100 mg/L	Active
Nitrate (NO_3^-) HR	Chromotropic acid	410	TNT835/TNT836	DR3900	0.2–30.0 mg/L NO_3-N	1 mL	15 min	Cl^- above 500 mg/L	*TBD*
Nitrite (NO_2^-)	Diazotization	371	2107169 (pillows)	DR3900, DR900	0.002–0.300 mg/L NO_2-N	10 mL	20 min	Strong oxidizers	*TBD*
Ammonia LR	Salicylate	385	2668000 (pillows)	DR3900, DR900	0.01–0.50 mg/L NH_3-N	10 mL	20 min	Ca above 1000 mg/L $CaCO_3$	Active
Ammonia HR	Salicylate HR	343	TNT832	DR3900	0.4–50.0 mg/L NH_3-N	0.2 mL	15 min	Same as LR	*TBD*
Total Nitrogen	Persulfate dig. + chromotropic acid	350	TNT826/TNT827	DR3900 + DRB200	1–25 mg/L N	0.5 mL	30 min dig. + 15 min	–	*TBD*

8.2. Metals

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Range	Sample Vol.	Reaction Time	Key Interferences	Status
Iron (total)	FerroVer	265	2105669 (pillows)	DR3900, DR900	0.02–3.00 mg/L Fe	10 mL	3 min	Cu above 5 mg/L	Active
Iron (Fe^{2+})	1,10-Phenanthroline	255	2105769 (pillows)	DR3900, DR900	0.02–3.00 mg/L Fe	10 mL	3 min	Strong oxidizers	*TBD*
Manganese	PAN	290	Check specific kit	DR3900	0.006–0.700 mg/L Mn	10 mL	2 min	Fe above 5 mg/L	*TBD*
Copper	Bicinchoninate	135	2105969 (pillows)	DR3900, DR900	0.04–5.00 mg/L Cu	10 mL	2 min	Fe above 10 mg/L	*TBD*
Zinc	Zincon	780	Check specific kit	DR3900	0.02–3.00 mg/L Zn	10 mL	Immediate	Cu, Pb, Co above 5 mg/L	*TBD*
Lead	LeadTrak (dithizone)	277	2877200 (AccuVac)	DR3900	5–150 µg/L Pb	25 mL	1 min	Cu, Hg, Bi, Sn	*TBD*
Chromium (Cr^{6+})	DPC	090	Check specific kit	DR3900, DR900	0.010–0.700 mg/L Cr	10 mL	5 min	Fe above 1 mg/L	*TBD*

8.3. Other Parameters

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Range	Sample Vol.	Reaction Time	Key Interferences	Status
Hardness (total)	CalMagVer 3	105	2429800 (pillows)	DR3900, DR900	10–4000 mg/L as $CaCO_3$	2 mL	Immediate	Mn above 4 mg/L	*TBD*
Chloride	Mercuric thiocyanate	070	2268500 (pillows)	DR3900, DR900	0.1–25.0 mg/L Cl^-	10 mL	Immediate	High turbidity	*TBD*
Silica	Silicomolybdate	656	2429600 (pillows)	DR3900	1–100 mg/L SiO_2	10 mL	10 min	PO_4; sulfide	*TBD*
Sulfate	SulfaVer 4	680	2106769 (pillows)	DR3900, DR900	2–70 mg/L SO_4	10 mL	5 min	Silica	*TBD*

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Range	Sample Vol.	Reaction Time	Key Interferences	Status
COD	Dichromate reactor	430	TNT821/822/823	DR3900 + DRB200	3–1500 mg/L	2 mL	2 hr dig.	Cl^- above 2000 mg/L	*TBD*

8.4. Adding a New Method to the Registry

1. Obtain the Hach procedure document for the method (from the kit box or hach.com).
2. Review the SDS for all reagents in the kit.
3. Fill in a new row in the appropriate table above with all required fields.
4. Run the method on at least 3 known standard concentrations spanning the method range to verify that the instrument's stored program produces results within ±10% of the standard value.
5. Run DI water blanks to confirm zero reading.
6. Change the status from *TBD* to **Active** once validation is complete.
7. Record the validation data, date, and analyst in the QC log.

9. Quality Control and Acceptance Criteria

9.1. Every Analytical Session

- **Blank:** Run a DI water blank at the start of each session; must read within the method MDL of zero (APHA et al., 2012).
- **Standard check:** Run one mid-range Hach standard at the start and end of each session; acceptance: ±10% of the stated value (Hach Company, 2024a).
- **Duplicates:** Analyze at least 10% of samples in duplicate; acceptable RPD less than 20% above 5 × the detection limit.

9.2. Calibration Verification

The DR3900 and DR900 use factory-programmed calibration curves that do not require user calibration under normal conditions (Hach Company, 2024a, 2024b). However:

- Run a multi-point verification using Hach-supplied standards at least annually or when the instrument is serviced.
- If a standard check deviates by more than 10%, clean the cell, re-zero, and re-read. If the problem persists, contact Hach technical support.

10. Waste Disposal

Waste Type	Disposal Method
Used powder pillow packets and caps	Standard trash (verify SDS; most are non-hazardous)
Cadmium-containing reagent waste	Hazardous waste; EH&S pickup
Reactor block digestion waste (COD)	Collect in labeled acid waste container
Used sample cells (glass)	Rinse thoroughly; reuse or recycle
Rinse water (DI)	Drain

11. References

- Hach Company (2024a)
- Hach Company (2024b)
- Hach Company (2024c)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2024a). *DR3900 benchtop spectrophotometer: Procedures manual*. https://www.hach.com/dr3900-benchtop-spectrophotometer/product?id=56440352466

Hach Company. (2024b). *DR900 portable colorimeter: Procedures manual*. https://www.hach.com/dr900-portable-colorimeter/product?id=56440352476

Hach Company. (2024c). *Water analysis guide* (7th ed.). https://www.hach.com/wag

12. Related SOPs

SOP	Title	Relationship
F208	Portable Colorimeter (Field)	Field use of the DR900
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Reagent waste disposal
L215	Hach DR3900 Instrument	Instrument startup and shutdown

13. Training Record

Before running analyses on the DR3900 or DR900 independently, personnel must:

1. Read this SOP and SOP-L215 in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the SDS for all reagent kits to be used
4. Observe a trained operator perform startup, blank zeroing, sample analysis, and a standard check for at least two analytes

5. Run a full session (blank, standard check, samples, duplicate) under supervision with acceptable QC results
6. Add at least one new method to the registry and validate it before using it on real samples
7. Be signed off by the lab manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L305

IDEXX Quanti-Tray 2000 for *E. coli*/Total Coliform Enumeration

Standard Operating Procedure

SOP Number	SOP-L305
Revision	4.3
Author(s)	Marc Los Huertos
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1. Purpose

This procedure describes the collection and analysis of water samples for the enumeration of *Escherichia coli* (*E. coli*) and total coliform bacteria using the IDEXX Colilert-18 / Quanti-Tray 2000 method. Results are reported as Most Probable Number (MPN) per 100 mL (APHA et al., 2012).

This method is applicable for analyzing drinking water, surface water, groundwater, and wastewater to assess microbial contamination levels.

2. Scope

- **Applies to:** Enumeration of *E. coli* and total coliform bacteria in drinking water, surface water, groundwater, and wastewater using the IDEXX Colilert-18 defined substrate method with Quanti-Tray 2000
- **Does not apply to:** Non-coliform bacterial analysis, viral or parasitic pathogens, or samples requiring membrane filtration methods
- **Location:** Biogeochemistry Lab and field sampling sites
- **Detection limit:** 1 MPN per 100 mL

3. Responsibilities

Role	Responsibility
Lab PI / Lab Director	Approve SOP, verify and sign off on results
QA Manager	Review completed bench sheets, sign lab notebook
Trained Personnel	Collect samples, perform analysis, record results
New Students	Complete training before independent use

4. Definitions

- **Colilert:** A proprietary IDEXX defined-substrate technology that detects total coliforms and *E. coli* simultaneously by enzymatic hydrolysis of ONPG (for coliforms) and MUG (for *E. coli*).

- **Coliform bacteria (total coliforms):** A group of facultatively anaerobic, gram-negative, rod-shaped bacteria that ferment lactose; includes *E. coli* and many non-fecal genera; used as an indicator of sanitary water quality.
- **Defined substrate technology (DST):** A quantitative detection method based on the enzymatic hydrolysis of a chromogenic or fluorogenic substrate specific to the target organism.
- **E. coli:** *Escherichia coli*; a fecal indicator bacterium detected by Colilert via MUG hydrolysis, which produces a fluorescent product visible under UV light at 365 nm.
- **MPN (Most Probable Number):** A statistical estimate of the number of organisms per 100 mL, derived from the pattern of positive and negative wells in the Quanti-Tray/2000 using the IDEXX MPN table.
- **Quanti-Tray/2000:** A sealed 97-well tray into which the sample- reagent mixture is sealed and incubated; provides positive/negative results for 49 large wells and 48 small wells, allowing MPN calculation up to 2,419 per 100 mL.
- **Total holding time:** The maximum time from sample collection to the start of incubation; for Colilert, the sample must be incubated within 6 hours of collection or 30 hours if held at 4 °C.

5. Safety and Hazards

⚠ Hazards

- **Biological:** Water samples may contain live microorganisms and pose a threat of infection. Treat all samples as potentially infectious.
- **Physical:** UV lamp (365 nm) can cause eye damage with prolonged exposure.
- **Chemical:** Colilert-18 reagent is not classified as hazardous per the manufacturer's SDS, but gloves and safety glasses are recommended.

5.1. Required PPE

☐ Clean disposable nitrile gloves
☐ Safety glasses (especially during UV reading and reagent handling)
☐ Lab coat
☐ Closed-toed shoes

5.2. Emergency Procedures

Water quality can range from potable to highly contaminated with assorted pathogens. Take every precaution to protect yourself. Wash hands frequently. In case of exposure to potentially contaminated water, wash affected area with soap and water immediately. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Quanti-Tray Sealer	Lab bench	IDEXX Model B9-10894-04, Serial QT1757
UV light source (365 nm)	Lab bench	For *E. coli* fluorescence reading
Incubator	Lab	Set to 35 +/- 0.5 C; Catalog 150152632, Serial IMC40490982
Comparator	Lab refrigerator	Catalog 2100Q-01; stored in red zip-lock bag

6.2. Reagents and Consumables

Item	Catalog No.	Storage
IDEXX Colilert-18 reagent	98-27164-00	Room temperature
Sterile 100 mL IDEXX bottles (shrink-wrapped)	WV120SBT-200	Room temperature
Sterile 100 mL IDEXX bottles with sodium thiosulfate (for chlorinated samples)	WV120SBT-200	Room temperature
IDEXX Quanti-Tray 2000	–	Room temperature
Pipettes and sterile pipette tips	–	–
Disposable gloves and lab coat	–	–
Deionized water (for reagent preparation)	–	–

7. Procedure

7.1. Definitions

The following definitions apply throughout this SOP:

- **Analytical batch:** The set of samples processed at the same time.
- **Control cultures:** Known positive and negative cultures used to verify each lot of medium. *E. coli* serves as the positive control; *Staphylococcus aureus* serves as the negative control.
- **Field duplicate (FD):** Two samples taken at the same time and place under identical circumstances, treated identically through field and laboratory procedures. Analysis of FDs indicates the precision associated with sample collection, preservation, storage, and laboratory procedures.
- **Laboratory reagent blank (LRB):** An aliquot of sterilized water treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if analytes or interferences are present in the laboratory environment, reagents, or apparatus.

- **Laboratory duplicate (LD):** Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of LDs indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage.

7.2. Biases and Interferences

- Water samples containing humic material or other colored substances may interfere with visual readings. If there is background color, compare inoculated trays to a control tray containing only water (SM, 9223 A) (APHA et al., 2012).
- Chlorinated water may inhibit the growth of coliform bacteria. If the water has been chlorinated, use the sodium thiosulfate bottles to neutralize the chlorine.

7.3. Sample Collection

1. Arrive at the site and record site number, date, and time
2. Label the bottle with: location (geographic area and stream or lake name), date, time, water temperature, and sampler's initials. Label the bottle before immersion using a black permanent marker or pre-printed labels.
3. Put on latex or nitrile gloves before handling bottles. Fingers contain contaminants such as nitrates; bug repellent and sunscreen are particularly troublesome. Once gloves are on, do not touch your face, the ground, or anything other than the bottles.
4. Collect the sample from flowing (not stagnant) water, facing upstream
5. Immerse the bottle completely, approximately 10 cm (4 inches) deep, with the mouth pointing upstream so no water flows over your hand into the bottle. Do not allow the bottle to contact the stream bottom where sediments can be disturbed. Collect water 6–12 inches below the surface.
6. Fill the bottle to the 100 mL line on the first immersion. Pour off any excess and cap immediately. Do not underfill, overfill, or re-dunk. If too much water is poured off, use a new 100 mL container.
7. Do not touch the bottle mouth or inside of the cap. Be careful not to contaminate the sample with surface film, contact with human skin, or breathing into the bottle or cap. If the stream is too shallow to fully immerse the bottle, collect as much as possible while avoiding the bottom, and note the depth in field notes.

> **Tip**
>
> Collect one field duplicate (FD) sample every two weeks of sampling. Duplicate sites are selected at random. The duplicate is the second sample when two samples are collected at the same location. Duplicates document repeatability of sample collection and reproducibility of laboratory results.

7.4. Sample Preservation and Storage

1. Keep samples cool during transport using a cooler with ice

2. Store at 4 C until analysis; do not freeze
3. Analyze within 6 hours of collection
4. Record the location number, bottle numbers, and collection time in the field sample log
5. Samples must remain in the possession of the researcher who both collects and analyzes them

7.5. Sample Processing

1. Turn on the Quanti-Tray sealer
2. Set the incubator to 35 C
3. Allow samples to reach room temperature before analysis
4. Aseptically add one packet of Colilert-18 reagent to the 100 mL water sample
5. Replace the lid and shake well until the reagent has fully dissolved
6. Label the Quanti-Tray with a permanent marker, recording information that identifies the sample
7. Open the Quanti-Tray by holding it at the top with the well side facing your palm. Push the top edge inward with your free hand while squeezing the tray into a circle. Gently pull the foil tab away from the plastic side. Be careful not to tear the tab. Do not touch the inside of the tray.
8. Pour the sample mixture into the Quanti-Tray, avoiding contact with the foil tab
9. Tap the small wells 2–3 times to release air bubbles. Allow foam to settle.
10. Place the filled Quanti-Tray on the rubber insert, making sure each well fits its corresponding hole
11. Push the insert with the tray into the sealer, non-foil-tab end first, until the sealer grabs the tray and pulls it through. If you need to reverse the motor, press and hold the reverse button. However, do not reverse the motor if the rubber insert is completely inside the sealer.
12. The sealer distributes the sample mixture into the wells, seals them, and partially ejects the sealed tray
13. Remove the rubber insert and tray from the sealer

> **!** Important
>
> If the sealer jams or malfunctions, do not force the tray. Contact the lab manager for assistance.

7.6. Incubation

1. Incubate the sealed tray at 35 +/- 0.5 C for 18 hours
2. Do not stack trays or block airflow around them in the incubator

7.7. Reading Results – Total Coliforms

1. After 18 hours of incubation, remove the tray from the incubator
2. Count the number of large wells and small wells that are yellow and equal to or greater than the comparator, including the large overflow well at the top
3. Mark positive wells with a permanent marker as you count
4. Record the number of positive small wells and positive large wells in the lab notebook

7.8. Reading Results – *E. coli*

1. In a darkened area, view the Quanti-Tray under the UV lamp (365 nm)

> ⚠ UV Safety
>
> Be careful to avoid eye damage when using the UV lamp. Do not look directly at the UV light source. Wear UV-protective safety glasses if available.

2. Count the number of fluorescent wells that are equal to or greater than the comparator
3. Record the number of fluorescent small wells and fluorescent large wells in the lab notebook

7.9. Determining MPN

1. Use the IDEXX MPN table (see Appendix) to look up the MPN based on the number of positive large wells and positive small wells
2. Cross-check results using the IDEXX MPN Generator software: https://www.idexx.com/en/water/resources/mpn-generator/ (Windows only)
3. Record results on the bench sheet for both total coliforms and *E. coli*

7.10. Interpreting Results

MPN/100 mL	Risk Level
1–10	Low risk
11–100	Medium risk
Greater than 100	High risk

7.11. Review and Sign-Off

1. The completed bench sheet should be reviewed by the researcher, the laboratory director, and the QA manager
2. The Lab Director or QA Manager must sign the lab notebook to verify that the results are accurate

7.12. Expected Results for Control Organisms

Organism	Expected Result
Escherichia coli	Yellow wells, fluorescence
Klebsiella pneumoniae	Yellow wells, no fluorescence
Pseudomonas aeruginosa	Clear wells, no fluorescence
Method blank	Clear wells, no fluorescence

8. Quality Control and Acceptance Criteria

8.1. QA/QC for Each New Lot

For initial analyst demonstration of capability and for each new lot of Quanti-Tray 2000, perform the following checks:

1. **Reagent lot check:** Shine the UV lamp on the Colilert media snap packs. If the unopened lot is fluorescent, discard it.
2. **Blank control:** Dissolve one packet of Colilert media in 100 mL of distilled water. Check for fluorescence. The blank must show no fluorescence.
3. **Positive control:** Dissolve one packet of Colilert media and 1–2 Control Culture vials (stored in freezer) in 100 mL of distilled water. Incubate for 18 hours. Confirm fluorescence.
4. **Reagent water blank:** Analyze a sterile reagent water blank with each batch of samples to verify a negative result after 18–22 hours.
5. **Sterile bottle check:** Gravimetrically check each new lot of sterile bottles to confirm they are transparent and non-fluorescing.

8.2. Precision

Calculate precision for duplicate analyses using relative percent difference (RPD):

$$\text{RPD} = \frac{(A - B) \times 100}{(A + B)/2}$$

where A = MPN from aliquot A and B = MPN from aliquot B.

8.3. Acceptance Criteria

- Desired precision: +/- 20% RPD
- Desired detection limit: 1 MPN/100 mL
- If a precision value exceeds 20%, record in the comments section of the bench sheet: "These data are associated with an out-of-control duplicate analysis. The UCL = 20%."
- If a blank value exceeds 1 MPN/100 mL, record in the comments section: "These data are associated with a blank value that exceeds the detection limit of 1 MPN/100 mL."
- Samples cannot be reanalyzed because the sample volume is depleted after the initial analysis
- If data are unacceptable for any reason, the analyst should review their technique before conducting this analysis again

9. Waste Disposal

Waste Type	Disposal Method
Used Quanti-Trays and media	Autoclave before disposal, then standard trash
Used sample bottles	Autoclave before disposal, then standard trash

Waste Type	Disposal Method
Workspace surfaces	Disinfect thoroughly after each session

> **!** Important
>
> All materials that have contacted water samples or culture media must be autoclaved prior to disposal. Workspaces must be thoroughly disinfected after each analytical session.

10. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-F001 Field Safety training
3. Review the Colilert-18 manufacturer's instructions
4. Review sampling and analysis procedures with the Lab Manager
5. Observe a trained operator perform the complete procedure (collection through enumeration)
6. Perform the procedure under supervision with acceptable QC results
7. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

TBD – add troubleshooting guidance for common issues (e.g., trays not sealing properly, ambiguous well color, incubator temperature deviations).

13. Appendix B: IDEXX MPN Tables

The original LaTeX document included two pages of MPN lookup tables (scanned PDFs). These should be included here as linked PDF files or reproduced as tables. The tables can also be accessed via the IDEXX MPN Generator at https://www.idexx.com/en/water/resources/mpn-generator/.

14. Appendix C: Estimated Time

Task	Time Required
Sample setup	Approximately 12 minutes
Incubation	18 hours
Reading and enumeration	Approximately 10 minutes

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L306

Biochemical Oxygen Demand (BOD_5)

Standard Operating Procedure

SOP Number	SOP-L306
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of five-day biochemical oxygen demand (BOD_5) in water and wastewater samples using the manometric method (WTW OxiTop system). BOD_5 is a measure of the dissolved oxygen consumed by microorganisms during the aerobic decomposition of organic matter over a five-day incubation period at 20 °C and is the standard regulatory indicator of organic pollution load in water (APHA et al., 2012; U.S. Environmental Protection Agency, 1978).

2. Scope

- **Applies to:** Surface water, wastewater, and effluent samples analyzed for BOD_5 using the WTW OxiTop manometric method in the EA Program laboratory
- **Does not apply to:** Carbonaceous BOD (CBOD) with nitrification inhibitor; BOD by Winkler titration or dissolved oxygen probe methods; chemical oxygen demand (COD)
- **Location:** Biogeochemistry Laboratory, Pomona College

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Approve SOP; review QC data; maintain equipment
Trained Personnel	Prepare reagents, run analyses, record results
New Students	Complete training before independent use

4. Definitions

- **Biochemical oxygen demand (BOD):** The mass of dissolved oxygen consumed per unit volume of sample by microbial oxidation of organic matter under aerobic conditions at 20 °C; expressed in mg/L (APHA et al., 2012).
- **BOD_5:** BOD measured over a 5-day incubation period at 20 °C; the standard regulatory measure of organic pollution load in surface water and effluent (APHA et al., 2012; U.S. Environmental Protection Agency, 1978).
- **Carbonaceous BOD (CBOD):** BOD attributable to carbon-containing organic compounds; measured in the presence of a nitrification inhibitor (allylthiourea) to prevent oxygen consumption by nitrifying bacteria from contributing to the result.

- **Dilution water:** Aerated, buffered, nutrient-supplemented deionized water used to dilute concentrated samples to bring the expected BOD_5 into the measurable range of the OxiTop system (APHA et al., 2012).
- **Glucose-glutamic acid (GGA) check standard:** A standard seed-check solution prepared at 150 mg/L glucose + 150 mg/L glutamic acid; expected BOD_5 of 198 ±30.5 mg/L; used to verify seed quality and method performance (APHA et al., 2012, Method 5210 B).
- **Manometric method (OxiTop):** A closed-system BOD measurement technique in which O_2 consumption is tracked by measuring the resulting decrease in headspace pressure; CO_2 produced during microbial respiration is absorbed by NaOH pellets, ensuring that pressure changes reflect only oxygen consumption (WTW (Xylem Analytics), 2019).
- **Nitrification:** Microbial oxidation of ammonia to nitrate by nitrifying bacteria; consumes dissolved oxygen and can inflate BOD values if not inhibited in carbonaceous BOD analyses.
- **Seeding:** Addition of a microbial inoculum (e.g., diluted wastewater or settled sewage) to dilution water to ensure adequate microbial activity when samples lack native microorganisms (APHA et al., 2012).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Nutrient buffer solutions contain phosphate and ammonium salts; NaOH pellets (CO_2 absorbent) are corrosive. Handle with gloves and avoid skin or eye contact.
- **Biological:** Wastewater samples may contain pathogens. Treat all samples as potentially infectious.
- **Physical:** Glass BOD bottles are fragile. Handle with care to avoid breakage.

5.1. Required PPE

☐ Safety glasses or goggles
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

In case of chemical spill or exposure, refer to SOP-L004 Chemical Spill Response or call Campus Safety. For NaOH contact with skin or eyes, flush immediately with copious water for at least 15 minutes and seek medical attention.

6. Equipment and Materials

6.1. Equipment

Item	Notes
WTW OxiTop manometers (3 per run)	One blank + two samples minimum; check battery (WTW (Xylem Analytics), 2019)
BOD_5 incubation bottles (nominal 500 mL)	Brown glass with neck adapter
Rubber quivers (CO_2 absorber holders)	One per bottle
Incubator at 20.0 ±1.0 °C	Verify temperature daily
Magnetic stirrer platform (in incubator)	Low-speed continuous stirring
Magnetic stir bars	One per bottle
500 mL graduated cylinder	
Micropipette, 100–1000 µL	With disposable tips
pH meter	Calibrated per SOP-L300
Air pump with diffuser stone	For aerating dilution water

6.2. Consumables

Item	Storage	Notes
NaOH pellets	Desiccator	CO_2 absorbent; 2 pellets per bottle
Phosphate buffer stock	Refrigerator	See §6
$MgSO_4$, $CaCl_2$, $FeCl_3$ solutions	Shelf	See §6
Seed inoculum (if needed)	Fresh	Settled domestic wastewater or commercial seed

7. Reagents and Dilution Water

7.1. Nutrient Stock Solutions

Prepare the following four stock solutions; add 1 mL of each per liter of dilution water (APHA et al., 2012):

1. **Phosphate buffer:** Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl in ~500 mL deionized water and dilute to 1 L (pH 7.2).
2. **Magnesium sulfate:** Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in deionized water; dilute to 1 L.
3. **Calcium chloride:** Dissolve 27.5 g $CaCl_2$ in deionized water; dilute to 1 L.
4. **Ferric chloride:** Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in deionized water; dilute to 1 L.

7.2. Dilution Water Preparation

1. Fill a clean 1 L vessel with deionized water.
2. Add 1 mL of each stock solution.
3. Aerate with an air pump for at least 1 hour to bring DO near saturation.
4. Verify pH is 6.8–7.2; adjust with dilute HCl or NaOH if necessary.
5. Allow to equilibrate to 20 °C before use.

7.3. Measuring Range and Sample Volume Selection

Table 1: Volume selection for WTW OxiTop BOD_5 analysis based on expected concentration (WTW (Xylem Analytics), 2019).

Expected BOD_5 (mg/L)	Total volume (mL)	Sample (mL)	DI water (mL)
0–40	432	216	216
0–80	365	182.5	182.5
0–200	250	125	125
0–400	164	82	82
0–800	97	48.5	48.5
0–2000	43.5	21.75	21.75

For most EA Program surface water samples, the 0–200 mg/L range is a reasonable starting point. If the expected concentration is unknown, run samples at two dilutions.

8. Procedure

8.1. Preparation (Day 0)

1. Calibrate the pH meter per SOP-L300.
2. Prepare dilution water as described above; make enough for all blanks and samples.
3. Allow refrigerated samples to equilibrate to 20 °C. Add seed inoculum to both sample and blank bottles if seeding is required.
4. Verify that all OxiTop manometers are functional (battery check, reset previous readings) (WTW (Xylem Analytics), 2019).

8.2. Bottle Setup

1. Place a clean magnetic stir bar in each BOD_5 bottle.
2. Measure the appropriate sample volume into each sample bottle (see Table 1).
3. Add the corresponding volume of dilution water.
4. For the blank bottle, add dilution water only (same total volume as sample bottles).
5. Place 2 NaOH pellets in each rubber quiver. Insert the quiver into the bottle neck above the liquid surface.
6. Screw the OxiTop manometer head onto each bottle; ensure a gas-tight seal.

> **Labeling**
>
> Label each bottle (Blank, Sample A-1, Sample A-2, etc.) on both the bottle and the manometer before starting incubation. Record bottle assignments in the lab notebook.

8.3. Incubation

1. Place all bottles on the magnetic stirrer platform inside the incubator at 20.0 ±1.0 °C.
2. Activate the OxiTop manometers to begin recording pressure.
3. Incubate for five days (120 ±2 hours) in the dark; keep the incubator door closed except for daily temperature checks (APHA et al., 2012).
4. Verify incubator temperature at least once daily; record in the instrument logbook.

8.4. Reading Results (Day 5)

1. Read the BOD_5 value from each OxiTop manometer; the instrument converts pressure decrease to mg/L automatically based on the selected measuring range (WTW (Xylem Analytics), 2019).
2. Record all readings in the lab notebook: setup and reading dates/ times, incubator temperature log, measuring range, sample IDs, and replicate values.
3. Remove bottles from the incubator. Discard sample water per §9. Clean bottles and stir bars thoroughly with hot water and detergent; rinse with deionized water.

9. Data Analysis and Calculations

9.1. OxiTop Direct Reading

The OxiTop system calculates BOD_5 internally from the headspace pressure decrease (WTW (Xylem Analytics), 2019). Record the displayed mg/L value directly.

9.2. Blank Correction

$$\mathrm{BOD}_{5,\mathrm{corrected}} = \mathrm{BOD}_{5,\mathrm{sample}} - \mathrm{BOD}_{5,\mathrm{blank}}$$

The blank must be less than 0.2 mg/L for unseeded dilution water (APHA et al., 2012). If the blank exceeds 0.2 mg/L, investigate dilution water quality and consider re-running the analysis.

9.3. Dilution Factor

For highly concentrated samples diluted beyond the standard OxiTop protocol:

$$\mathrm{BOD}_{5,\mathrm{final}} = \mathrm{BOD}_{5,\mathrm{corrected}} \times \frac{V_{\mathrm{total}}}{V_{\mathrm{sample}}}$$

For standard OxiTop runs, the dilution factor is already incorporated into the displayed result.

10. Quality Control

- Run at least one blank per analytical batch (APHA et al., 2012).
- Run duplicate sample bottles for each sample; RPD must be less than 20%.
- If available, include a GGA check standard at 300 mg/L combined (150 mg/L each); expected BOD_5 = 198 ±30.5 mg/L (APHA et al., 2012, Method 5210 B).
- Blank BOD_5 must be less than 0.2 mg/L (unseeded) or less than 0.6 mg/L (seeded) (APHA et al., 2012).
- Verify incubator temperature is 20.0 ±1.0 °C throughout incubation.
- Log all QC results; flag and consult the PI if criteria are not met.

11. Troubleshooting

Problem	Possible Cause	Corrective Action
No pressure change after 5 days	Dead battery; poor seal	Check battery; reseat manometer
Blank BOD_5 too high	Contaminated dilution water	Reclean bottles; prepare fresh dilution water
Replicate RPD > 20%	Heterogeneous sample	Mix samples well; check pipette calibration
BOD_5 exceeds range	Sample more concentrated than expected	Re-run at higher range (lower volume)
Negative BOD_5	Leak in bottle seal; NaOH saturated	Inspect seal; use fresh NaOH pellets

12. Waste Disposal

Waste Type	Disposal Method
Analyzed sample water	Drain with copious water (if non-hazardous)
NaOH pellets (used)	Dissolve in water, neutralize, drain
Dilution water	Drain
Broken glass	Sharps container

13. References

- APHA et al. (2012)
- U.S. Environmental Protection Agency (1978)
- WTW (Xylem Analytics) (2019)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

U.S. Environmental Protection Agency. (1978). *Method 405.1: Biochemical oxygen demand (5 days, 20 °C)*. U.S. EPA, Environmental Monitoring; Support Laboratory. https://www.epa.gov/sites/default/files/2015-08/documents/method_405-1_1978.pdf

WTW (Xylem Analytics). (2019). *OxiTop control system: Operating manual*. https://www.xylem.com/en-us/brands/wtw/

14. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L300	pH and Conductivity (Bench)	pH meter calibration for dilution water
F209	Dissolved Oxygen Meter (Field)	Related field DO measurement
F300	Surface Water Sampling	Sample collection context

15. Training Record

Before performing BOD_5 analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the WTW OxiTop operating manual (WTW (Xylem Analytics), 2019)
4. Observe a trained operator perform the complete procedure (setup through Day 5 reading)
5. Perform the analysis under supervision with acceptable QC results (duplicate RPD within 20%; blank within criteria)
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L307

ELISA: Bisphenol A, Bisphenol B, and Pesticides in Water

Standard Operating Procedure

SOP Number	SOP-L307
Revision	1.0
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the use of competitive enzyme-linked immunosorbent assay (ELISA) kits to screen water samples for bisphenol A (BPA), bisphenol B (BPB), and selected pesticides (atrazine, glyphosate, and 2,4-D). ELISA provides a rapid, cost-effective immunochemical screening method for detecting trace-level organic contaminants in environmental water samples without the need for extensive sample preparation or chromatographic instrumentation (Aga, 2013; Goda et al., 2000; Voller et al., 1978). Results exceeding the kit detection threshold should be considered presumptive and confirmed by an instrumental method (e.g., LC-MS/MS or GC-MS) when data will be used for regulatory reporting (US Environmental Protection Agency, 1996).

2. Scope

- **Applies to:** Screening of surface water, groundwater, treated effluent, and drinking water source water for BPA, BPB, atrazine, glyphosate, and 2,4-D using commercially available competitive ELISA plate kits (96-well microplate format)
- **Does not apply to:** Confirmatory quantitative analysis by LC-MS/MS or GC-MS (see SOP-L309); soil or sediment extracts (matrix effects require separate validation); clinical or food-safety testing
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Select analytes and approve kit vendors; review QC data
Trained Personnel	Run ELISA assays, record results, flag exceedances
New Students	Complete training before independent use

4. Definitions

- **Absorbance:** The logarithm of the ratio of incident to transmitted light through a colored solution, measured at the wavelength of maximum absorption; the raw output of spectrophotometric ELISA readers.

- **Bisphenol A (BPA):** An industrial chemical used in polycarbonate plastics and epoxy resins; an endocrine-disrupting compound detected in environmental water samples.
- **Competitive ELISA:** An enzyme-linked immunosorbent assay format in which analyte in the sample and a fixed amount of enzyme-labeled analyte compete for binding to a limited number of antibody sites; signal decreases as analyte concentration increases (inverse response).
- **Cross-reactivity:** The degree to which an antibody recognizes structurally similar compounds other than the target analyte; expressed as a percentage and listed in the kit insert; high cross-reactivity with related compounds (e.g., BPB with a BPA kit) can produce false positives.
- **ELISA (Enzyme-Linked Immunosorbent Assay):** A plate-based immunoassay that uses antibody-antigen binding and an enzyme-substrate color reaction to detect and quantify target analytes.
- **Matrix effect:** Interference from sample components (suspended solids, humic acids, pH, salinity) that alter antibody-antigen binding or enzyme activity; minimized by sample dilution, solid-phase extraction, or pH adjustment.
- **Standard curve:** A set of calibration solutions of known analyte concentration used to convert absorbance readings to concentrations; ELISA curves are typically sigmoidal (4-PL fit).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** ELISA kits contain dilute acids (stop solution, typically 1 N HCl or H_2SO_4), enzyme conjugates, and substrate solutions (typically 3,3',5,5'-tetramethylbenzidine, TMB). Stop solution is corrosive at working concentration. Review the Safety Data Sheet (SDS) provided with each kit.
- **Biological:** Environmental water samples may contain pathogens. Treat all samples as potentially infectious.
- **Physical:** Microplate wells are fragile. Handle plates carefully to avoid cracking wells or cross-contaminating adjacent wells.

5.1. Required PPE

☐ Safety glasses or goggles
☐ Lab coat
☐ Nitrile gloves (change between samples to prevent cross-contamination)

5.2. Emergency Procedures

For stop solution (acid) contact with skin or eyes, flush immediately with copious water for at least 15 minutes. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Microplate reader (450 nm filter)	Absorbance mode; confirm filter is installed
Microplate washer (optional)	Manual wash with squeeze bottle is acceptable
Adjustable micropipettes (10–100 μL, 100–1000 μL)	Calibrated per manufacturer schedule
Multichannel micropipette (optional)	For 8- or 12-channel dispensing
Plate shaker or orbital incubator	Low speed (200–300 rpm)
Timer	
Incubator or water bath (if kit requires temperature control)	Some kits specify 25 °C or 37 °C

6.2. Consumables

Item	Notes
ELISA kit (analyte-specific)	See Section 6 for kit selection
Disposable pipette tips	Use fresh tips for every transfer
Deionized water (Type II or better)	For dilutions and wash steps
Absorbent paper towels	For blotting plates after washing
Aluminum foil	For covering plates during incubation

7. ELISA Kit Selection

The EA Program uses commercial competitive ELISA kits. The table below lists the kits currently validated for use. All kits follow the same general competitive ELISA workflow (Section 7) but differ in incubation times, dilution requirements, and detection ranges. Always consult the kit insert for analyte-specific instructions.

Table 1: ELISA kits validated or under evaluation for EA Program use.

Analyte	Manufacturer	Catalog No.	Detection Range	Sample Volume	Status
Bisphenol A	Abraxis	530701	0.05–10 μg/L	50 μL	Active
Bisphenol B	Abraxis	*TBD*	*TBD*	50 μL	*TBD*
Atrazine	Abraxis	500001	0.04–5.0 μg/L	50 μL	Active
Glyphosate	Abraxis	500086	0.075–4.0 μg/L	50 μL	*TBD*
2,4-D	Abraxis	500201	0.05–5.0 μg/L	50 μL	*TBD*

> **Note**
>
> Catalog numbers and detection ranges are based on Abraxis (Eurofins) kits as of 2025. Verify against the current kit insert before use. Other manufacturers (EnviroLogix, Neogen) produce equivalent kits that may be substituted after validation.

8. Procedure

8.1. General Competitive ELISA Workflow

All competitive ELISA kits in this SOP share the same principle: the analyte in the sample competes with an enzyme-labeled analyte conjugate for a limited number of antibody binding sites coated on the microplate well. Higher analyte concentrations in the sample result in less conjugate binding and therefore lower color development after substrate addition. The relationship between absorbance and concentration is inversely proportional (Goda et al., 2000; Voller et al., 1978).

8.2. Preparation

1. Remove the ELISA kit from the refrigerator and allow all reagents to equilibrate to room temperature (20–25 °C) for at least 30 minutes before opening.
2. Prepare wash buffer by diluting the concentrate per kit instructions (typically 1:10 with deionized water). Mix thoroughly.
3. If samples require dilution (e.g., wastewater with expected high concentrations), prepare dilutions in deionized water or the sample diluent provided with the kit. Record all dilution factors.
4. Label a plate map in the lab notebook showing the position of standards, controls, blanks, and samples. Each standard and sample should be run in duplicate.

8.3. Assay Procedure

Follow the specific kit insert for exact volumes and incubation times. The general sequence is:

1. **Add standards and samples.** Pipette the specified volume (typically 50 μL) of each calibration standard, positive control, negative control (zero standard), and sample into the appropriate wells. Change tips between every addition.
2. **Add enzyme conjugate.** Pipette the conjugate solution into each well. The conjugate competes with any analyte present in the sample for antibody binding sites.
3. **Incubate.** Cover the plate with foil and incubate on the plate shaker at the specified temperature and duration (typically 60 minutes at room temperature for BPA; consult kit insert for other analytes).
4. **Wash.** Aspirate or decant the well contents. Wash each well 3–5 times with wash buffer (approximately 250 μL per wash). After the final wash, invert the plate and tap firmly on absorbent paper to remove residual liquid. Incomplete washing is the most common source of high background and poor precision.

5. **Add substrate.** Pipette the TMB substrate solution into each well. Incubate for the specified time (typically 20–30 minutes at room temperature) in the dark. A blue color develops in wells with bound conjugate.
6. **Add stop solution.** Pipette the stop solution (acid) into each well. The blue color turns yellow. Read the plate within 15 minutes of adding stop solution.
7. **Read absorbance.** Measure absorbance at 450 nm using the microplate reader.

> **Tip**
>
> Dispense reagents quickly and consistently across the plate. Use a multichannel pipette when possible to minimize time differences between the first and last wells. Stagger plates if running more than one analyte simultaneously.

8.4. Plate Washing Details

Inadequate washing is the most frequent source of assay failure in ELISA. Follow these guidelines:

1. Aspirate well contents completely (manual flicking or vacuum aspiration)
2. Fill each well with approximately 250 μL wash buffer
3. Allow wash buffer to soak for 5–10 seconds
4. Aspirate again
5. Repeat for the total number of wash cycles specified by the kit (typically 3–5)
6. After the final aspirate, invert the plate and tap firmly on a pad of clean paper towels 3–4 times to remove residual buffer

9. Data Analysis and Calculations

9.1. Standard Curve Construction

1. Calculate the mean absorbance of the duplicate wells for each standard concentration.
2. Express each standard as a percentage of the zero standard (maximum binding, B_0):

$$\%B/B_0 = \frac{\bar{A}_{\text{standard or sample}}}{\bar{A}_{B_0}} \times 100$$

3. Plot %B/B_0 (y-axis) against the log of the standard concentration (x-axis). Fit a four-parameter logistic (4PL) curve, which is the standard model for competitive immunoassay calibration (Findlay et al., 2000):

$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

where A = maximum asymptote (zero dose), D = minimum asymptote, $C = \text{IC}_{50}$ (inflection point), and B = slope factor.

4. Interpolate sample concentrations from the 4PL curve. Most microplate reader software includes 4PL curve fitting. Alternatively, use R:

```r
# Four-parameter logistic (4PL) curve fitting for ELISA data
library(drc)

# Example data: concentration (ug/L) and %B/B0
standards <- data.frame(
  conc = c(0.05, 0.1, 0.5, 1.0, 5.0, 10.0),
  pct_b_b0 = c(92.3, 84.1, 55.7, 38.2, 12.4, 6.8)
)

# Fit 4PL model
model_4pl <- drm(pct_b_b0 ~ conc, data = standards,
                 fct = LL.4(names = c("Slope", "Lower", "Upper", "IC50")))
summary(model_4pl)

# Predict concentration for a sample with %B/B0 = 45.0
sample_pct <- 45.0
pred_conc <- ED(model_4pl, sample_pct, type = "absolute", display = FALSE)
cat(sprintf("Sample concentration: %.3f ug/L\n", pred_conc[1]))
```

9.2. Dilution Correction

If samples were diluted before analysis, multiply the interpolated concentration by the dilution factor:

$$C_{\text{original}} = C_{\text{interpolated}} \times DF$$

9.3. Reporting

Report results as μg/L (ppb) for water samples. Indicate whether the result is a screening (ELISA) value and whether it exceeds any applicable regulatory threshold. Flag any result that falls outside the linear range of the standard curve (below the lowest standard or above the highest standard) and re-run with appropriate dilution if above range.

10. Quality Control

10.1. Within-Assay QC

- Run all standards and samples in duplicate. The coefficient of variation (CV) between duplicates should be less than 15%. If a duplicate pair exceeds 15% CV, re-run those samples.
- Include the kit-supplied positive control on every plate. The control must fall within the range stated on the certificate of analysis.

- Include at least one reagent blank (deionized water) per plate.
- The zero standard (B_0) absorbance should typically be greater than 0.8 (consult kit insert for acceptable range). Low B_0 values indicate degraded antibody or conjugate.

10.2. Between-Assay QC

- Run a laboratory-prepared quality control sample (spiked DI water at a known concentration within the linear range) with each batch. Recovery should be 80–120%.
- Track control chart performance (mean and +/- 2 SD limits) over time.
- Record kit lot numbers and expiration dates. Do not mix reagents from different kit lots.

10.3. Method Detection Limits

The method detection limit (MDL) for each analyte is kit-specific and is defined as the concentration producing a $\%B/B_0$ response statistically distinguishable from the zero standard at the 95% confidence level. MDLs are typically provided in the kit insert. Report results below the MDL as "less than MDL."

10.4. Cross-Reactivity

Competitive ELISA antibodies may cross-react with structurally related compounds. For example, BPA antibodies may exhibit partial cross-reactivity with BPB, BPS, or BPF. Atrazine antibodies may cross-react with simazine, propazine, or other triazine herbicides. Consult the cross-reactivity table in the kit insert and report results accordingly (e.g., "total triazines" rather than "atrazine" if significant cross-reactivity is known) (Aga, 2013).

11. Troubleshooting

Problem	Possible Cause	Corrective Action
High background (all wells yellow)	Incomplete washing; contaminated wash buffer	Increase wash cycles; prepare fresh buffer
Poor standard curve ($r^2 < 0.95$)	Reagent degradation; pipetting error	Check kit expiration; verify pipette calibration
Sample absorbance above B_0	Matrix interference; very low analyte	Dilute and re-run; consider sample cleanup
Sample absorbance below lowest standard	Concentration exceeds range	Dilute sample and re-run
High CV between duplicates ($> 15\%$)	Bubbles in wells; inconsistent pipetting	Tap plate to remove bubbles; use multichannel pipette
Positive control out of range	Kit degradation; incorrect storage	Check storage conditions; use new kit
Edge effects (outer wells differ)	Temperature gradient across plate	Use plate shaker; avoid outer wells for critical samples

12. Waste Disposal

Waste Type	Disposal Method
Used microplate and wells	Standard trash (after stop solution is added)
Stop solution waste (acid)	Neutralize with $NaHCO_3$; drain with copious water
TMB substrate waste	Standard liquid waste; drain
Conjugate and antibody waste	Standard liquid waste; non-hazardous at kit dilutions
Sample waste	Drain with copious water (non-hazardous surface water)
Pipette tips	Standard trash

13. References

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14. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials Handling	Waste and chemical safety
L303	Hach Colorimetric Analysis	Complementary screening for nutrients/metals
L308	Microplastics in Water	Related contaminant screening
L309	Pesticide Analysis (GC-MS/LC-MS)	Confirmatory instrumental method
F300	Surface Water Sampling	Sample collection context

15. Training Record

Before performing ELISA analyses independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the SDS for the ELISA kit reagents (especially stop solution)
4. Review the kit insert for the specific analyte(s) to be analyzed
5. Observe a trained operator perform a complete assay (plate setup through data analysis)
6. Perform the assay under supervision with acceptable QC results (duplicate CV less than 15%; positive control within range; standard curve $r^2 > 0.98$)
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L308

Microplastics in Water – Extraction and Enumeration

Standard Operating Procedure

SOP Number	SOP-L308
Revision	1.1
Author(s)	Clare Flynn
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP provides methods for extracting, staining, and enumerating microplastics (particles less than 5 mm) from water samples. Microplastics are stained with Nile Red dye, vacuum-filtered onto glass-fiber filters, and counted under fluorescence using either a Crime-Lite forensic light source with goggles or the Echo Revolve microscope (Erni-Cassola et al., 2017; Maes et al., 2017).

This SOP is intended as an internal teaching and research protocol. Where EA Program data will be submitted to external programs or compared with regulatory monitoring datasets, researchers should consult the emerging standardized methods described in Appendix B and adapt procedures accordingly (California State Water Resources Control Board, 2022; Thornton Hampton et al., 2025).

2. Scope

- **Applies to:** Extraction and enumeration of microplastics from drinking water, surface water, stormwater, and other aqueous samples
- **Does not apply to:** Microplastics in soil, sediment, or biological tissue (see SCCWRP Technical Report #1410.A (Thornton Hampton et al., 2025)); chemical identification of polymer type (requires FTIR or Raman spectroscopy)
- **Regulatory alignment:** This SOP provides a Nile Red fluorescence screening method suitable for research and teaching. It does not replace the FTIR- or Raman-based methods required for California regulatory drinking water monitoring (California State Water Resources Control Board, 2022). Consult Appendix B and the PI before submitting data to SWAMP, CEDEN, or other regulatory programs.
- **Location:** EA Program laboratory, SGM 133 and SGM 134

3. Acknowledgements

This document builds on the work of Kyle Wees (PO ’20), Baile (PO ’22), and Jon Gunasti (PO ’20). Clare Flynn developed the first SOP, further modified by Marc Los Huertos and Sidarth (PO ’23).

4. Responsibilities

Role	Responsibility
Lab PI	Approve methods, review QC data
Trained Personnel	Prepare samples, perform extraction and enumeration
New Students	Complete training before independent use

5. Definitions

- **FTIR (Fourier Transform Infrared Spectroscopy):** An analytical technique that identifies polymer type by comparing a sample's infrared absorption spectrum to reference spectra; the gold standard for confirmatory microplastic characterization (California State Water Resources Control Board, 2022).
- **Microplastic (MP):** A synthetic polymer particle or fiber with at least one dimension between 1 µm and 5 mm; includes fragments, fibers, films, beads, and foam; classified as primary (manufactured at micro-scale) or secondary (degradation products of larger plastic items) (Maes et al., 2017).
- **Nanoplastic:** A plastic particle smaller than 1 µm; not quantifiable by standard visual methods; an emerging concern for ecological and human health research.
- **Nile Red:** A fluorescent dye that selectively stains hydrophobic plastic surfaces, enabling detection under blue-light excitation; used as a screening tool to distinguish plastic particles from organic debris (Erni-Cassola et al., 2017; Maes et al., 2017).
- **Procedural blank:** A sample of laboratory-grade water carried through the complete analytical procedure alongside field samples; used to quantify airborne and procedural contamination; required for every analytical batch.
- **Raman spectroscopy:** A spectroscopic technique that identifies molecular composition from inelastic light scattering; complementary to FTIR; particularly useful for particles smaller than 10 µm (California State Water Resources Control Board, 2022).
- **Visual sorting:** The initial enumeration step in which particles on a filter are examined under a stereomicroscope or the Revolve fluorescence microscope and candidate plastic particles are identified by color, shape, and texture before any confirmatory chemical analysis.

6. Safety and Hazards

⚠ Hazards

- **Chemical:** Acetone is flammable (flash point -20 °C) and potentially toxic by inhalation. All Nile Red solution preparation and sample staining must be performed in a fume hood. Review the SDS for acetone before use.
- **Optical:** The Crime-Lite 2BG2735 is a high-intensity 450–510 nm light source. Never shine it into anyone's eyes. Always wear the orange filter goggles when the Crime-Lite is on.

6.1. Required PPE

- ☐ Safety glasses
- ☐ Laboratory coat (cotton preferred – minimize synthetic fibers)
- ☐ Nitrile gloves
- ☐ Orange filter goggles (Crime-Lite enumeration only)

6.2. Emergency Procedures

For acetone spills, extinguish all ignition sources, ventilate the area, and clean up per SOP-L003 Hazardous Materials Handling. For skin or eye contact, flush with water for at least 15 minutes.

7. Required Materials

7.1. Equipment

Item	Location	Notes
Vacuum filtration apparatus	SGM 134	Glass funnel and fritted base
Vacuum pump	SGM 134	
Drying oven	SGM 134	Set to 60 °C for filter drying
Crime-Lite 2BG2735 (450–510 nm)	SGM 133	Store power supply and goggles together
Orange filter goggles	SGM 133	Must be used with Crime-Lite
Echo Labs Revolve microscope	SGM 133	See SOP-L211
Dissecting scope with 550 nm filter	SGM 133	Alternative to Revolve
Watch glasses	SGM 134	For filter drying in oven
Fume hood	SGM 134	Required for all Nile Red/acetone work

7.2. Reagents and Consumables

Item	Notes
Nile Red	*TBD catalog number*
Acetone (ACS grade)	Stored in flammables cabinet
Whatman 934-AH glass-fiber filters	42.5 mm, 1.5 µm pore
Glass sample bottles (500 mL)	Pre-cleaned; avoid plastic
Glass pipettes or syringes	For Nile Red injection

Glassware Decontamination

A standard protocol for removing residual micro- and nanoplastics from glassware before use has not yet been finalized. This is a critical gap that must be addressed before results can be

considered quantitative (see Appendix A).

8. Reagent Preparation

8.1. Nile Red Stock Solution

1. Working in a fume hood, dissolve Nile Red in acetone to 1 mg/mL.
2. Store in a dark amber glass bottle at room temperature.
3. Label with concentration, date, and analyst.

8.2. Working Concentration

The working concentration is 10 µg/mL, achieved by a 100:1 dilution of the stock. For a 500 mL water sample, add 0.5 mL of the 1 mg/mL stock solution (Maes et al., 2017).

9. Estimated Time

Task	Time
Nile Red staining and incubation	30 minutes
Vacuum filtration (per sample)	10–15 minutes
Filter drying (oven at 60 °C)	10 minutes
Enumeration (per sample, 3–10 fields)	30–60 minutes
Total per sample (excluding wait times)	Approximately 90–150 minutes

10. Procedure

10.1. Sample Preparation and Staining

1. Minimize synthetic fiber contamination: wear a cotton lab coat, keep samples covered, and work in a clean area.
2. Prepare samples in 500 mL volumes in glass bottles.
3. Working in a fume hood, inject the appropriate volume of Nile Red stock solution (e.g., 0.5 mL for a 500 mL sample) to achieve 10 µg/mL. Cap immediately (Maes et al., 2017).
4. Incubate with the injected dye for at least 30 minutes at room temperature.

10.2. Vacuum Filtration

5. Place a Whatman 934-AH glass-fiber filter (42.5 mm, 1.5 µm pore) on the fritted base of the vacuum filtration apparatus.
6. Vacuum-filter each sample through the filter.
7. Dry the filter on a watch glass in the oven at 60 °C for 10 minutes.

10.3. Enumeration Option A: Crime-Lite with Goggles

> **Laser Safety**
>
> Do not shine the Crime-Lite into anyone's eyes. Never observe illuminated samples without the orange filter goggles.

8. Place the dried filter on a dark, non-reflective surface.
9. Put on the orange filter goggles.
10. Illuminate the filter with the Crime-Lite (450–510 nm).
11. Count fluorescing particles through the goggles, or mount the filter under the dissecting scope with a 550 nm emission filter.
12. Record particle counts and morphology (fiber, fragment, bead, film).

10.4. Enumeration Option B: Echo Revolve Microscope (Fluorescence)

13. Mount the dried filter on a glass slide per SOP-L211.
14. Use the fluorescence channel (excitation ~470 nm, emission ~550 nm long-pass).
15. Photograph and count fluorescent particles in at least 3 randomly selected fields of view.
16. Import images into the Revolve app; use the annotation tools to mark and count particles.

11. Quality Control

- Include at least one procedural blank per analytical batch; blank particle counts inform the detection threshold for field samples (Erni-Cassola et al., 2017; Maes et al., 2017).
- Analyze at least 3 (preferably 10) randomly selected fields of view per filter; report mean, standard deviation, and coefficient of variation.
- Include at least one spiked filter (known number of plastic particles added before staining) per batch to verify Nile Red staining performance.

> **Contamination Controls Are Essential**
>
> Every batch must include procedural blanks processed identically to samples. Without blanks, microplastic counts cannot be meaningfully interpreted (Maes et al., 2017).

12. Troubleshooting

Problem	Probable Cause	Corrective Action
High blank counts	Airborne contamination; dirty glassware	Improve lab cleanliness; decontaminate glassware
No fluorescence on spiked samples	Nile Red concentration too low; degraded dye	Verify stock; prepare fresh solution

Problem	Probable Cause	Corrective Action
Fluorescence fades during counting	Photobleaching	Minimize illumination time; work quickly
Organic matter fluoresces	Nile Red binding to natural organics	Consider H_2O_2 digestion pre-treatment

13. Waste Disposal

Waste Type	Disposal Method
Acetone waste (Nile Red solution)	Collect in halogenated/organic waste; EH&S pickup
Used glass-fiber filters	Standard trash
Sample water (post-filtration)	Drain to sink

14. References

- Maes et al. (2017)
- Erni-Cassola et al. (2017)
- California State Water Resources Control Board (2022)
- Thornton Hampton et al. (2025)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

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15. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Acetone waste disposal
L211	Revolve Microscope	Fluorescence enumeration
L114	Vacuum Filtration	Filtration system operation

16. Training Record

Before performing microplastics analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 and SOP-L003 training
3. Review the SDS for acetone and Nile Red
4. Complete SOP-L211 Revolve Microscope training (if using the Revolve for enumeration)
5. Demonstrate safe Crime-Lite operation under supervision
6. Process at least one procedural blank and one spiked sample under supervision with acceptable results
7. Be signed off by the PI or lab manager

17. Appendix A: Open Questions and Development Needs

Item	Status	Notes
Glassware decontamination protocol	*TBD*	Critical for quantitative results
Organic matter interference assessment	*TBD*	Needed for high-organic samples
Polymer identity confirmation (FTIR access)	*TBD*	Lab does not currently have FTIR
Microplastic size class definition	*TBD*	Original SOP noted “DEFINE SIZE”
Nile Red and acetone catalog numbers	*TBD*	Add to materials table
Hydrogen peroxide digestion protocol	*TBD*	For removing organic matter before staining

18. Appendix B: Regulatory and Standards Context

In 2022, the California State Water Resources Control Board approved a policy requiring drinking water agencies to monitor microplastics, specifying FTIR and Raman spectroscopy as the two approved analytical methods (California State Water Resources Control Board, 2022). Standardized collection procedures for aquatic biota and sediment are described in SCCWRP Technical Report #1410.A (Thornton Hampton et al., 2025).

The Nile Red fluorescence screening method in this SOP is appropriate for teaching and exploratory research but does not meet California regulatory requirements (California State Water Resources Control Board, 2022). For data intended for submission to SWAMP or CEDEN:

1. Use microplastics-analysis-grade water for all blanks and rinses
2. Adopt SCCWRP contamination control procedures
3. Confirm polymer identity on a subset of particles by FTIR or Raman
4. Consult the PI before beginning any study intended for regulatory submission



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L309

Pesticide Analysis via Gas Chromatography

Standard Operating Procedure

SOP Number	SOP-L309
Revision	1.1
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP defines the protocols for extraction verification, instrument parameters, operation, and safety requirements for analyzing trace pesticide residues using Gas Chromatography (GC) coupled with specialized detectors (ECD or MS) (McNair et al., 2019; United States Environmental Protection Agency, 2007).

2. Scope

- **Applies to:** Targeted pesticide residue analysis in environmental water, soil extracts, and plant tissue digests using GC-ECD or GC-MS
- **Does not apply to:** LC-amenable pesticides, non-targeted screening, or preparative-scale separations
- **Location:** Environmental analysis laboratories

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Autosampler:** A robotic device that withdraws a precise volume from each GC vial and injects it into the inlet without manual intervention; reduces injection variability and allows unattended overnight sequences.
- **Capillary column:** A fused-silica column (typically 15–60 m × 0.18–0.32 mm ID) coated on its interior with a thin film of stationary phase; separates pesticides by their differential affinity for the stationary phase relative to the carrier gas (McNair et al., 2019).
- **Carrier gas:** An inert gas (helium, nitrogen, or hydrogen) that moves sample vapor through the column; flow rate and mode (constant pressure or constant flow) affect retention times and peak shape (McNair et al., 2019).
- **CCV (continuing calibration verification) standard:** A mid-range calibration standard analyzed every 10 injections to confirm that instrument response has not drifted; must recover within ±15% of the true value (United States Environmental Protection Agency, 2007).
- **Column bleed:** Gradual decomposition and volatilization of the GC stationary phase at high temperature; produces a rising baseline at the end of a temperature program; distinguished from analyte peaks by reproducible retention and broad peak shape.
- **ECD (electron capture detector):** A selective detector that measures current suppression caused by electron-capturing compounds (organochlorine pesticides, polychlorinated biphenyls); extremely sensitive (pg range) for halogenated compounds (United States Environmental Protection Agency, 2007).

- **GC-MS (gas chromatography-mass spectrometry):** Coupling of a GC with a mass spectrometer; provides compound identification by mass spectrum matching in addition to retention time; the confirmatory technique for pesticide analysis (United States Environmental Protection Agency, 2018).
- **Inlet liner:** A deactivated glass tube inside the split/splitless inlet; protects the column from non-volatile matrix residues; replaced daily or after every 50–100 injections to prevent sample carry-over and peak degradation.
- **MDL (method detection limit):** The minimum analyte concentration that can be detected with 99% confidence under the stated method conditions; determined from replicate analyses of low-level standards; listed per analyte in the method SOP.
- **RPD (relative percent difference):** |A - B| / ((A + B) / 2) × 100; used to assess duplicate precision; acceptance criterion is RPD 20% for environmental matrices (United States Environmental Protection Agency, 2007).
- **Septum:** A silicone or PTFE-faced silicone disc that seals the inlet and is penetrated by the autosampler needle; replaced daily or after 50–100 injections to prevent coring fragments from entering the liner.
- **Splitless injection mode:** An inlet mode in which the split vent is closed during injection to transfer nearly all of the sample onto the column; standard for trace pesticide analysis to maximize sensitivity (McNair et al., 2019).
- **Standby method:** A GC method programmed after sequence completion that drops oven, inlet, and detector temperatures while maintaining a reduced carrier gas flow; prevents column oxidation and extends stationary phase lifetime.

5. Safety and Hazards

⚠ Hazards

- **Chemical toxicity:** Pesticide standards, target analytes, and extraction solvents (hexane, dichloromethane, acetone) are highly toxic, carcinogenic, or neurotoxic. Inhalation of vapors or skin absorption poses a severe chronic health risk.
- **Thermal:** The GC injection port (200–250 °C) and column oven (50–320 °C) operate at extreme temperatures causing instant severe burns. Ensure the inlet is below 50 °C before opening it.
- **High-pressure gas cylinders:** Carrier gas cylinders (helium, nitrogen, hydrogen) pose crush and high-pressure hazards if dropped or if regulators fail. Hydrogen carrier gas presents an explosion risk if allowed to accumulate in the oven.

5.1. Required PPE

☐ Safety glasses or chemical splash goggles
☐ Laboratory coat (fully fastened)
☐ Nitrile or solvent-resistant gloves (Viton or Silver Shield for bulk chlorinated solvents)

5.2. Emergency Procedures

In case of chemical spill, gas leak, or equipment failure, refer to SOP-L002 Laboratory Safety or call campus safety. For compressed gas emergencies, evacuate the area and contact EH&S.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Gas chromatograph	With autosampler, split/splitless capillary inlet, and detector (ECD or MS)
Fused-silica capillary column	e.g., 30 m × 0.25 mm ID, 0.25 µm film, semi-volatile optimized (McNair et al., 2019)
Carrier gas cylinders	Helium or nitrogen (carrier); nitrogen (ECD makeup)
Inline gas traps	Moisture, oxygen, and hydrocarbon traps

6.2. Consumables

Item	Notes
GC vials (2 mL amber glass)	With PTFE-lined septa (McNair et al., 2019)
Replacement injection port liners	Deactivated split/splitless with quartz wool plug
Low-bleed septa	Replace daily or every 50–100 injections
Certified pesticide reference standards	Spanning expected concentration range (United States Environmental Protection Agency, 2007)
Extraction solvents	Hexane, dichloromethane, acetone (GC grade)

7. Procedure

7.1. Gas Cylinder and Inlet Verification

1. Verify carrier gas cylinders have sufficient pressure (> 500 psi).
2. Confirm secondary regulator delivery pressure meets manufacturer specifications (typically 60–80 psi).
3. Check moisture, oxygen, and hydrocarbon traps; replace if indicator dyes signal saturation.

7.2. Injection Port Maintenance (Pre-Run Check)

> **!** Hot Inlet
>
> Ensure the inlet temperature has dropped below 50 °C before opening the inlet assembly.

1. Replace the injection port septum daily or every 50–100 injections (McNair et al., 2019).
2. Inspect the glass liner; replace if charring, particulate matter, or matrix deposits are visible.

7.3. Sequence Setup and Sample Loading

1. Prepare calibration curves from certified pesticide reference materials spanning expected concentration intervals (e.g., 10–1000 µg/L) (United States Environmental Protection Agency, 2007).
2. Aliquot extracts into clean 2 mL amber GC vials; cap with PTFE-faced septa.
3. Program the injection sequence including:
   - Solvent blanks (hexane/acetone matrix check)
   - Multi-point calibration curve
   - CCV standards every 10 injections
   - Duplicate sample matrices

7.4. Initiating the GC Run

1. Load the analytical method and verify parameters (United States Environmental Protection Agency, 2007):
   - **Inlet:** Splitless mode; 250 °C
   - **Oven program:** 50 °C (1 min hold) → ramp 10 °C/min → 300 °C (5 min hold)
   - **Carrier gas:** Constant flow, 1.2 mL/min helium
2. Allow all zones to reach setpoint; confirm `Ready` status in software.
3. Initiate the sequence; monitor the first injection cycle to confirm the autosampler syringe articulates without bending the needle.

7.5. Post-Analysis and Standby Shutdown

1. Review chromatogram baselines for peak symmetry, column bleed spikes, or retention time shifts.

> **Retention Time Drift**
>
> If retention times shift, trimming 10–20 cm from the front of the analytical column can restore performance. Document the trim in the instrument logbook (McNair et al., 2019).

2. Upon sequence completion, load the Standby method to drop oven and detector temperatures while maintaining a reduced carrier gas purge to prevent column phase oxidation.

8. Quality Control and Acceptance Criteria

- Initial calibration: $r^2 \geq 0.995$ (United States Environmental Protection Agency, 2007).
- CCV standards every 10 injections: recovery within ±15%; halt and re-calibrate if outside this window (United States Environmental Protection Agency, 2007).
- Method blanks: no target analyte peaks above the MDL.
- Duplicate RPD: 20% (United States Environmental Protection Agency, 2007).

9. Waste Disposal

Waste Type	Container	Disposal Method
Spent solvents and rinses	Halogenated or non-halogenated jug (as appropriate)	EH&S pickup
Used septa, liners, vials	Solid chemical waste	Hazardous waste
Expired pesticide standards	Hazardous waste container	EH&S pickup

10. Maintenance

- **Daily:** Replace septum; inspect liner.
- **Weekly:** Check gas trap indicators; verify carrier gas pressure.
- **As needed:** Trim or replace the analytical column if baseline degradation or retention time shifts persist (McNair et al., 2019).
- **Annually:** Full instrument preventive maintenance per manufacturer schedule.

11. References

- United States Environmental Protection Agency (2007)
- United States Environmental Protection Agency (2018)
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12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L307	ELISA Pesticide Screening	Complementary screening method

13. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer



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Pomona College
Biogeochemistry Laboratory

SOP-L310

Chlorophyll *a* Extraction and Fluorometric Analysis

Standard Operating Procedure

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1. Purpose

This procedure describes the in vitro determination of chlorophyll *a* and pheophytin *a* concentrations in marine and freshwater phytoplankton by fluorescence. Chlorophyll-containing cells are concentrated on a glass fiber filter, extracted in 90% acetone, and measured on the Turner Designs Trilogy Fluorometer before and after acidification with 0.1 N HCl. The method is modified from US EPA Method 445.0 and APHA Standard Methods 10200 H (APHA et al., 2012; Arar & Collins, 1997).

2. Scope

- **Applies to:** Chlorophyll *a* and pheophytin *a* in surface water, freshwater, and marine water samples collected per SOP-F300
- **Does not apply to:** In vivo chlorophyll measurements with field multiprobes (see SOP-F200); benthic algae unless filtration volumes are adjusted; HPLC-based pigment analysis
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure standards are current
Lab Manager	Maintain fluorometer, stock reagents and filters
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Chlorophyll a:** The primary photosynthetic pigment in phytoplankton, algae, and higher plants; absorbs red light (~665 nm) and blue light (~430 nm); the standard proxy for phytoplankton biomass in aquatic systems.
- **Chlorophyll b:** An accessory pigment found in green algae and higher plants; absorbs at ~647 nm; distinguishable from chlorophyll a by spectrophotometry or fluorometry.
- **Fluorometric method:** Measurement of chlorophyll a by fluorescence emission (~685 nm) after excitation (~430 nm); more sensitive than spectrophotometry; requires acidification to distinguish chlorophyll a from pheophytin a.
- **Pheophytin a:** The magnesium-free degradation product of chlorophyll a; retains similar absorption properties; distinguished from chlorophyll a by the acidification step, which converts chlorophyll a to pheophytin a and causes a characteristic decrease in fluorescence.

- **Trichromatic equation:** Spectrophotometric calculation of chlorophyll a, b, and c concentrations in an extract from absorbance readings at three wavelengths (665, 647, 630 nm); corrects for spectral overlap among pigments.
- **Turbid extract correction:** Subtraction of non-specific absorbance (measured at 750 nm, where chlorophyll does not absorb) from all pigment wavelength readings to correct for turbidity and colored dissolved compounds in the extract.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Acetone (HPLC grade) is highly flammable and an irritant. Work in the fume hood and away from ignition sources. Concentrated HCl is severely corrosive. 0.1 N HCl is a mild irritant.
- **Physical:** Glass filtration apparatus and cuvettes pose breakage risk. Sonicator tip generates heat and noise.
- **Noise:** The sonicator requires hearing protection (ear plugs or ear muffs) whenever it is in use.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves
☐ Hearing protection (when using sonicator)

5.2. Emergency Procedures

For acetone spills, extinguish all ignition sources, ventilate the area, and clean up per SOP-L003 Hazardous Materials Handling. For HCl splashes, flush with water for at least 15 minutes. Dispose of all acetone waste in the designated organic waste collection bottle.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Turner Designs Trilogy Fluorometer (P/N 998-7210)	With Chlorophyll Acidification (Chlor-A) module; see SOP-L203
Millipore glass filtration unit	With vacuum pump and 47 mm fritted glass base
Sonicator	With probe tip; set to 70% amplitude

Item	Notes
Tissue grinder (alternative)	Teflon-tipped pestle with grooved surface, 30 mL glass grinding tube
Clinical benchtop centrifuge	Capable of 1,000 x g with fixed-angle rotor; see *SOP-L109*
Fume hood	Required for all acetone and HCl work

6.2. Consumables

Item	Notes
Whatman GF/F glass fiber filters (47 mm, 0.7 um)	For concentrating phytoplankton
Glass cuvettes (10 x 10 mm)	For fluorometer; see SOP-L203
15 mL glass or polypropylene centrifuge tubes	Screw-cap; for extraction
Plastic petri dishes	For storing filters before extraction
Aluminum foil	For wrapping samples to exclude light
Kimwipes	For blotting filters and cleaning cuvettes
Forceps (flat-tipped)	For handling filters
Pasteur pipettes or medicine droppers	For dispensing HCl
Class A volumetric pipettes	Assorted sizes
Class A volumetric flasks (50 mL, 100 mL, 1 L)	For standard preparation

6.3. Reagents and Standards

Item	Notes
Acetone, HPLC grade (CAS 67-64-1)	Stored in flammables cabinet
Concentrated HCl (sp. gr. 1.19; CAS 7647-01-0)	Stored in acid cabinet
Chlorophyll *a* standard (free of chlorophyll *b*)	Commercial source (e.g., Sigma); stored at –20 C
Milli-Q water (Type I)	From *SOP-L11*

7. Procedure

7.1. Reagent Preparation

0.1 N HCl: Add 0.85 mL of concentrated HCl to approximately 50 mL of Milli-Q water in a 100 mL volumetric flask. Bring to volume with Milli-Q water. Mix thoroughly.

90% Aqueous Acetone: Measure 100 mL of Milli-Q water into a 100 mL volumetric flask, then transfer to a 1 L volumetric flask. Fill to the 1 L mark with HPLC-grade acetone. Mix and transfer to a dark amber bottle. Label and store in the flammables cabinet.

Chlorophyll *a* Stock Standard Solution (SSS): Working in subdued light, transfer the contents of the chlorophyll *a* ampoule into a 50 mL volumetric flask and dilute to volume with 90% acetone. Determine the concentration spectrophotometrically. Wrap in foil and label with the lot number. Stable for at least six months in an airtight container at room temperature.

Primary Dilution Standard (PDS): Add 1 mL of SSS to a 100 mL volumetric flask and dilute to volume with 90% acetone. If the SSS was prepared from exactly 1 mg of pure chlorophyll *a*, the PDS concentration is 200 ug/L. Prepare fresh before use. Wrap in foil.

Quality Control Standards (QCs): Prepare from the PDS at the following concentrations: 5, 20, 50, and 200 ug/L. Wrap in foil.

> **!** Important
>
> All photosynthetic pigments are light- and temperature-sensitive. Perform all work in subdued light. Store all standards, QC materials, and filter samples in the dark at –20 C when not in use.

7.2. Filtration

1. Invert the sample bottle gently several times to suspend particles
2. Assemble the glass filtration unit: place the fritted glass base on the Erlenmeyer flask, position a GF/F filter on the frit using forceps, seat the glass funnel on top, and secure with the spring clamp
3. Connect the vacuum hose and turn on the vacuum
4. Working in subdued light, filter the sample in 20 mL increments using a graduated cylinder. The volume required depends on algal density:

Sample Type	Typical Volume
Benthic samples	approximately 5 mL
Freshwater	20–300 mL
Seawater	100–1000+ mL

5. Stop filtering when a brown or green tint is visible on the filter. Record the exact filtered volume. If the filter becomes too darkly colored, the sample may exceed the linear dynamic range and require dilution during analysis.
6. Using forceps and a micro spatula, fold the filter in half with the retained material on the inside
7. Gently blot excess water from the outside of the filter with a Kimwipe
8. Place the folded filter in a clean 15 mL centrifuge tube or a plastic petri dish
9. Include a laboratory reagent blank (100 mL Milli-Q water through a GF/F filter) and a field duplicate every 10–15 samples
10. Wrap the tubes or dishes in aluminum foil and store at –20 C. Filters may be stored frozen for up to 28 days before extraction.

7.3. Extraction – Sonication Method

1. Remove samples and QCs from the freezer and allow to reach room temperature
2. Add 25 mL of 90% aqueous acetone to each tube, ensuring the filter is fully submerged. Cap the tubes and invert several times.
3. Secure the sonicator probe in its clamp stand
4. **Put on hearing protection**
5. Set the sonicator to 70% amplitude
6. Submerge the tip halfway into the acetone solution and sonicate, moving the tip around the sample to break up the filter completely
7. After sonication, shake the tube vigorously, wrap in foil, and steep in the refrigerator (4 C) for a minimum of 2 hours

> **!** Important
>
> Never operate the sonicator outside of solution. The tip will overheat and be damaged.

7.4. Extraction – Tissue Grinder Method (Alternative)

1. Using forceps, transfer the filter into a 30 mL glass grinding tube and push it to the bottom with the pestle or a glass rod
2. Add 4 mL of 90% acetone and grind with the pestle until the filter is converted to a slurry. Avoid overheating.
3. Pour the slurry into a 15 mL screw-cap centrifuge tube
4. Rinse the pestle and grinding tube with 6 mL of 90% acetone and add the rinse to the centrifuge tube
5. Cap, shake vigorously, wrap in foil, and steep at 4 C for 2 hours
6. Clean the grinder and pestle with water followed by acetone between samples; wipe dry with a Kimwipe

7.5. Post-Extraction Processing

1. Remove samples from the refrigerator and allow to reach room temperature (approximately 30 minutes)
2. Shake each tube vigorously
3. Centrifuge at 1,000 x g for 5 minutes using the fixed-angle rotor until all filter material is pelleted
4. Handle tubes carefully after centrifugation; do not resuspend the pellet

7.6. Fluorometric Analysis

1. Transfer 2.5 mL of clear supernatant from each tube into a clean glass cuvette, taking care not to disturb the pellet
2. Turn on the Trilogy Fluorometer and allow the 10-minute warm-up to complete (see SOP-L203)

3. Select the **Chlor-A** (Chlorophyll Acidification) module on the touch screen
4. Calibrate using the QCs prepared from the PDS, or load a saved calibration. For a new calibration, follow the Direct Multipoint Calibration procedure in SOP-L203, Section 7.3.
5. Run a 90% acetone blank
6. Wipe the outside of each cuvette with a Kimwipe before inserting into the fluorometer
7. Measure and record the pre-acidification fluorescence (F_b)

> **Note**
>
> If the chlorophyll *a* reading exceeds 90% of the upper limit of the linear dynamic range (LDR = 1,000 ug/L), dilute the sample before proceeding. For a 1:2 dilution, add 1 mL of sample to 1 mL of 90% acetone. For a 1:100 dilution, add 50 uL of sample to 4.95 mL of 90% acetone.

8. Add 75 uL of 0.1 N HCl to the cuvette. Seal with parafilm and invert several times to mix. Wait 90 seconds.
9. Measure and record the post-acidification fluorescence (F_a)
10. Repeat for all samples, QCs, and blanks

8. Quality Control and Acceptance Criteria

8.1. Calculations

Variables:

- $[Std]$ = concentration of the calibration standard used (ug/L)
- S_b = standard fluorescence reading before acidification
- S_a = standard fluorescence reading after acidification
- F_b = sample fluorescence before acidification
- F_a = sample fluorescence after acidification
- V_e = volume of extract (mL)
- V_s = volume of sample filtered (mL)

Equations:

$$AR = \frac{S_b - \text{Blank}}{S_a - \text{Blank}}$$

$$\text{InterpB} = [Std] \times \frac{F_b - \text{Blank}}{S_b - \text{Blank}}$$

$$\text{InterpA} = [Std] \times \frac{F_a - \text{Blank}}{S_b - \text{Blank}}$$

$$\text{Chlorophyll } a \text{ } (\mu\text{g/L}) = \frac{AR}{AR - 1} \times (\text{InterpB} - \text{InterpA}) \times \frac{V_e}{V_s}$$

$$\text{Pheophytin } a \text{ } (\mu\text{g/L}) = (AR \times \text{InterpA} - \text{InterpB}) \times \frac{V_e}{V_s}$$

8.2. QC Requirements

- Run a laboratory reagent blank (LRB) every 10–15 samples. The LRB should read below the calculated lower detection limit.
- QC standards should recover within +/- 20% of the known value
- Relative standard deviation (RSD) of replicate measurements should be within +/- 20%
- Field duplicates should agree within +/- 20% relative percent difference
- Samples, standards, LRBs, and QCs must all be at ambient room temperature during analysis. Fluorescence is temperature-dependent; cooler samples produce artificially high readings.

9. Waste Disposal

Waste Type	Disposal Method
Acetone extracts and rinses	Collect in the designated organic waste bottle in the fume hood; EH&S pickup
Acidified sample waste (HCl)	Add to the organic waste bottle (small volumes of dilute HCl in acetone matrix)
Used GF/F filters	Standard trash (if non-hazardous samples)
Used cuvettes	Rinse with acetone, then DI water; reuse or recycle

10. References

- USEPA. (1997). Method 445.0: *In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence*. Revision 1.2. National Exposure Research Laboratory, Cincinnati, OH.
- APHA, AWWA, WEF. (2012). Standard Methods 10200 H: Chlorophyll. In: *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.
- Turner Designs. *Trilogy Laboratory Fluorometer: User's Manual*.

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll textita and pheophytin textita in marine and freshwater algae by fluorescence*. U.S. Environmental Protection Agency, Office of Research; Development, National Exposure Research Laboratory. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309417

11. Training Record

Before performing chlorophyll analysis independently, personnel must:

1. Read this SOP, SOP-L203 Trilogy Fluorometer, and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for acetone and HCl
3. Demonstrate proper filtration technique under supervision
4. Perform at least one complete extraction-through-analysis cycle on QC standards with acceptable recovery (+/- 20%)
5. Demonstrate proper fluorometer calibration
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation

```
# Corrected chlorophyll a and pheophytin a calculation
# Per EPA Method 445.0

# Calibration standard
Std_conc <- 50       # ug/L (QC standard concentration)
S_b <- 485           # Standard fluorescence before acid
S_a <- 210           # Standard fluorescence after acid
blank <- 5           # Blank fluorescence

# Sample readings
F_b <- 320           # Sample fluorescence before acid
F_a <- 140           # Sample fluorescence after acid

# Volumes
V_e <- 25            # mL of extract (acetone)
V_s <- 100           # mL of sample filtered

# Calculations
AR <- (S_b - blank) / (S_a - blank)
InterpB <- Std_conc * (F_b - blank) / (S_b - blank)
InterpA <- Std_conc * (F_a - blank) / (S_b - blank)

chl_a <- (AR / (AR - 1)) * (InterpB - InterpA) * (V_e / V_s)
pheo_a <- (AR * InterpA - InterpB) * (V_e / V_s)

cat(sprintf("Acid ratio (AR):        %.3f\n", AR))
cat(sprintf("Chlorophyll a:          %.2f ug/L\n", chl_a))
cat(sprintf("Pheophytin a:           %.2f ug/L\n", pheo_a))
```

13. Appendix B: Interferences

Interference	Effect	Mitigation
Chlorophyll *b*	Overestimates chlorophyll *a*	Optical filters in the Chlor-A module minimize this; if severe, use HPLC
Pheophytin *a*	Overestimates chlorophyll *a* if not acidified	Acidification step corrects for this
Other fluorescent compounds	Positive bias	Laboratory reagent blanks detect this
Quenching (concentrated samples)	Underestimates concentration	Dilute samples approaching 90% of LDR
Temperature differences	Higher sensitivity at lower temperatures	Ensure all materials are at ambient temperature
Light exposure	Degrades pigments	Work in subdued light; wrap in foil



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L311

Dissolved Organic Carbon in Water – Shimadzu TOC-L (NPOC Mode)

Standard Operating Procedure

SOP Number	SOP-L311
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1. Purpose

This procedure describes the determination of dissolved organic carbon (DOC) in natural water samples (surface water, groundwater, stormwater, treated effluent) by the non-purgeable organic carbon (NPOC) method on the Shimadzu TOC-L analyzer. DOC is a fundamental water quality parameter that influences aquatic ecosystem productivity, light penetration, metal complexation, disinfection byproduct formation, and nutrient cycling (Thurman, 1985; Wetzel, 2001).

The NPOC method acidifies the sample and sparges with carrier gas to remove inorganic carbon (CO_2, HCO_3^-, CO_3^{2-}) before combustion. The remaining carbon is measured as NPOC, which for most natural waters is operationally equivalent to DOC when the sample has been filtered through 0.45 µm (APHA et al., 2012, Section 5310B).

For instrument operation, startup, and shutdown, see SOP-L204 Shimadzu TOC-L. For water-extractable DOC from soils, see SOP-L418.

2. Scope

- **Applies to:** Filtered (0.45 µm) water samples from surface water, groundwater, stormwater, effluent, and precipitation, analyzed for DOC in NPOC mode on the Shimadzu TOC-L
- **Does not apply to:** Total organic carbon in unfiltered water (use TOC mode on the TOC-L); particulate organic carbon (requires separate filtration and combustion); soil carbon by SSM (see SOP-L407); soil extract DOC (see SOP-L418); microbial biomass carbon (see SOP-L409)
- **Location:** EA Program analytical laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Instrument Manager	Maintain the TOC-L per SOP-L204
Trained Personnel	Prepare samples and standards, run analysis, export data
New Students	Complete training before independent use

4. Definitions

- **Calibration blank:** Deionized water (18.2 MΩ · cm) of verified DOC less than 0.05 mg C/L; used as the zero point for calibration and as the system blank to verify background carbon from the instrument and reagents.

- **CCV (continuing calibration verification):** A mid-range KHP standard analyzed at regular intervals during an analytical run to confirm calibration stability; acceptance criterion is 90–110% recovery; if outside this range, re-calibrate and re-run affected samples (APHA et al., 2012).
- **Chromophoric dissolved organic matter (CDOM):** The fraction of DOC that absorbs UV/visible light; derived primarily from aromatic humic and fulvic acids; a surrogate indicator of DOC concentration in optically characterized waters (Thurman, 1985).
- **Dissolved organic carbon (DOC):** Organic carbon that passes through a 0.45 µm filter; in natural waters, DOC includes humic substances, fulvic acids, amino acids, carbohydrates, and other soluble organic compounds; the operationally defined quantity measured as NPOC by the Shimadzu TOC-L (APHA et al., 2012; Thurman, 1985).
- **Field blank:** A sample of deionized water processed through the field collection equipment (bottles, filters, cooler) and returned to the laboratory for analysis; used to detect contamination introduced during sample collection and transport (Sharp, 1993).
- **Humic substances:** High-molecular-weight, polydisperse aromatic compounds derived from the decomposition of plant and microbial material; typically comprise 40–80% of DOC in natural fresh waters (Thurman, 1985).
- **IC (inorganic carbon):** Carbon present as dissolved CO_2, bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}); removed from water samples by acidification and sparging before NPOC measurement.
- **KHP (potassium hydrogen phthalate, $KC_8H_5O_4$):** The primary calibration standard for TOC analysis; a pure, non-hygroscopic organic acid weighed accurately to prepare standards of known carbon concentration; 1.0000 g KHP = 0.4711 g C.
- **Method blank:** Deionized water acidified and processed identically to field samples, including any filtration and preservation steps; used to detect contamination from reagents and equipment; must read less than 0.2 mg C/L (APHA et al., 2012).
- **NPOC (non-purgeable organic carbon):** The organic carbon remaining in a sample after acidification (typically with HCl or H_3PO_4) and sparging with carrier gas to remove IC and volatile organic carbon (VOC); the quantity reported as DOC by the Shimadzu TOC-L (APHA et al., 2012, Section 5310B).
- **Particulate organic carbon (POC):** Organic carbon retained on a 0.45 µm filter; not measured by this SOP; requires separate filter collection and combustion analysis.
- **Pre-combusted glass vial:** A TOC autosampler vial heated to 450 °C for at least 4 hours to destroy residual organic carbon from the glass or manufacturing process; used to prevent contamination of samples from the container.
- **PVDF (polyvinylidene fluoride) filter:** A 0.45 µm syringe filter with low organic carbon leaching; preferred for DOC sample filtration; cellulose acetate filters must not be used because they leach DOC (Kaplan, 1994).
- **TOC (total organic carbon):** The sum of DOC and POC; in this SOP, only DOC (the filtered, NPOC fraction) is measured.

5. Safety and Hazards

All safety requirements from SOP-L204 Section 4 apply.

Additional hazards specific to this method:

> ⚠ Hazards
>
> - **Chemical:** Hydrochloric acid (2 M HCl) is used for sample acidification and preservation. Avoid skin and eye contact.
> - **Glassware:** Pre-combusted glass vials are fragile. Handle with care to avoid cuts.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

6. Required Materials

6.1. Equipment

Item	Notes
Shimadzu TOC-L with ASI-L autosampler	Operated per SOP-L204
TNM-L (optional)	If simultaneous TN analysis is desired
Vacuum filtration apparatus	Per SOP-L114
Analytical balance (0.001 g)	Per SOP-L200

6.2. Consumables

Item	Notes
Pre-combusted glass vials (40 mL, PTFE-lined caps)	Combust at 450 °C for 4 hours per SOP-L111
Autosampler vials (8 mL or 40 mL)	Pre-combusted or acid-washed
0.45 µm filters (PVDF or PES)	Do NOT use cellulose acetate (Kaplan, 1994)
Syringe filters (0.45 µm, 25 mm, PVDF)	Discard first 2–3 mL of filtrate

6.3. Reagents

Reagent	Notes
KHP stock standard (1000 mg C/L)	2.125 g KHP per 1 L DI water; prepare fresh monthly; store at 4 °C (APHA et al., 2012)
2 M HCl (Trace Metal grade)	For sample acidification and preservation

Reagent	Notes
Deionized water (18.2 MΩ · cm)	Verify DOC less than 0.05 mg/L before each session

> **!** Important
>
> Prepare the 1000 mg C/L KHP stock fresh monthly or verify against a certified reference material (CRM) before use. Degraded stock causes a systematic low bias across the calibration.

7. Sample Collection and Preservation

Proper sample handling is critical; contamination from fingers, plastic containers, and atmospheric CO_2 can bias results (Sharp, 1993).

- Collect samples in pre-combusted glass bottles. Do not use plastic bottles for DOC samples.
- Filter through 0.45 µm PVDF syringe filters within 4 hours of collection; discard the first 2–3 mL of filtrate.
- Acidify filtered samples to pH 2 with 2 M HCl (approximately 100 µL per 40 mL sample) to suppress microbial activity.
- Store acidified samples at 4 °C in the dark; analyze within 28 days (APHA et al., 2012).
- Cap vials immediately after filling to minimize atmospheric CO_2 contamination.

8. Procedure

8.1. Calibration

Prepare a 5-point calibration curve in the expected sample concentration range (APHA et al., 2012):

Level	Concentration (mg C/L)	Preparation
0	0.00	Acidified DI water blank
1	0.50	Dilute from 1000 mg/L KHP
2	1.00	Dilute from 1000 mg/L KHP
3	5.00	Dilute from 1000 mg/L KHP
4	10.00	Dilute from 1000 mg/L KHP
5	25.00	Dilute from 1000 mg/L KHP

Adjust the range to match expected sample concentrations. Load standards into pre-combusted autosampler vials and enter into the Sample Table in LabSolutions TOC.

8.2. Setting Up the Sample Table

1. Open LabSolutions TOC and start a new analysis.
2. Enter calibration standards (type = Standard), blanks (type = Blank), CCV standards, and samples.
3. Set the measurement mode to **NPOC** for all samples and blanks.
4. Set the number of injections per sample to 3; the software reports the mean and coefficient of variation (CV).
5. Enter dilution factors for any samples that have been diluted before the run.

8.3. Running the Analysis

1. Load all vials into the autosampler; verify rack positions match the Sample Table.
2. Confirm the ASI-L rinse reservoir is full.
3. Click **Start** and monitor the first few injections for normal peak shape and baseline stability.
4. The run proceeds unattended; the autosampler advances automatically.

8.4. Data Review and Export

1. After the run, open the Data Browser.
2. Check each result:
   - Replicate injection CV must be less than 2%.
   - Blank values must be less than 0.1 mg C/L.
   - CCV recovery must be 90–110%; re-calibrate and re-run affected samples if outside this range.
3. Dilute and re-run any sample exceeding the calibration range.
4. Export results to CSV per SOP-L204 Section 7.9.
5. Record the run in the instrument logbook.

9. Calculations

The TOC-L software reports NPOC concentration directly in mg C/L, corrected for dilution factor if entered in the Sample Table. Report as DOC:

$$\text{DOC (mg C/L)} = \text{NPOC}_{\text{reported}} - \text{NPOC}_{\text{method blank}}$$

If samples were diluted before analysis, apply the dilution factor:

$$\text{DOC (mg C/L)} = (\text{NPOC}_{\text{reported}} - \text{NPOC}_{\text{blank}}) \times D$$

where D is the dilution factor.

9.1. R Code for Batch Processing

```
# SOP-L311: DOC (NPOC) -- batch calculation from TOC-L CSV export
raw <- read.csv("TOC-L_export.csv", stringsAsFactors = FALSE)

blanks <- raw[grepl("^Blank|^DI|^MQ", raw$SampleID, ignore.case = TRUE), ]
ccv    <- raw[grepl("^CCV|^CVS|^Check", raw$SampleID, ignore.case = TRUE), ]

mean_blank <- mean(blanks$NPOC_mg_L, na.rm = TRUE)
cat(sprintf("Mean method blank: %.3f mg C/L\n", mean_blank))

samples <- raw[!grepl("^Blank|^DI|^MQ|^CCV|^CVS|^Check|^Std",
                      raw$SampleID, ignore.case = TRUE), ]
samples$DOC_mg_L <- (samples$NPOC_mg_L - mean_blank) * samples$Dilution
samples$DOC_mg_L[samples$DOC_mg_L < 0] <- 0
samples$QC_flag <- ifelse(samples$CV_pct > 2, "CV>2%", "")

print(samples[, c("SampleID", "NPOC_mg_L", "Dilution", "DOC_mg_L", "QC_flag")],
      row.names = FALSE)
```

10. Quality Control

10.1. Instrument QC

Per SOP-L204:

- System blank less than 0.1 mg C/L
- Calibration $r^2 \geq 0.999$
- Replicate injection CV less than 2%
- CCV recovery 90–110%

10.2. Method QC

QC Element	Frequency	Acceptance Criteria
Method blank	1 per batch	Less than 0.2 mg C/L
Field blank	1 per sampling event	Less than 0.5 mg C/L
Laboratory duplicate	1 per 10 samples	RPD less than 10%
CRM (when available)	1 per batch	Recovery 90–110%
Matrix spike	1 per 20 samples	Recovery 80–120%

10.3. Common Contamination Sources

Source	Prevention
Fingers touching vial interiors	Always wear gloves; handle by exterior only
Cellulose acetate filters	Use PVDF or PES filters exclusively (Kaplan, 1994)
Detergent residue on glassware	Pre-combust glass; acid-wash and rinse with DI
Atmospheric CO_2 absorption	Minimize headspace; cap immediately
Degraded DI water	Verify DI DOC less than 0.05 mg/L before each session
Old or degraded KHP stock	Prepare fresh monthly; refrigerate

11. Waste Disposal

Waste Type	Disposal Method
Acidified sample waste	Neutralize and drain, or collect per SOP-L006
Calibration standard waste	Drain (dilute KHP, non-hazardous)
Used syringe filters	Standard trash
Pre-combusted glass vials	Rinse and reuse, or glass recycling

12. References

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

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13. Related SOPs

SOP	Title	Relationship
L204	Shimadzu TOC-L Instrument	Instrument operation, startup, shutdown
L020	Reagent Preparation	KHP stock and standard preparation
L111	Muffle Furnace	Pre-combustion of glass vials
L114	Vacuum Filtration	Sample filtration
L407	Total Carbon (Soils)	SSM solid carbon companion
L409	Microbial Biomass Carbon	CFE extract DOC companion
L418	Water-Extractable DOC	Soil extract DOC companion
L802	Quality Control	QC requirements

SOP	Title	Relationship
F300	Surface Water Sampling	Sample collection and preservation

14. Training Record

Before performing this analysis independently, personnel must:

1. Read this SOP in full
2. Complete instrument training per SOP-L204 Section 14
3. Demonstrate competence preparing KHP calibration standards
4. Demonstrate proper sample filtration technique (PVDF filter, first-rinse discard)
5. Run a complete batch (calibration, blanks, CCV, samples) under supervision with acceptable QC results
6. Be signed off by the PI or instrument manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L312

Total Suspended Solids (TSS) and Total Dissolved Solids (TDS)

Standard Operating Procedure

SOP Number	SOP-L312
Revision	1.1
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the gravimetric determination of Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) in environmental water samples (e.g., surface water, groundwater, runoff). TSS represents the portion of solids retained by a glass fiber filter, while TDS represents the portion that passes through the filter and is dried to constant weight in a vessel (APHA et al., 2012).

2. Scope

- **Applies to:** Environmental water samples analyzed for TSS and TDS by gravimetric methods.
- **Does not apply to:** Settleable solids, volatile suspended solids, or non-aqueous matrices.
- **Location:** Environmental analysis laboratories.

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Constant mass:** Successive weighings (after additional drying cycles of at least 1 hour) agree within ±0.5 mg; the criterion confirming complete desiccation.
- **Detection limit (MDL):** The minimum concentration that can be reliably distinguished from a blank; for gravimetric TSS/TDS, the practical lower limit is approximately 5 mg/L, set by the precision of the analytical balance and filter tare variability (APHA et al., 2012).
- **Fixed residue:** The fraction of total solids remaining after ignition at 550 °C; equivalent to inorganic (mineral) solids.
- **Relative percent difference (RPD):** A measure of duplicate precision: RPD (%) = |A - B| / ((A + B) / 2) × 100; acceptance criterion for TSS/TDS duplicates is 10%.
- **Total dissolved solids (TDS):** The mass of material passing through a 0.45 µm filter per unit volume of sample, determined gravimetrically after evaporation at 180 °C; expressed in mg/L (APHA et al., 2012, Method 2540 C).
- **Total solids (TS):** The residue remaining after evaporation of a sample at 103–105 °C; TS = TSS + TDS.
- **Total suspended solids (TSS):** The mass of material retained on a pre-weighed glass fiber filter per unit volume of sample; expressed in mg/L (APHA et al., 2012, Method 2540 D).
- **Volatile suspended solids (VSS):** The fraction of TSS lost on ignition at 550 °C; approximates the organic content of suspended solids (APHA et al., 2012).

5. Safety and Hazards

⚠ Hazards

- **Thermal:** Drying ovens (103–180 °C) and muffle furnaces can cause severe thermal burns. Always use heat-resistant crucible tongs and thermal gloves when handling hot apparatus.
- **Vacuum/Implosion:** Vacuum filtration assemblies operate under reduced pressure. Ensure filtration flasks are borosilicate glass and free of visible cracks to eliminate implosion risks.

5.1. Required PPE

☐ Safety glasses
☐ Laboratory coat
☐ Nitrile gloves
☐ Heat-resistant thermal gloves (when handling hot dishes, filters, or oven contents)

5.2. Emergency Procedures

In case of burn or equipment failure, refer to SOP-L002 Laboratory Safety or call campus safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Vacuum filtration apparatus	Buchner funnel, flask, and vacuum pump
Analytical balance	Capable of weighing to ±0.1 mg
Drying oven	Regulated at 103–105 °C (TSS) and 180 ±2 °C (TDS)
Desiccator	With active color-indicating silica gel
Blunt forceps	For filter handling
Graduated cylinder	For measuring sample volume

6.2. Consumables

Item	Notes
Glass microfiber filter disks	e.g., Whatman 934-AH, 1.5 µm nominal pore size (APHA et al., 2012)
Evaporating dishes	Porcelain or high-silica glass
Aluminum weigh pans	For filter drying

Item	Notes
Deionized (DI) water	For filter washing and volume adjustment

7. Procedure

7.1. Filter and Dish Preparation

1. **TSS filter prep:** Place a glass fiber filter on the filtration apparatus. Apply vacuum and wash with three successive 20 mL volumes of DI water. Continue vacuum until all trace water is removed. Transfer the filter to an aluminum weigh pan and dry in an oven at 103–105 °C for 1 hour. Cool in a desiccator to room temperature and weigh to determine the tare mass (B_{TSS}) (APHA et al., 2012).
2. **TDS dish prep:** Clean an evaporating dish and heat in an oven at 180 ±2 °C for 1 hour. Cool completely in a desiccator and record the stable tare mass (B_{TDS}).

7.2. Filtration and Sample Analysis

1. Assemble the pre-weighed filter pad into the filtration funnel and wet with a small volume of DI water to seal it against the porous plate. Turn on the vacuum.
2. Shake the sample bottle vigorously to achieve homogeneous particle distribution. Measure a known sample volume (V, typically 100 mL to 500 mL depending on turbidity) using a graduated cylinder.
3. Pour the measured sample into the filter funnel. Wash the cylinder and funnel walls with three successive 10 mL aliquots of DI water, allowing total drainage between washings.
4. **For TSS:** Carefully remove the filter pad from the funnel using blunt forceps. Place it back in its aluminum pan and dry in the oven at 103–105 °C for at least 1 hour. Cool in the desiccator and weigh to constant mass (A_{TSS}).
5. **For TDS:** Transfer the entire filtrate volume from the filter flask quantitatively into the pre-weighed evaporating dish. Place the dish into the drying oven at 180 ±2 °C until all liquid evaporates completely. Dry for at least an additional hour, cool inside the desiccator, and weigh to find the final mass (A_{TDS}).

> **Constant Mass**
>
> "Constant mass" means that successive weighings (after additional drying cycles of at least 1 hour) agree within ±0.5 mg (APHA et al., 2012).

7.3. Shutdown and Cleanup

1. Discard used filters in solid waste.

2. Rinse filtration apparatus thoroughly with DI water.
3. Return evaporating dishes and tongs to storage after cooling.
4. Turn off the vacuum pump and drying oven if no other users need them.

8. Calculations

$$\text{TSS (mg/L)} = \frac{(A_{\text{TSS}} - B_{\text{TSS}}) \times 1{,}000{,}000}{V \text{ (mL)}}$$

$$\text{TDS (mg/L)} = \frac{(A_{\text{TDS}} - B_{\text{TDS}}) \times 1{,}000{,}000}{V \text{ (mL)}}$$

Where:

- A = weight of dry filter or dish plus residue (g)
- B = tare weight of clean filter or dish (g)
- V = sample volume (mL)

If the calculated TSS residue mass is less than 2.5 mg, increase the sample volume and repeat (APHA et al., 2012).

9. Quality Control and Acceptance Criteria

- Run a method blank (DI water through the full procedure) with each batch. The blank result must be less than the method detection limit (APHA et al., 2012).
- Duplicate samples should agree within ±10% RPD.
- If the measured residue mass is less than 2.5 mg, use a larger sample volume.

10. Waste Disposal

Waste Type	Container	Disposal Method
Used glass fiber filters	Solid waste bin	Standard solid waste
Filtered water samples	Drain	If no hazardous analytes

11. References

- APHA et al. (2012)
- SOP-L002 Laboratory Safety
- SOP-L114 Vacuum Filtration

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Emergency procedures
L114	Vacuum Filtration	Filtration system operation

13. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L313

Metals in Water (ICP-MS Prep and Acidification)

Standard Operating Procedure

SOP Number	SOP-L313
Revision	1.1
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the acidification, trace metal preservation, and digestion protocols for aqueous environmental samples prior to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis. It covers dissolved metals (filtration + acidification) and total recoverable metals (acid digestion) (Creed et al., 1994).

2. Scope

- **Applies to:** Environmental water samples (surface water, groundwater, runoff, wastewater) requiring dissolved or total recoverable metals analysis by ICP-MS.
- **Does not apply to:** Solid matrices (soils, sediments, tissues), total metals by XRF, or organic analyte preparation.
- **Location:** Environmental analysis laboratories.

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Acid digestion (water samples):** The addition of concentrated HNO_3 (typically 0.5–2% v/v final) to preserved water samples to dissolve any particulate metal and stabilize dissolved metals; a prerequisite for ICP-MS analysis (Creed et al., 1994).
- **Blank (method blank):** A reagent water sample carried through the entire preparation procedure; used to assess and correct for metal contamination introduced during sample processing (APHA et al., 2012).
- **Certified reference material (CRM):** A water sample with certified metal concentrations from an accredited producer (e.g., NIST, USGS); used to verify ICP-MS accuracy (Beauchemin, 2017).
- **Digestion blank:** A solution containing only the acid reagents (no sample) processed through the digestion procedure; detects contamination from reagents and vessels.
- **ICP-MS (Inductively Coupled Plasma Mass Spectrometry):** An analytical technique that vaporizes and ionizes a sample in an argon plasma and separates ions by mass-to-charge ratio to identify and quantify elements at parts-per-trillion concentrations (Beauchemin, 2017).
- **Method detection limit (MDL):** The minimum concentration of an analyte that can be detected with 99% confidence that the true concentration is greater than zero; determined from replicate blank analyses per 40 CFR Part 136 (Creed et al., 1994).
- **Preservation (acidification):** Addition of HNO_3 to a water sample at the time of collection to prevent metal adsorption to container walls and to maintain dissolved metals in solution; required for regulatory compliance samples (Creed et al., 1994).
- **Relative percent difference (RPD):** $|A - B| / ((A + B) / 2) \times 100$; used to assess duplicate precision; acceptance criterion is RPD 20% for environmental matrices (Creed et al., 1994).
- **Spike recovery:** The percentage of a known added concentration of a metal that is measured by the instrument; acceptable range is typically 85–115%; used to check for matrix interferences (Creed et al., 1994).

- **Total recoverable metals:** Metals determined in an unfiltered sample after acid digestion; includes both dissolved and particulate-bound fractions (Creed et al., 1994).

5. Safety and Hazards

⚠ Hazards

- **Chemical burns and corrosive vapors:** Concentrated nitric acid (HNO_3, 67–70%) is an extremely corrosive oxidizing agent. Contact causes severe chemical burns. Vapors are highly damaging to respiratory tissue.
- **Hydrochloric acid:** Concentrated HCl produces corrosive fumes and can cause burns on contact.

! Engineering Controls

All acid dispensing, sample acidification, and hot block heating must be performed inside an active chemical fume hood.

5.1. Required PPE

☐ Safety glasses or face shield
☐ Laboratory coat
☐ Acid-resistant nitrile gloves (double-gloving recommended)

5.2. Emergency Procedures

In case of acid spill or chemical burn, refer to SOP-L002 Laboratory Safety or call campus safety. For acid contact with skin or eyes, flush immediately with water for at least 15 minutes and seek medical attention.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Automated sample digestion block	Hot block, programmable to 85–95 °C
Chemical fume hood	Required for all acid work
Analytical balance	If gravimetric volume verification is needed

6.2. Reagents and Consumables

Item	Notes
Nitric acid (HNO_3)	Ultra-pure, trace metal grade
Hydrochloric acid (HCl)	Trace metal grade; used when target analytes include Ag, Sb, or high Fe (Creed et al., 1994)
Certified metal-free membrane filters	0.45 µm DigiFILTERS or syringe filters
Metal-free polypropylene tubes	15 mL or 50 mL autosampler tubes
Ribbed watch glasses or reflux caps	To prevent evaporation loss during digestion
Narrow-range pH strips	To verify pH < 2
High-purity DI water	For volume adjustment and blanks
Multi-element stock solution	Certified reference material for LFB spikes (Beauchemin, 2017)

7. Procedure

7.1. Sample Preservation and Dissolved Metals Fraction

1. **Filtration:** Filter the raw, unacidified sample immediately upon collection or arrival through a certified 0.45 µm metal-free membrane filter. Discard the first 5 mL of filtrate to rinse the filter matrix. Collect remaining filtrate into a clean polypropylene tube.
2. **Acidification:** Add high-purity trace metal grade HNO_3 dropwise to the sample container to achieve a final matrix concentration of 2% (v/v). For example, add 1.0 mL concentrated HNO_3 per 50 mL sample matrix (Creed et al., 1994).
3. Verify pH is < 2 using a narrow-range pH strip. Store acidified samples for at least 16 hours prior to analysis to ensure proper metal desorption from container walls (Creed et al., 1994).

7.2. Total Recoverable Metals Digestion (Hot Block)

1. Transfer a well-mixed 50.0 mL portion of unfiltered sample into a clean digestion tube.
2. Working inside a fume hood, add 1.0 mL of trace metal grade HNO_3 and 0.5 mL of trace metal grade HCl (Creed et al., 1994).
3. Place the digestion tube into the hot block. Fit with a ribbed watch glass or vapor reflux cap to prevent evaporation loss.
4. Program the block heater to 85–95 °C. Heat the sample for 2 hours without allowing the solution to boil (United States Environmental Protection Agency, 2007).
5. Remove from heat, allow to cool to room temperature, and adjust back to the original 50.0 mL volume mark using high-purity DI water. If visible particulates remain, filter the digestate to prevent ICP-MS nebulizer clogging.

Avoiding Boil-Over

Monitor samples during the first 15–20 minutes of heating. If the solution begins to bump or boil, reduce the block temperature by 5 °C. Loss of sample volume through boiling invalidates the digestion (United States Environmental Protection Agency, 2007).

7.3. Shutdown and Cleanup

1. Allow the hot block to cool before removing tubes.
2. Rinse all non-disposable glassware and equipment with 10% (v/v) HNO_3 followed by three rinses of DI water.
3. Dispose of acid waste per §8 below.

8. Quality Control and Acceptance Criteria

QC Element	Requirement
Method blank	Process 50 mL DI water through full procedure; analyte concentrations must be below the MDL (Creed et al., 1994)
Laboratory fortified blank (LFB)	Spike multi-element stock into DI water and carry through digestion; recovery must be 85–115% (Creed et al., 1994)
Laboratory fortified matrix (LFM)	Spike a field sample; recovery 70–130% (APHA et al., 2012)
Duplicate samples	RPD 20% (Creed et al., 1994)
Calibration verification	Initial and continuing calibration per instrument SOP

See Creed et al. (1994) and APHA et al. (2012) for additional QC guidance.

9. Waste Disposal

Waste Type	Container	Disposal Method
Acid-preserved samples	Acid waste carboy	EH&S pickup
Spent acid rinses	Acid waste carboy	EH&S pickup
Used filters	Solid chemical waste	Standard hazardous waste
Empty acid bottles	Triple-rinsed, labels defaced	Glass recycling

10. Maintenance

- **Each use:** Wipe down the hot block surface and fume hood sill after acid work.
- **Monthly:** Inspect digestion tubes for etching or discoloration; replace as needed.
- **As needed:** Replace pH strips and membrane filters before expiration dates.

11. References

- Creed et al. (1994)
- United States Environmental Protection Agency (2007)
- Beauchemin (2017)
- APHA et al. (2012)
- SOP-L002 Laboratory Safety

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

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12. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L315

eDNA Laboratory Processing

Standard Operating Procedure

SOP Number	SOP-L315
Revision	1.1
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes contamination-controlled processing protocols for environmental DNA (eDNA) from aquatic collection matrices. It outlines vacuum membrane filtration, total genomic extraction, and subsequent PCR inhibition testing steps (Goldberg et al., 2016).

2. Scope

- **Applies to:** Laboratory processing of aquatic eDNA samples, including filtration, DNA extraction, and inhibition screening prior to PCR amplification.
- **Does not apply to:** Field collection of eDNA water samples, PCR amplification and sequencing workflows, or bioinformatic analysis of sequence data.
- **Location:** Dedicated Pre-PCR clean room, physically separated from post-amplification facilities.

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Amplification inhibition:** Suppression of PCR or ddPCR amplification efficiency by co-extracted substances such as humic acids, tannins, heavy metals, or residual ethanol; detected by comparing amplification of a known-concentration internal positive control (Jane et al., 2015; McKee et al., 2015).
- **Contamination control:** Practices that prevent introduction of exogenous DNA into eDNA samples, including use of UV-decontaminated surfaces, negative-pressure rooms, dedicated pre-PCR equipment, and frequent glove changes (Goldberg et al., 2016).
- **eDNA (environmental DNA):** Genetic material shed into the environment by organisms through mucus, feces, skin cells, gametes, and other biological material; collected from water, sediment, or air samples without direct organism capture (Ficetola et al., 2008).
- **Environmental DNA extraction:** The process of lysing filter-captured cells or particles and purifying nucleic acids from co-extracted inhibitors; produces a DNA extract suitable for PCR-based detection (Qiagen, 2024).
- **Extraction blank (negative control):** A reagent-only sample carried through the full extraction procedure without biological material; detects reagent or cross-contamination; must yield no amplification of target sequences (Goldberg et al., 2016).
- **Field blank:** A filter processed through the collection and preservation steps at the site but with no water sample passed through it; detects airborne or handling contamination in the field (Goldberg et al., 2016).
- **Inhibition testing:** A diagnostic assay in which a known-positive control is co-amplified with the sample to detect PCR inhibition; common approaches include an internal amplification control (IAC) or serial dilution (McKee et al., 2015).
- **Sterivex filter:** A polysulfone capsule filter (0.22 or 0.45 µm) used to concentrate eDNA from large water volumes by inline pumping; sealed and stored at -20 °C or -80 °C for later extraction.

- **Vacuum filtration pressure:** Vacuum applied to the filter manifold during eDNA filtration; should be kept below 150 mmHg to minimize cell lysis and DNA shearing on the filter membrane.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** 10% bleach solution (0.5% sodium hypochlorite) is corrosive to skin and eyes. Lysis buffers contain chaotropic salts (e.g., guanidinium thiocyanate) that are harmful if ingested or inhaled. Ethanol-based wash buffers are flammable.
- **Biological:** Environmental water samples may harbor unknown pathogens. Treat all samples as potentially infectious.
- **Physical:** Vacuum filtration apparatus operates under reduced pressure; ensure flasks are borosilicate and crack-free.

5.1. Required PPE

☐ Safety glasses
☐ Dedicated Pre-PCR laboratory coat (do not wear in post-amplification areas)
☐ Clean nitrile gloves (change frequently between sample groups)

5.2. Emergency Procedures

In case of chemical spill or exposure, refer to SOP-L002 Laboratory Safety or call campus safety. For bleach or lysis buffer contact with eyes, flush immediately with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Vacuum filtration manifold	Dedicated to Pre-PCR room
Vacuum pump or line	Regulated to < 150 mmHg
Digital dry bath or shaker	Capable of 56 °C incubation
Microcentrifuge	10,000 × g
qPCR thermocycler	For inhibition testing (Phase C)
Freezer (–20 °C)	For deferred extraction storage

6.2. Reagents and Consumables

Item	Notes
Sterile membrane filters	0.45 µm cellulose nitrate or glass fiber
Sterile single-use plastic forceps	
2.0 mL microcentrifuge tubes	Sterile, DNA-free
Genomic extraction kit	e.g., DNeasy Blood & Tissue or PowerWater (Qiagen, 2024)
Proteinase K	Supplied with extraction kit
Lysis buffer	Supplied with extraction kit
Ethanol-based wash buffers	Supplied with extraction kit
Elution buffer	10 mM Tris-HCl or molecular-grade water
10% bleach solution	0.5% sodium hypochlorite final
70% ethanol	For surface decontamination
Molecular-grade water	
IPC primer/probe set	Internal Positive Control for inhibition testing (McKee et al., 2015)
Inhibitor removal columns	e.g., Zymo OneStep PCR Inhibitor Removal
Silica gel desiccant	If filters are stored dry before extraction

7. Procedure

7.1. Contamination Control and Spatial Separation

! Contamination Prevention

Environmental DNA exists at extremely low fragment densities and is exceptionally vulnerable to cross-contamination. Strict spatial separation and decontamination protocols are mandatory (Goldberg et al., 2016; Turner et al., 2015).

1. All eDNA filtration and extraction work must occur in the dedicated Pre-PCR room, structurally separated from post-amplification thermocycling facilities.
2. **Decontamination regimen:** Before and after each protocol sequence, wipe all benchtops, pipettes, and manifold bodies with 10% bleach solution. Allow a 10-minute contact window, then rinse with 70% ethanol and molecular-grade water.
3. Wear a lab coat dedicated to the Pre-PCR room. Put on clean nitrile gloves and replace them frequently (e.g., between processing separate filtration manifolds or extraction groups).

7.2. Phase A: Membrane Vacuum Filtration

1. Sanitize the filter manifold station completely using 10% bleach solution and rinse as described in §6.1.
2. Using sterile, single-use plastic forceps, center a sterile 0.45 µm cellulose nitrate or glass fiber filter disc onto the clean manifold support pad. Mount the upper funnel collar tightly.

3. Agitate the water sample bottle and pour a precise volume (typically 500 mL to 2 L) into the funnel. Apply low vacuum pressure (< 150 mmHg) to avoid cell lysis on the filter.

> **Vacuum Pressure**
>
> Keeping vacuum pressure below 150 mmHg minimizes mechanical disruption of captured cells and reduces DNA loss through the filter membrane. If the filter clogs before the target volume is processed, record the actual volume filtered and proceed to step 4.

4. Once filtration completes, use sterile forceps to roll or fold the filter disc inward (preventing target loss on exterior surfaces) and insert it into a sterile 2.0 mL microcentrifuge tube containing lysis buffer or silica gel desiccant. Freeze at –20 °C if extraction is deferred.

7.3. Phase B: Genomic eDNA Extraction

This phase uses standard commercial spin-column kits (e.g., DNeasy Blood & Tissue or PowerWater kits (Qiagen, 2024)).

1. Add 50 µL Proteinase K and the target volume of lysis buffer directly to the microcentrifuge tube containing the folded filter disc. Incubate on a digital dry bath shaker at 56 °C for at least 2–3 hours (or overnight) to thoroughly digest captured cellular structures.
2. Centrifuge to pellet filter debris. Pass the clear supernatant lysate through a silica spin column. Wash sequentially with ethanol-based buffers to remove protein impurities. Elute clean genomic fragments using a low-ionic-strength buffer (10 mM Tris-HCl or molecular-grade water).

> **Elution Volume**
>
> A smaller elution volume (e.g., 50–100 µL) increases DNA concentration but may reduce total yield. Choose based on downstream assay sensitivity requirements.

7.4. Phase C: Internal Control Inhibition Testing

Environmental matrices contain co-extracted PCR inhibitors (humic acids, fulvic acids, tannic compounds) that can produce false negative results (Jane et al., 2015; McKee et al., 2015).

1. Run a dual-plex qPCR assay using an artificial Internal Positive Control (IPC) DNA target sequence and matched primer/probe set alongside your environmental samples.
2. **Analysis threshold:** If the IPC amplification curve shifts to the right ($\Delta C_t > 3.0$ cycles) relative to the control baseline water standard, inhibition is present (McKee et al., 2015).
3. **Remediation:** For inhibited extracts, either pass the genomic sample through a secondary commercial inhibitor-removal column (e.g., Zymo OneStep PCR Inhibitor Removal) or perform a 1:10 dilution with molecular-grade water before final target gene amplification. Re-test IPC performance after remediation (McKee et al., 2015).

7.5. Cleanup and Storage

1. Dispose of used lysis buffer, wash buffer, and flow-through in the appropriate chemical waste stream (§8).
2. Wipe down all work surfaces with 10% bleach, followed by 70% ethanol and molecular-grade water.
3. Store extracted DNA at –20 °C (short-term) or –80 °C (long-term archival).

8. Quality Control and Acceptance Criteria

QC Element	Requirement
Field blank	Filtered molecular-grade water collected alongside field samples; must show no target amplification (Goldberg et al., 2016)
Extraction blank	Lysis buffer processed through full extraction without a filter; must show no target amplification
IPC ΔC_t	Must be 3.0 cycles relative to baseline; samples exceeding this threshold require inhibitor remediation (McKee et al., 2015)
Positive extraction control	Known positive tissue or synthetic DNA standard carried through extraction; must amplify at expected C_t
No-template control (NTC)	Molecular-grade water in place of template during qPCR; must show no amplification

9. Waste Disposal

Waste Type	Container	Disposal Method
Lysis buffer and flow-through	Chemical waste carboy	EH&S pickup
Used filters and tubes	Biohazard bag	Autoclave and solid waste
Bleach rinse waste	Drain (if neutralized)	Per local discharge rules
Ethanol wash waste	Flammable waste container	EH&S pickup

10. Maintenance

- **Each use:** Decontaminate manifold, benchtops, and pipettes per §6.1.
- **Weekly:** Check vacuum pump oil level and integrity of tubing connections.
- **Monthly:** Verify dry bath temperature accuracy with a calibrated thermometer.
- **As needed:** Replace UV bulbs in the Pre-PCR room (if UV decontamination is used).

11. References

- Goldberg et al. (2016)

- Turner et al. (2015)
- McKee et al. (2015)
- Jane et al. (2015)
- Ficetola et al. (2008)
- Qiagen (2024)
- SOP-L002 Laboratory Safety
- SOP-F700 eDNA Field Sampling

Ficetola, G. F., Miaud, C., Pompanon, F. ccois, & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS ONE*, *12*(6), e0179251. https://doi.org/10.1371/journal.pone.0179251

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow, and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216–227. https://doi.org/10.1111/1755-0998.12285

McKee, A. M., Spear, S. F., & Pierson, T. W. (2015). The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation*, *183*, 70–76. https://doi.org/10.1016/j.biocon.2014.11.031

Qiagen. (2024). *DNeasy blood and tissue kit handbook*. https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, *183*, 93–97. https://doi.org/10.1016/j.biocon.2014.11.017

⚠ Missing bibliography entry: hinlo2017

The previous version of this SOP cited `@hinlo2017` for the < 150 mmHg vacuum pressure recommendation. This key does not exist in `references.bib`. The full citation is:
Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLOS ONE*, *12*(6), e0179251. https://doi.org/10.1371/journal.pone.0179251
Please add this entry to `references.bib` using the BibTeX block in the supplemental bib additions file, then replace this callout with the inline citation `[@hinlo2017]` in the Phase A filtration step.

12. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L400

Soil Moisture – Gravimetric Method

Standard Operating Procedure

SOP Number	SOP-L400
Revision	0.3
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the gravimetric determination of soil moisture content. Gravimetric moisture is a fundamental soil property required for expressing analytical results on a dry-weight basis (e.g., microbial biomass carbon, nutrient concentrations) and for characterizing site conditions (Gardner, 1986).

2. Scope

- **Applies to:** All soil samples collected per SOP-F400 Soil Sampling and Processing that require moisture correction or moisture characterization.
- **Does not apply to:** Volumetric soil moisture measured with in-situ probes (TDR, capacitance sensors); soil water potential measurements.
- **Location:** EA Program laboratory.

3. Definitions

- **Air-dry moisture content:** The moisture content of soil equilibrated with ambient laboratory air (typically 20–40 °C, 30–60% relative humidity); generally a few percent and varies with humidity (Gardner, 1986).
- **Constant mass:** The criterion for complete drying: successive weighings separated by at least 2 hours of additional oven time agree within 0.01 g.
- **Dry-weight correction factor (f):** The ratio of moist soil mass to oven-dry soil mass for the same sample; used to convert results expressed per gram of moist soil to per gram of oven-dry soil: $f = (W_{\text{moist}} - W_{\text{container}})/(W_{\text{dry}} - W_{\text{container}})$.
- **Field-moist soil:** Soil at the moisture content it had when collected from the field; the reference state for biological and enzyme activity measurements (Robertson et al., 1999).
- **Gravimetric moisture content (θ_g):** Mass of water per mass of oven-dry soil: $\theta_g = (M_{\text{wet}} - M_{\text{dry}})/M_{\text{dry}}$; expressed as g g^{-1} or as a percentage (Gardner, 1986).
- **Oven-dry soil:** Soil dried at 105 °C to constant mass; the standard reference state for expressing soil chemical and physical properties (Gardner, 1986).
- **Soil moisture:** The amount of water contained in the pore spaces of soil; may be expressed as gravimetric content (θ_g, g g^{-1}), volumetric content (θ_v, m^3 m^{-3}), or as a percentage (Gardner, 1986).
- **Volumetric moisture content (θ_v):** Volume of water per volume of soil: $\theta_v = \theta_g \times \rho_b/\rho_w$ where ρ_b is bulk density and ρ_w is the density of water (1.0 g cm^{-3}).

4. Safety and Hazards

⚠ Hazards

- **Thermal:** Drying ovens operate at 105 °C. Use heat-resistant gloves and tongs when removing samples.
- **Physical:** Hot aluminum tins retain heat for several minutes after removal from the oven. Do not handle bare-handed.

4.1. Required PPE

☐ Safety glasses
☐ Laboratory coat
☐ Heat-resistant gloves (when handling hot tins)

5. Equipment and Materials

5.1. Equipment

Item	Notes
Drying oven	Regulated at 105 ±2 °C; verify with a calibrated thermometer (Gardner, 1986)
Analytical balance	Resolution 0.01 g
Desiccator	With active color-indicating silica gel
Crucible tongs	For safe removal of hot tins

5.2. Consumables

Item	Notes
Aluminum tins or ceramic crucibles	Pre-labeled with permanent marker or engraved
Spatula	For transferring soil to tins

6. Estimated Time

Task	Time Required
Sample preparation and weighing	5–10 minutes per batch
Oven drying	24 hours at 105 °C
Cooling and re-weighing	30–45 minutes per batch (including cooling)
Total per batch	Approximately 25 hours (mostly unattended)

7. Procedure

7.1. Preparation

1. Print copies of the Soil Moisture Datasheet (Appendix A) for recording measurements.
2. Label aluminum tins or ceramic crucibles with a permanent marker or engraving tool.
3. Pre-dry the empty containers at 105 °C for at least 1 hour to remove any residual moisture (Gardner, 1986).
4. Cool the containers in a desiccator to room temperature.
5. Weigh each empty container to the nearest 0.01 g and record as Tin Weight on the datasheet.

7.2. Sample Weighing

1. Using a spatula, transfer approximately 10–20 g of field-moist soil into a pre-weighed container.

2. Record the mass of the container plus moist soil to the nearest 0.01 g as Wet Weight on the datasheet.
3. Avoid compacting the soil; a loose arrangement promotes even drying.

> **Tip**
>
> Process moisture subsamples at the same time as other subsamples (e.g., for microbial biomass or respiration) to ensure the moisture value accurately reflects the condition of the soil used in those analyses (Robertson et al., 1999).

7.3. Drying

1. Place the uncovered containers in the drying oven set to 105 °C (Gardner, 1986).
2. Dry for a minimum of 24 hours.
3. After 24 hours, remove one or two containers, cool in the desiccator, and re-weigh. If the mass has decreased by more than 0.01 g compared to a check 2 hours earlier, return all samples to the oven for an additional 4 hours and re-check.
4. Drying is complete when the mass is constant (change of less than 0.01 g between successive weighings).

7.4. Cooling and Final Weighing

1. Transfer all containers from the oven to a desiccator.
2. Allow to cool to room temperature (approximately 20–30 minutes).
3. Weigh each container plus dry soil to the nearest 0.01 g and record as Dry Weight on the datasheet.

7.5. Calculations

Gravimetric soil moisture content (%):

$$\theta_g(\%) = \left(\frac{W_{\text{moist}} - W_{\text{dry}}}{W_{\text{dry}} - W_{\text{container}}} \right) \times 100$$

where:

- W_{moist} = mass of container + moist soil (g)
- W_{dry} = mass of container + oven-dried soil (g)
- $W_{\text{container}}$ = mass of empty container (g)

Dry-weight correction factor (for converting field-moist analytical results to a dry-weight basis):

$$f = \frac{W_{\text{moist}} - W_{\text{container}}}{W_{\text{dry}} - W_{\text{container}}}$$

To convert a result expressed per gram of moist soil to per gram of dry soil, multiply by f (Gardner, 1986).

8. Quality Control

- Run analytical duplicates for at least 10% of samples; the coefficient of variation between duplicates should be less than 5% (Robertson et al., 1999).
- Verify oven temperature with a calibrated thermometer at the start of each drying cycle.
- Ensure the desiccator contains active (indicating) desiccant; replace or regenerate when the indicator changes color.
- Re-weigh a subset of samples after an additional 2 hours of drying to confirm mass constancy (Gardner, 1986).

9. Waste Disposal

Waste Type	Disposal Method
Oven-dried soil	Standard trash or return to field site
Aluminum tins	Clean and reuse, or recycle

10. References

- Gardner (1986)
- Robertson et al. (1999)

Gardner, W. H. (1986). Water content. In A. Klute (Ed.), *Methods of soil analysis, part 1: Physical and mineralogical methods* (2nd ed., pp. 493–544). Soil Science Society of America.

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research*. Oxford University Press.

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Demonstrate safe operation of the drying oven
3. Demonstrate proper use of the analytical balance and desiccator
4. Perform the procedure under supervision with acceptable duplicate precision
5. Be signed off by the PI or lab manager

12. Appendix A: Soil Moisture Datasheet

Print this page and use it to record measurements in the lab.

Project / Soil Location: _____

Sampling Date (YYMMDD): _____

Researcher Name: _____

Oven Temperature (°C): _____

Date of Entry (YYMMDD): _____

Soil ID	Soil Tin #	Tin Weight (g)	Wet Weight (Tin + Wet Soil) (g)	Dry Weight (Tin + Dry Soil) (g)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L401

Soil Texture Analysis – Hydrometer Method

Standard Operating Procedure

SOP Number	SOP-L401
Revision	0.1
Author(s)	Marc Los Huertos, Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil particle size distribution using the hydrometer method (ASTM D422). Soil texture (the relative proportions of sand, silt, and clay) is a fundamental physical property that controls water-holding capacity, drainage, nutrient retention, and soil structure. The hydrometer method measures the density of a soil-water suspension at timed intervals as progressively finer particles settle, allowing calculation of the percent sand, silt, and clay and assignment of a USDA textural class.

For a rapid field estimate of texture without laboratory equipment, see SOP-F401 Texture by Feel.

2. Scope

- **Applies to:** Mineral soils with less than approximately 20% coarse fragments (greater than 2 mm). Soils with high organic matter or carbonates require pre-treatment (see Section 6.4).
- **Does not apply to:** Pure gravel, organic soils (histosols), or soils where only a textural class estimate is needed (use SOP-F401 instead)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain equipment, prepare HMP solution
Trained Personnel	Perform the analysis over multiple sessions
New Students	Complete training before independent use

4. Definitions

- **Clay:** Soil mineral particles with an equivalent spherical diameter less than 0.002 mm (2 µm); slow-settling; comprises the finest soil texture fraction.
- **Dispersion:** The process of separating soil aggregates into individual particles by chemical treatment (sodium hexametaphosphate) and mechanical agitation; essential for accurate hydrometer analysis.

- **ASTM 151H hydrometer:** A standardized glass hydrometer calibrated in g L^{-1} for use in soil texture analysis; reads the density of the suspension at the center of the hydrometer bulb.
- **Sand:** Soil mineral particles with an equivalent spherical diameter of 0.05–2.0 mm; collected on a 0.05 mm sieve after sedimentation.
- **Sedimentation:** The settling of soil particles in suspension under gravity; particle settling velocity follows Stokes' Law: $v = 2gr^2(\rho_s - \rho_l)/(9\eta)$, where r is particle radius, ρ_s is particle density, ρ_l is liquid density, and η is dynamic viscosity.
- **Silt:** Soil mineral particles with an equivalent spherical diameter of 0.002–0.05 mm; intermediate settling rate; calculated as silt = 100% - sand% - clay%.
- **Stokes' Law:** The physical law governing particle sedimentation velocity in a viscous fluid; the basis for hydrometer texture analysis; assumes spherical particles of uniform density (2.65 g cm^{-3}).
- **Texture class:** A USDA textural classification (e.g., sandy loam, clay loam, silty clay) based on the relative percentages of sand, silt, and clay; determined from the USDA textural triangle.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Sodium hexametaphosphate (HMP) is a mild skin and eye irritant. Hydrogen peroxide (30%) is a strong oxidizer and causes burns on contact. Hydrochloric acid (1 N) is corrosive.
- **Physical:** The milkshake mixer creates a vigorous vortex; keep loose clothing and hair clear. Hot plates and drying ovens pose burn risk.

5.1. Required PPE

☐ Safety glasses (chemical-safety goggles when using H_2O_2 or HCl)
☐ Lab coat
☐ Nitrile or latex gloves
☐ Hearing protection not required but recommended near the mixer

5.2. Emergency Procedures

For H_2O_2 or HCl splashes, flush with water for at least 15 minutes. For HMP powder inhalation, move to fresh air. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
No. 10 sieve (2 mm mesh)	For removing gravel
No. 200 sieve (0.075 mm mesh)	For sand fraction recovery
Milkshake mixer and dispersion cup	For mechanical dispersion
151H hydrometer	ASTM standard
1 L sedimentation cylinder with No. 13 stopper	Glass or polycarbonate
Stopwatch or timer	For recording settling times
Thermometer (0.5 C resolution)	For temperature corrections
Analytical balance (0.01 g)	See SOP-L102
Drying oven (110 C)	See SOP-L105
Hot plate	For pre-treatment heating
250 mL and 400 mL beakers	For pre-treatment and weighing
Soil tins (pre-weighed)	For moisture determination
Mortar and pestle	For breaking up air-dried aggregates
Squeeze bottle (DI water)	For quantitative transfers

6.2. Reagents

Reagent	Preparation / Notes
Sodium hexametaphosphate (HMP)	40 g/L solution in DI water; prepare fresh weekly
Hydrogen peroxide, 30% (CAS 7722-84-1)	For organic matter removal (pre-treatment)
Hydrochloric acid, 1 N (CAS 7647-01-0)	For carbonate removal (pre-treatment)
Distilled or deionized water	From *SOP-L11*

7. Procedure

The analysis spans multiple lab sessions. Plan accordingly:

Session	Tasks	Time Required
1	Air-dry 200 g of soil	24+ hours (passive)
2	Sieve through No. 10; subsample for moisture	1–2 hours
3	Calculate DW_e; pre-treat and/or disperse	2–3 hours + 16 h soak
4	Conduct hydrometer readings	24+ hours (intermittent)
5	Recover sand on No. 200 sieve; complete data entry	1–2 hours

7.1. Air-Drying the Soil

1. Spread approximately 200 g of field-moist soil in a labeled tin or beaker

2. Allow to equilibrate with laboratory air for 24 hours or more. Very wet soils may require several days.
3. Break up large clods periodically to promote even drying

7.2. Sieving Through the No. 10 Sieve

Soil is defined as particles less than 2 mm. Gravel must be removed before analysis but quantified.

1. Homogenize the air-dried soil by thorough mixing
2. Weigh approximately 65 g (silt/clay soils) or 120 g (sandy soils) of air-dried soil and record the mass
3. Using a mortar and pestle, gently break up aggregates so that individual particles pass the No. 10 sieve. Do not crush primary mineral grains.
4. Sieve the soil through the No. 10 sieve (2 mm)
5. Weigh and record the mass of soil that passed through the sieve
6. Calculate the percent passing:

$$\text{Percent passing No. } 10 = \frac{M_{\text{passed}}}{M_{\text{total}}} \times 100$$

7.3. Determining Hygroscopic Moisture Content

Air-dried soil still contains hygroscopic water adsorbed to clay surfaces, salts, and organic matter. This must be measured and corrected for.

1. Select a soil tin; record the tin number and tare mass
2. Transfer a 10–15 g subsample of the air-dried, sieved soil to the tin and record the air-dried mass (tin + soil)
3. Dry in the oven at 110 C for approximately 24 hours
4. Cool in a desiccator and record the oven-dried mass (tin + soil)
5. Calculate hygroscopic moisture:

$$\text{Moisture}_H = \frac{M_{\text{air-dry w/ tin}} - M_{\text{oven-dry w/ tin}}}{M_{\text{air-dry w/ tin}} - M_{\text{tin}}}$$

> **Note**
>
> The standard drying temperature of 105–110 C is somewhat arbitrary. Clay minerals may retain 10–15% water (dry basis) at 400 C. Organic components also release volatiles over a range of temperatures. The key is to specify and consistently use the same temperature.

7.4. Calculating Oven-Dry Equivalent Mass (DW_e)

The target oven-dry equivalent mass for the hydrometer test is 50 g for fine-textured soils or 100 g for coarse-textured soils (greater than approximately 70% sand).

Since air-dried soil contains hygroscopic water, the actual mass of air-dried soil to weigh out is:

$$DW_{\text{air}} = \frac{DW_e}{1 - \text{Moisture}_H}$$

where DW_e = 50 g or 100 g.

7.5. Pre-Treatment (If Required)

Pre-treatment is necessary for soils high in organic matter, carbonates, iron oxides, or soluble salts. If the soil is low in all of these, proceed directly to Section 6.6.

1. Transfer DW_{air} g of soil to a 400 mL beaker
2. **Carbonate removal:** Add 50 mL DI water and sufficient 1 N HCl to reduce the pH to 3.0–4.0. Stir and allow to equilibrate for 10 minutes until effervescence ceases.
3. **Organic matter removal:** Carefully add 30% H_2O_2 a few milliliters at a time, allowing frothing to subside between additions. If necessary, acidify the suspension with a few drops of 1 N HCl (H_2O_2 oxidation requires an acidic medium). When frothing subsides, heat to 90 C and continue adding H_2O_2 until no further reaction occurs. Rinse down the beaker walls and continue heating until excess H_2O_2 is consumed.
4. **Iron oxide removal (if needed):** Add 150 mL citrate-bicarbonate buffer. Add 3 g sodium hydrosulfite ($Na_2S_2O_4$) gradually (may froth). Heat in a water bath at 80 C for 20 minutes.
5. **Soluble salt removal (if needed):** Transfer soil to a centrifuge tube, add 100 mL DI water, and centrifuge at 1,500 rpm for 10 minutes. Decant the supernatant. Repeat until the supernatant is clear.

> ⚠ Warning
>
> Handle 30% H_2O_2 in the fume hood. It is a strong oxidizer that causes skin burns on contact. Add it slowly to avoid violent frothing.

7.6. Dispersing the Soil Sample

1. Weigh DW_{air} g of pre-treated (or untreated) soil into the dispersion cup and record the mass to 0.1 g
2. Add 125 mL of 40 g/L sodium hexametaphosphate solution
3. Stir until the slurry is thoroughly wetted
4. Cover and soak for at least 16 hours (overnight)
5. Add distilled water to the first indentation mark on the dispersion cup
6. Attach the cup to the milkshake mixer and mix for 5 minutes (sandy soils) or 15 minutes (fine-textured soils)

7.7. Conducting the Hydrometer Test

Blank preparation:

1. Prepare a blank cylinder: add 125 mL of HMP solution and fill to the 1,000 mL mark with distilled water
2. Record the blank hydrometer reading and temperature. If the zero mark is below the meniscus surface, record the correction as a negative number. Read at the top of the meniscus (the suspension is opaque).

Sample measurement:

1. Transfer the dispersed soil suspension to a sedimentation cylinder, using a squeeze bottle to rinse all material from the dispersion cup
2. Fill the cylinder to the 1,000 mL mark with distilled water
3. Stopper the cylinder (No. 13) and invert 180 degrees and back in a steady 2-second cycle for one full minute. Vigorously agitate during the first two inversions to loosen settled material.
4. Set the cylinder upright and start the timer immediately
5. At 20 seconds elapsed, slowly lower the hydrometer into the suspension (spinning gently to dislodge air bubbles) and read at 40 seconds. Remove, rinse, and dry the hydrometer.
6. Repeat the inversion and 40-second reading two more times (three total readings at 40 s) for accuracy
7. After the final 40-second reading, lower a thermometer into the suspension and record the temperature

> **!** Important
>
> The hydrometer must be removed, rinsed, and dried between every reading. Particles accumulate on the glass surface and progressively bias subsequent readings if not cleaned.

8. Continue taking hydrometer and temperature readings at the following schedule:

Reading	Time After Inversion	Temperature?	Blank Reading?
0	Before inverting	Yes	Yes
1	40 seconds (x3)	Yes (after)	–
2	2 minutes	–	–
3	5 minutes	Yes	Yes
4	15 minutes	–	–
5	30 minutes	–	–
6	60 minutes	Yes	Yes
7	2 hours	Yes	Yes
8	3 hours (optional)	Yes	Yes
9	6–8 hours (optional)	Yes	Yes
10	24 hours	Yes	Yes

9. Place the cylinder in a stable, undisturbed location between readings

7.8. Recovering Sand on the No. 200 Sieve

The hydrometer method does not resolve sand subfractions well. Sieve analysis of the sand fraction provides this detail.

1. After the final hydrometer reading, pour the cylinder contents through a No. 200 sieve (0.075 mm)
2. Using tap water (with a sediment trap in the sink), wash the material on the sieve until the rinse water is clear
3. Select a soil tin; record the tin number and tare mass
4. Carefully wash the retained sand from the sieve into the tin using a squeeze bottle. Do not use a brush or metal tool on the sieve mesh.
5. Dry at 110 C for 24 hours
6. Cool and weigh the tin plus dried sand; record the mass

> **Tip**
>
> The No. 200 sieve screens are delicate. Forcing particles through or scrubbing with a brush can stretch or distort the mesh. Always use a gentle water stream or squeeze bottle.

8. Quality Control and Acceptance Criteria

8.1. Calculations

The key calculated quantities for each hydrometer reading are:

Corrected hydrometer reading:

$$R_c = R_{\text{actual}} - R_{\text{blank}}$$

Effective particle diameter (D_e, mm) at each reading time is calculated from Stokes' Law, accounting for temperature-corrected viscosity and density of the suspension fluid.

Percent finer at each reading:

$$PF = \frac{R_c \times a}{DW_e} \times 100$$

where a is a correction factor based on the specific gravity of soil particles (typically 2.65 for mineral soils).

Textural class is determined by plotting the calculated percent sand, silt, and clay on the USDA textural triangle.

8.2. Bias and Interference Sources

Source	Effect	Correction
Temperature and viscosity	Cold water increases viscosity, slowing settling	Temperature correction factor applied to each reading
Temperature and density	Water density varies with temperature	Density correction in Stokes' Law
Hygroscopic moisture	Air-dried soil mass overestimates oven-dry mass	DW_e correction (Section 6.4)
Incomplete dispersion	Aggregates settle as single large particles; underestimates clay	Use HMP + mechanical mixing; pre-treat if needed
Meniscus reading	Opaque suspension prevents reading at bottom of meniscus	Read at top of meniscus; apply meniscus correction
Flocculation	Ca^{2+} and Al^{3+} bridge clay particles	HMP displaces polyvalent cations; Na^{+} hydrates clay surfaces

8.3. QC Requirements

- Run a duplicate analysis on at least 10% of samples
- Duplicate results should agree within one textural class
- Include a blank cylinder (HMP + water, no soil) with every batch
- Record all temperatures; temperature drift greater than 2 C during the reading series indicates an unstable environment
- Cross-check a subset of hydrometer results against field texture-by- feel estimates (SOP-F401)

9. Waste Disposal

Waste Type	Disposal Method
Soil suspension (no pre-treatment chemicals)	Settle, decant water, dispose of soil in standard trash
HMP solution (spent)	Drain with copious water (low hazard)
H_2O_2 waste (from pre-treatment)	Dilute and drain, or collect for EH&S if concentrated
HCl waste (from pre-treatment)	Neutralize with $NaHCO_3$ and drain, or collect per SOP-L003
Sieve washings	Use a sediment trap; dispose of trapped soil in standard trash

10. References

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- Soil Science Division Staff. (2017). *Soil Survey Manual*. USDA Handbook No. 18. USDA-NRCS.

11. Training Record

Before performing hydrometer analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety, SOP-L003 Hazardous Materials Handling, and SOP-L400 Soil Moisture training
3. Observe a trained operator perform the full multi-session analysis
4. Demonstrate proper soil dispersion, hydrometer reading technique, and temperature recording under supervision
5. Complete one full analysis (all sessions) with acceptable duplicate precision
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculations in R

```r
library(tidyverse)

# --- Hygroscopic moisture correction ---
tin_mass_g <- 15.32
air_dry_w_tin_g <- 27.45
oven_dry_w_tin_g <- 27.18

moisture_H <- (air_dry_w_tin_g - oven_dry_w_tin_g) /
              (air_dry_w_tin_g - tin_mass_g)

cat(sprintf("Hygroscopic moisture: %.4f g/g\n", moisture_H))

# --- Oven-dry equivalent mass ---
DW_e <- 50  # g; use 100 for sandy soils
```

```r
DW_air <- DW_e / (1 - moisture_H)

cat(sprintf("Air-dried soil to weigh: %.2f g\n", DW_air))

# --- Hydrometer readings ---
# Example data: time (min), hydrometer reading, temp (C), blank reading
readings <- tibble(
  time_min = c(0.67, 2, 5, 15, 30, 60, 120, 1440),
  R_actual = c(38, 32, 27, 22, 19, 16, 13, 8),
  temp_C = c(23, 23, 23, 22, 22, 22, 22, 21),
  R_blank = c(5, 5, 5, 5, 5, 5, 5, 5)
)

# Corrected readings
readings <- readings %>%
  mutate(
    R_corrected = R_actual - R_blank,
    # Simplified percent finer (a = 1 for Gs = 2.65)
    percent_finer = (R_corrected / DW_e) * 100
  )

# Sand, silt, clay from key readings
# Percent sand: 100 - PF at 40 sec (0.67 min)
# Percent clay: PF at 1440 min (24 h)
pct_sand <- 100 - readings$percent_finer[readings$time_min == 0.67]
pct_clay <- readings$percent_finer[readings$time_min == 1440]
pct_silt <- 100 - pct_sand - pct_clay

cat(sprintf("\nSand: %.1f%%  Silt: %.1f%%  Clay: %.1f%%\n",
            pct_sand, pct_silt, pct_clay))

# Plot particle size distribution curve
ggplot(readings, aes(x = time_min, y = percent_finer)) +
  geom_point(size = 3) +
  geom_line() +
  scale_x_log10() +
  labs(
    x = "Settling time (minutes, log scale)",
    y = "Percent finer (%)",
    title = "Hydrometer Particle Size Distribution"
  ) +
  theme_minimal()
```

13. Appendix B: USDA Textural Classes

Once percent sand, silt, and clay are determined, assign the textural class using the USDA textural triangle. The 12 classes are:

Class	Sand (%)	Silt (%)	Clay (%)
Sand	85–100	0–15	0–10
Loamy sand	70–90	0–30	0–15
Sandy loam	43–85	0–50	0–20
Loam	23–52	28–50	7–27
Silt loam	0–50	50–88	0–27
Silt	0–20	80–100	0–12
Sandy clay loam	45–80	0–28	20–35
Clay loam	20–45	15–53	27–40
Silty clay loam	0–20	40–73	27–40
Sandy clay	45–65	0–20	35–55
Silty clay	0–20	40–60	40–60
Clay	0–45	0–40	40–100

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
Clay content seems too low	Incomplete dispersion	Extend soaking time; increase mixer time; verify HMP concentration
Readings inconsistent at 40 s	Insufficient mixing; uneven settling	Ensure vigorous, consistent inversions; repeat three times
Temperature drifts more than 2 C	Lab temperature unstable	Move cylinder away from heat sources; consider a water bath
Blank reading changes over time	HMP solution degrading	Prepare fresh HMP solution weekly
Sand fraction sums do not match hydrometer	Soil lost during sieve transfer	Rinse quantitatively; check for cracks in the sieve
Percent finer exceeds 100% or is negative	Data entry or calculation error	Double-check raw readings, blank correction, and DW_e

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L402

Soil pH and Salinity

Standard Operating Procedure

SOP Number	SOP-L402
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the measurement of soil pH and electrical conductivity (EC, as a proxy for salinity) on soil-water and soil-$CaCl_2$ extracts. Soil pH controls nutrient availability, metal solubility, microbial activity, and the fate of contaminants. Soil salinity (estimated from EC) affects plant growth, soil structure, and is a primary criterion for classifying saline, sodic, and saline-sodic soils. The methods follow Thomas (1996) for pH and Rhoades (1996) for EC, as described in the SSSA *Methods of Soil Analysis, Part 3* (Rhoades, 1996; Thomas, 1996).

For meter operation, calibration, and electrode maintenance, see SOP-L300 pH and Conductivity – Bench-Top Measurements.

2. Scope

- **Applies to:** Air-dried, sieved (less than 2 mm) mineral soils from all EA Program sites; compost and potting media (with appropriate extraction ratios noted in Section 6)
- **Does not apply to:** In situ field pH measurements with portable probes (see SOP-F200); water sample pH and conductivity (see SOP-L300); saturated paste extraction for formal salinity classification of irrigation water
- **Location:** EA Program laboratory, SGM 133

3. Definitions

- **Cation exchange capacity (CEC):** The total capacity of a soil to hold exchangeable cations; closely related to soil pH buffering capacity (see SOP-L416).
- **Electrical conductivity (EC):** A measure of a soil extract's ability to conduct electricity, proportional to total dissolved ion concentration; expressed in dS m^{-1} or mS cm^{-1}; used as a surrogate for soluble salt content.
- **Paste extract (saturated paste):** A soil-water mixture prepared at the saturation moisture content; the USDA standard extract for measuring soil salinity.
- **pH:** The negative base-10 logarithm of the hydrogen ion activity; soil pH is measured in a defined soil-to-water or soil-to-$CaCl_2$ suspension and controls nutrient availability and microbial activity.
- **Salinity:** The total concentration of dissolved salts in soil solution; high salinity reduces plant-available water and can be toxic to salt-sensitive crops; typically characterized by EC of the saturation extract (EC_e).

- **Soil-to-water ratio:** The ratio of soil mass to water volume used in pH and EC measurements (e.g., 1:1, 1:2, 1:5); must be reported alongside the result because pH and EC values differ between ratios.

4. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain meter and electrodes, stock reagents
Trained Personnel	Prepare extracts, measure and record results
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Chemical:** 0.01 M $CaCl_2$ is a mild irritant. pH buffer solutions and conductivity standards are low-hazard but should not contact eyes.
- **Physical:** No significant physical hazards beyond standard glassware handling.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For buffer or chemical splashes, flush with water. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
pH/conductivity meter	Calibrated per SOP-L300
pH combination electrode	Glass bulb with refillable KCl junction preferred
Conductivity cell	With automatic temperature compensation
Analytical balance (0.01 g)	See SOP-L102

Item	Notes
Beakers (50–100 mL)	Clean; one per sample plus blanks
Graduated cylinder or dispenser	For volumetric delivery of water or $CaCl_2$
Glass stirring rods	One per sample or rinsed between samples
Timer	For equilibration period

6.2. Reagents

Reagent	Preparation / Notes
Deionized water (Type I or II)	From *SOP-L11*
0.01 M $CaCl_2$ solution	Dissolve 1.47 g $CaCl_2$-$2H_2O$ in 1 L DI water
pH buffer solutions (4.01, 7.00, 10.01)	For pH meter calibration
Conductivity standard (1413 uS/cm)	For conductivity meter calibration

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature until constant mass (typically 24–48 hours)
2. Pass the dried soil through a 2 mm sieve. See SOP-F400 Soil Sampling for details on sieving and homogenization.
3. Store the sieved soil in a labeled bag or container at room temperature

7.2. Selecting the Extraction Ratio

Two standard soil-to-solution ratios are used. The choice depends on the project objectives and the need for comparability with published data:

Ratio	Method	When to Use
1:1	10 g soil : 10 mL water (or $CaCl_2$)	Most common in agronomic soil testing; closest to field conditions
1:5	10 g soil : 50 mL water (or $CaCl_2$)	Useful for saline soils, compost, or when more extract volume is needed for subsequent analyses

> **Note**
>
> Soil pH measured in 0.01 M $CaCl_2$ is typically 0.3–0.5 units lower than pH measured in DI water because the salt displaces H^+ and Al^{3+} from exchange sites. $CaCl_2$ pH is less sensitive to seasonal variation in soil salt content and is preferred for monitoring studies where consistency over time matters (Thomas, 1996). Record the extraction medium (water or $CaCl_2$) and ratio with every result.

7.3. Preparing the Soil Slurry (1:1 Method)

1. Weigh 10.0 g of air-dried, sieved soil into a clean beaker
2. Add 10 mL of DI water (for pH_{water} and EC) or 10 mL of 0.01 M $CaCl_2$ (for pH_{CaCl2})
3. Stir vigorously with a glass rod for 30 seconds
4. Allow to stand for 10 minutes
5. Stir briefly again before measurement

7.4. Preparing the Soil Extract (1:5 Method)

1. Weigh 10.0 g of air-dried, sieved soil into a clean beaker
2. Add 50 mL of DI water (or 50 mL of 0.01 M $CaCl_2$)
3. Stir vigorously for 30 seconds
4. Allow to stand for 30 minutes, stirring occasionally
5. For EC measurement on the 1:5 extract, allow the soil to settle and insert the conductivity cell into the clear supernatant. For pH, insert the electrode into the slurry while still partially suspended.

7.5. Measuring Electrical Conductivity

> **!** Important
>
> Always measure EC before pH on the same aliquot. The pH electrode's KCl reference junction leaks electrolyte into the sample and will artificially elevate the EC reading (APHA et al., 2012).

1. Calibrate the conductivity meter per SOP-L300, Section 6.3
2. Rinse the conductivity cell with DI water and blot dry
3. Insert the cell into the supernatant (1:5 method) or into the settled portion of the slurry (1:1 method). Tap gently to dislodge air bubbles.
4. Allow the reading to stabilize with automatic temperature compensation active
5. Record the specific conductance (uS/cm at 25 C), sample ID, and extraction ratio
6. Rinse the cell with DI water between samples

7.6. Measuring Soil pH

1. Calibrate the pH meter with a three-point calibration per SOP-L300, Section 6.2
2. Rinse the pH electrode with DI water and blot gently
3. Insert the electrode into the slurry so that the glass bulb and reference junction are fully immersed. For the 1:1 method, the electrode should contact the settled soil.
4. Allow the reading to stabilize (typically 30–60 seconds; may take 2–3 minutes for poorly buffered soils)
5. Record the pH value, extraction medium (water or $CaCl_2$), ratio, and sample ID
6. Rinse the electrode with DI water between samples

7.7. Converting 1:5 EC to Estimated Saturated Paste EC (EC_e)

The saturated paste extract (EC_e) is the standard for soil salinity classification, but the 1:5 extract is quicker to prepare. An approximate conversion can be made using the following relationship, though the factor varies with texture (Rhoades, 1996):

$$EC_e \approx EC_{1:5} \times f$$

where f is an empirical factor:

Soil Texture	f (approximate)
Sand	6 – 8
Loam	8 – 10
Clay	10 – 13

Warning

These conversion factors are approximate and site-specific. For formal salinity classification (e.g., land-use planning, irrigation suitability), prepare an actual saturated paste extract per USDA Handbook 60.

8. Quality Control and Acceptance Criteria

8.1. Interpretation of Soil pH

pH Range	Classification	Agronomic Significance
Less than 4.5	Extremely acidic	Toxic Al^{3+} levels; few crops tolerate
4.5 – 5.5	Strongly acidic	Low P availability; Mo deficiency; lime recommended
5.5 – 6.5	Moderately acidic	Suitable for most crops; some nutrient limitations
6.5 – 7.5	Neutral	Optimal for most crops and nutrient availability
7.5 – 8.5	Moderately alkaline	Common in arid soils; Fe, Mn, Zn may be limiting
Greater than 8.5	Strongly alkaline	Possible sodicity; Na toxicity

8.2. Interpretation of Soil Salinity (EC_e)

EC_e (dS/m)	Classification	Plant Response
0 – 2	Non-saline	No yield reduction for most crops

EC_e (dS/m)	Classification	Plant Response
2 – 4	Slightly saline	Sensitive crops affected
4 – 8	Moderately saline	Many crops affected
8 – 16	Strongly saline	Only tolerant crops survive
Greater than 16	Very strongly saline	Few plants survive

8.3. Saline, Sodic, and Saline-Sodic Classification

Class	EC_e (dS/m)	SAR / ESP	pH
Normal	Less than 4	SAR less than 13 / ESP less than 15	Variable
Saline	4 or greater	SAR less than 13 / ESP less than 15	Usually less than 8.5
Sodic	Less than 4	SAR 13 or greater / ESP 15 or greater	Usually greater than 8.5
Saline-sodic	4 or greater	SAR 13 or greater / ESP 15 or greater	Variable

SAR = sodium adsorption ratio; ESP = exchangeable sodium percentage. Full SAR/ESP determination requires cation analysis beyond the scope of this SOP.

8.4. QC Requirements

- Calibrate the pH meter before each measurement session (three-point). Acceptable slope: 90–102% of theoretical.
- Calibrate the conductivity meter before each session
- Re-read pH 7.00 buffer after every 10 samples; recalibrate if drift exceeds +/- 0.05 pH units
- Re-read the conductivity standard after every 10 samples; recalibrate if drift exceeds +/- 5%
- Run a DI water blank and a laboratory reference soil (if available) with each batch
- Run duplicate measurements on at least 10% of samples. Acceptable precision: +/- 0.1 pH units and +/- 5% relative percent difference for EC.
- Record extraction medium, ratio, calibration data, and QC results in the lab notebook

9. Waste Disposal

Waste Type	Disposal Method
Soil slurry waste (non-hazardous)	Standard trash once settled
$CaCl_2$ extract solutions	Drain with copious water (non-hazardous)
Used pH buffer solutions	Collect in waste container per SOP-L300
Used conductivity standard	Drain with copious water
DI water rinses	Drain

10. References

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11. Training Record

Before measuring soil pH and EC independently, personnel must:

1. Read this SOP and SOP-L300 in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate pH meter three-point calibration with acceptable slope
4. Demonstrate conductivity meter calibration
5. Prepare 1:1 and 1:5 extracts and measure a set of at least 5 practice soils under supervision with acceptable duplicate precision
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation and Reporting

```
library(tidyverse)

# Example data entry
soil_data <- tibble(
  sample_id = c("PV-01", "PV-02", "PV-03", "PV-04", "PV-05"),
  pH_water_1_1 = c(6.82, 7.45, 5.91, 8.12, 7.03),
  pH_CaCl2_1_1 = c(6.48, 7.10, 5.55, 7.78, 6.68),
  EC_1_5_uS_cm = c(245, 1820, 85, 3450, 310),
```

```
  texture = c("loam", "clay", "sand", "clay", "loam")
)

# Estimate EC_e from 1:5 EC using texture-based conversion factors
conversion <- c(sand = 7, loam = 9, clay = 11.5)

soil_data <- soil_data %>%
  mutate(
    EC_e_dS_m = (EC_1_5_uS_cm / 1000) * conversion[texture],
    salinity_class = case_when(
      EC_e_dS_m < 2 ~ "Non-saline",
      EC_e_dS_m < 4 ~ "Slightly saline",
      EC_e_dS_m < 8 ~ "Moderately saline",
      EC_e_dS_m < 16 ~ "Strongly saline",
      TRUE ~ "Very strongly saline"
    ),
    pH_class = case_when(
      pH_water_1_1 < 4.5 ~ "Extremely acidic",
      pH_water_1_1 < 5.5 ~ "Strongly acidic",
      pH_water_1_1 < 6.5 ~ "Moderately acidic",
      pH_water_1_1 < 7.5 ~ "Neutral",
      pH_water_1_1 < 8.5 ~ "Moderately alkaline",
      TRUE ~ "Strongly alkaline"
    )
  )

print(soil_data %>% select(sample_id, pH_water_1_1, pH_CaCl2_1_1,
                           EC_e_dS_m, salinity_class, pH_class))
```

13. Appendix B: Reagent Preparation

13.1. M $CaCl_2$ Solution

1. Weigh 1.47 g of $CaCl_2$-$2H_2O$ (MW 147.01) on the analytical balance
2. Dissolve in approximately 800 mL of DI water in a 1 L volumetric flask
3. Bring to 1 L with DI water and mix thoroughly
4. Label with concentration, preparation date, and preparer's initials
5. Store at room temperature in a capped polyethylene or borosilicate bottle. Stable indefinitely.

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
pH reading drifts continuously	Poorly buffered soil; electrode junction fouled	Allow longer equilibration (up to 5 min); clean electrode per SOP-L300
pH in $CaCl_2$ higher than pH in water	Measurement error; contaminated $CaCl_2$ solution	Verify $CaCl_2$ concentration; recalibrate meter; re-measure
EC reading unexpectedly high	Fertilizer residue; saline soil; contaminated beaker	Verify with a blank; use acid-washed glassware (SOP-L101)
EC reading near zero	Electrode not fully immersed; air bubble in cell	Reposition cell; tap to dislodge bubbles
Large difference between 1:1 and 1:5 EC	Normal; dilution effect	Report both with the ratio clearly stated; convert to EC_e using appropriate factor
pH calibration slope below 90%	Aged electrode; dirty glass bulb	Soak in cleaning solution or 0.1 M HCl for 15–30 min; if slope does not recover, replace electrode

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L403

Soil Nitrate and Ammonium – KCl Extraction

Standard Operating Procedure

SOP Number	SOP-L403
Revision	0.4
Author(s)	Aaron Rogers
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the extraction of inorganic nitrogen (nitrate, NO_3^-, and ammonium, NH_4^+) from soil using 2 M potassium chloride (KCl). The KCl extraction is the standard method for determining plant-available inorganic nitrogen in soils (Keeney & Nelson, 1982; Mulvaney, 1996). The high ionic strength of the KCl solution displaces NH_4^+ from cation exchange sites and brings NO_3^- into solution, producing a filtered extract suitable for colorimetric, flow-injection, or ion chromatographic analysis (Maynard et al., 2008).

2. Scope

- **Applies to:** Field-moist or air-dried soil samples collected per SOP-F400 Soil Sampling and Processing
- **Does not apply to:** Total nitrogen analysis (requires Kjeldahl or combustion digestion; see SOP-L407); dissolved nitrogen in water samples; semi-quantitative field screening (see SOP-L404)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Trained Personnel	Prepare extracts, record and report results
New Students	Complete training before independent use

3.1. Required Training

Students using this SOP should be trained on:

- SOP-F001 Field Safety
- SOP-L003 Hazardous Materials Handling
- SOP-L400 Soil Moisture

4. Definitions

- **Ammonium (NH_4^+):** The protonated form of ammonia; held on negatively charged cation exchange sites in soil; released into solution by the high ionic strength of the 2 M KCl extractant; the primary plant-available nitrogen pool in many soils.
- **Cation exchange capacity (CEC):** The total capacity of a soil to hold exchangeable positively charged ions; controls how much NH_4^+ can be stored and released; higher in clay-rich and organic-matter-rich soils.
- **KCl extraction:** Shaking soil with 2 mol L^{-1} potassium chloride solution at a defined soil-to-solution ratio (typically 1:10 w/v); the standard procedure for quantifying plant-available inorganic nitrogen in soils (Keeney & Nelson, 1982).
- **Mineral nitrogen (mineral N):** The sum of NH_4^+-N and NO_3^--N in the soil; the immediately plant-available nitrogen pool; expressed in mg N kg^{-1} dry soil.
- **Nitrate (NO_3^-):** The oxidized form of inorganic nitrogen; anion, so not held on cation exchange sites; highly mobile in soil solution; measured colorimetrically or by flow injection analysis after KCl extraction.
- **Soil-to-solution ratio:** The mass of soil (g) to volume of extractant (mL) used in the extraction; typically 1:10 for KCl extractions; must be consistent within and between studies for results to be comparable.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** 2 M KCl is a mild irritant; avoid contact with eyes and skin. Review the SDS before first use.
- **Physical:** Reciprocating shaker can pinch fingers if containers are not secured properly.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Lab coat
☐ Closed-toed shoes

6. Required Materials

6.1. Equipment

Item	Notes
Reciprocating shaker	For 2-hour extraction (Keeney & Nelson, 1982)
Magnetic stirrer with stir bar	For preparing KCl solution
Stir bar retriever	Magnetic wand

Item	Notes
Top-loading balance	0.1 g resolution
Graduated cylinder or volumetric flask	2 L capacity for KCl preparation
Repipet-type dispenser	For dispensing KCl solution (optional)
Drying oven	Set to 105 C (for moisture subsample)
Sorvall ST8R Centrifuge	Optional; see SOP-L109

6.2. Consumables

Item	Cat. No.	Notes
Whatman #42 filter paper		Ashless, 185 mm diameter
Filter funnels	09-855F	To fit filter paper
Test tubes or extraction bottles	S89476	50 mL capacity, with caps
Specimen containers or Nalgene bottles		For collecting filtered extracts
Aluminum weigh boats		For moisture subsamples
Pipette		For dispensing precise volumes

6.3. Reagents

Item	Cat. No.	Notes
Potassium chloride (KCl)	AA11595A1	ACS grade; 298.2 g per 2 L for 2 M solution
Distilled or deionized water		For preparing KCl solution

7. Estimated Time

Task	Time Required
KCl solution preparation	10 minutes active + 2 hours stirring
Moisture subsamples	5 minutes active + 24 hours drying
KCl extraction (per batch)	15 minutes setup + 2 hours shaking + 10 minutes settling
Filtering	20–30 minutes per batch

8. Procedure

8.1. Preparing 2 M KCl Solution

The 2 M KCl concentration is the standard for inorganic N extraction (Keeney & Nelson, 1982; Mulvaney, 1996). Lower concentrations (e.g., 1 M) may underestimate exchangeable NH_4^+ in

high-CEC soils (Maynard et al., 2008).

1. Weigh out 298.2 g KCl into a 2 L volumetric flask
2. Fill the flask approximately two-thirds full with distilled water
3. Place the flask on a magnetic stirrer and add a magnetic stir bar
4. Set the stirrer to low-medium speed and leave on for 4 hours until fully dissolved
5. Use a stir bar retriever to remove the magnetic stir bar
6. Fill the flask to the 2 L mark with distilled water and mix

> **Tip**
>
> Prepare the KCl solution the day before extraction. It can be stored at room temperature in a capped container indefinitely. Verify the solution with a conductivity meter if stored for more than 6 months.

8.2. Moisture Subsample

A companion gravimetric moisture determination is required to express results on an oven-dry soil basis (Keeney & Nelson, 1982).

1. Set aside 10 g of soil from each sample for moisture determination
2. Place each 10 g subsample into a labeled aluminum weigh boat
3. Dry at 105 C for 24 hours and record the dry weight
4. Calculate gravimetric moisture content per SOP-L400 Soil Moisture

8.3. KCl Extraction

The standard soil-to-solution ratio is approximately 1:5 to 1:10 (w/v) (Mulvaney, 1996). This SOP uses 7 g field-moist soil in 40 mL KCl, yielding an effective ratio of approximately 1:6 on an oven-dry basis for a typical soil at 15% gravimetric moisture.

1. Dispense 40 mL of 2 M KCl solution into labeled test tubes or extraction bottles
2. Add 7 g of field-moist soil to each tube (the total volume should reach approximately 50 mL). Record the exact mass of soil added to the nearest 0.01 g.
3. Cap the tubes securely
4. Place on the reciprocating shaker for 2 hours
5. Remove from the shaker and either:
    - Allow soil particles to settle for approximately 10 minutes (do not exceed 1 hour), or
    - Centrifuge for 3 minutes at 9,000 rpm (see SOP-L109: Sorvall ST8R Centrifuge)

> **Important**
>
> Record the exact mass of field-moist soil added to each tube. This value, together with the gravimetric moisture content, is needed to calculate results on an oven-dry basis (see Appendix

A).

8.4. Filtering

1. Set up filter funnels with Whatman #42 filter paper over labeled specimen containers or clean test tubes
2. Pour the settled or centrifuged solution into the funnels; do not fill above the filter paper line
3. Allow the solution to drain completely until only soil remains on the filter
4. Discard the used filter paper and soil

8.5. Extract Storage

1. Cap the filtered extract containers
2. If analysis will be completed within 48 hours, store at 4 C
3. If analysis will be delayed beyond 48 hours, freeze the extracts at -20 C until analysis (Keeney & Nelson, 1982)

! Important

Extracts should be analyzed as soon as possible. Prolonged storage, even frozen, can alter NH_4^+ and NO_3^- concentrations due to microbial activity or chemical transformations (Maynard et al., 2008).

9. Calculations

9.1. Converting Extract Concentration to Soil Concentration

The colorimetric or flow-injection analyzer reports the concentration of NO_3^--N or NH_4^+-N in the filtered extract (mg N/L). To convert this to mg N per kg oven-dry soil (ppm):

$$C_{\text{soil}}\ (\text{mg N kg}^{-1}) = \frac{C_{\text{extract}} \times V_{\text{KCl}}}{m_{\text{dry}}}$$

where:

- C_{extract} = concentration in the filtered extract (mg N/L)
- V_{KCl} = volume of KCl extractant added (L; typically 0.040 L)
- m_{dry} = oven-dry equivalent mass of soil extracted (kg), calculated as:

$$m_{\text{dry}} = \frac{m_{\text{moist}}}{1 + \theta_g}$$

where m_{moist} is the field-moist mass added (g, converted to kg) and θ_g is the gravimetric moisture content expressed as a decimal (g water / g oven-dry soil).

See Appendix A for a worked R code example.

9.2. Soil Texture Correction Factor (Optional)

When using the semi-quantitative quick test (SOP-L404), a texture-based correction factor may be applied. For the quantitative KCl extraction described here, no texture correction is needed because the exact soil mass and moisture content are measured directly.

Texture	Moist Soil Factor	Dry Soil Factor
Sand	2.3	2.6
Loam	2.0	2.5
Clay	1.7	2.2

These factors are retained here for reference when comparing quick-test results to KCl extraction results.

10. Quality Control

- Include a reagent blank (KCl solution only, no soil) in each extraction batch. The blank should read below the method detection limit for both NO_3^--N and NH_4^+-N.
- Run extraction duplicates for at least 10% of samples. Duplicate results should agree within 15% relative percent difference (RPD).
- Include a laboratory reference soil with known inorganic N content in each batch when available.
- Record the KCl solution preparation date and batch number.
- Verify that the analytical calibration curve for the colorimetric method has an $r^2 \geq 0.995$ before reporting results.
- Subtract the mean reagent blank concentration from all sample results if the blank reads above zero (Mulvaney, 1996).

11. Waste Disposal

Waste Type	Disposal Method
KCl extracts	Aqueous waste; drain with copious water if approved by EH&S, or collect for pickup
Used filter paper and soil	Standard trash
Unused KCl solution	Drain with water (non-hazardous at this concentration)

12. References

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13. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L400 Soil Moisture training
3. Demonstrate competence preparing KCl solution
4. Perform the extraction and filtering procedure under supervision
5. Calculate results for one batch using the R code in Appendix A and verify against the instructor's values
6. Be signed off by the PI or lab manager

14. Appendix A: Calculation of Soil NO_3^--N and NH_4^+-N (R Code)

The following R code converts extract concentrations (mg N/L from the colorimetric analyzer) to soil concentrations (mg N/kg oven-dry soil, equivalent to ppm) for a batch of samples. It corrects for the exact mass of field-moist soil added and the gravimetric moisture content of each sample.

```
# ============================================================================
# SOP-L403: KCl Extraction -- Convert extract concentration to soil ppm
# ============================================================================

# ---- 1. Enter your data ----------------------------------------------------
# Replace the example values below with your actual data.
# Each vector must have the same length (one entry per sample).

sample_id <- c("S01", "S02", "S03", "S04", "S05")

# Mass of field-moist soil added to the extraction tube (g)
soil_moist_g <- c(7.03, 7.10, 6.98, 7.05, 7.12)

# Gravimetric moisture content (proportion, g water / g oven-dry soil)
```

```
# From SOP-L400.  Example: 0.15 means 15% moisture.
theta_g <- c(0.152, 0.148, 0.161, 0.155, 0.143)

# Volume of 2 M KCl added (mL) -- typically 40 mL for all samples
vol_kcl_mL <- rep(40, length(sample_id))

# Extract concentrations from the colorimetric analyzer (mg N / L)
no3_extract_mg_L <- c(3.42, 5.18, 2.76, 4.01, 6.33)
nh4_extract_mg_L <- c(1.05, 0.87, 1.22, 0.93, 0.78)

# Reagent blank concentrations (mg N / L) -- mean of your blanks
blank_no3_mg_L <- 0.02
blank_nh4_mg_L <- 0.01

# ---- 2. Calculate oven-dry soil mass (kg) ---------------------------------
soil_dry_g  <- soil_moist_g / (1 + theta_g)
soil_dry_kg <- soil_dry_g / 1000

# ---- 3. Blank-correct extract concentrations ------------------------------
no3_corrected <- no3_extract_mg_L - blank_no3_mg_L
nh4_corrected <- nh4_extract_mg_L - blank_nh4_mg_L

# Ensure no negative values after blank correction
no3_corrected[no3_corrected < 0] <- 0
nh4_corrected[nh4_corrected < 0] <- 0

# ---- 4. Convert to mg N / kg oven-dry soil (ppm) --------------------------
vol_kcl_L <- vol_kcl_mL / 1000

no3_ppm <- (no3_corrected * vol_kcl_L) / soil_dry_kg
nh4_ppm <- (nh4_corrected * vol_kcl_L) / soil_dry_kg

# ---- 5. Assemble results table --------------------------------------------
results <- data.frame(
  Sample        = sample_id,
  Moist_g       = soil_moist_g,
  Moisture_pct  = round(theta_g * 100, 1),
  Dry_g         = round(soil_dry_g, 3),
  Vol_KCl_mL    = vol_kcl_mL,
  NO3_extract   = no3_extract_mg_L,
  NH4_extract   = nh4_extract_mg_L,
  NO3_ppm       = round(no3_ppm, 2),
  NH4_ppm       = round(nh4_ppm, 2),
  Total_inorgN  = round(no3_ppm + nh4_ppm, 2)
)

# ---- 6. Print results -----------------------------------------------------
```

```
cat("SOP-L403: KCl Extraction Results\n")
cat("================================\n\n")
print(results, row.names = FALSE)
cat("\n")
cat(sprintf("Reagent blank NO3-N: %.2f mg/L\n", blank_no3_mg_L))
cat(sprintf("Reagent blank NH4-N: %.2f mg/L\n", blank_nh4_mg_L))
cat("\nAll soil concentrations are mg N / kg oven-dry soil (ppm).\n")
```

14.1. Worked Example (Sample S01)

Using the example data above for sample S01:

- Field-moist soil added: 7.03 g
- Gravimetric moisture: 15.2% (0.152)
- Oven-dry equivalent: $7.03/(1 + 0.152) = 6.102$ g $= 0.006102$ kg
- KCl volume: 40 mL $= 0.040$ L
- NO_3^--N in extract: 3.42 mg/L (blank-corrected: 3.40 mg/L)
- Soil NO_3^--N: $(3.40 \times 0.040)/0.006102 = 22.29$ mg N/kg (ppm)
- NH_4^+-N in extract: 1.05 mg/L (blank-corrected: 1.04 mg/L)
- Soil NH_4^+-N: $(1.04 \times 0.040)/0.006102 = 6.82$ mg N/kg (ppm)
- Total inorganic N: $22.29 + 6.82 = 29.11$ mg N/kg (ppm)

15. Appendix B: Interpretation Guide

General interpretation thresholds for extractable inorganic N in agricultural and wildland soils. These are approximate and vary by crop, climate, and soil type (Hart et al., 1994; Mulvaney, 1996).

Extractable Inorganic N (mg N/kg)	Interpretation	Notes
Less than 10	Low	Nitrogen likely limiting plant growth
10–25	Moderate	Adequate for many crops and native vegetation
25–50	High	Sufficient N; fertilization generally not needed
Greater than 50	Very high	Excess N; potential leaching or denitrification concern

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L404

Soil Nitrate Quick Test – Test Strip Method

Standard Operating Procedure

SOP Number	SOP-L404
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes a rapid, semi-quantitative field or laboratory method for estimating soil nitrate (NO_3^-) concentration using colorimetric test strips in a calcium chloride ($CaCl_2$) extracting solution (Bronikowski & Thomason, 2022; Hartz, 2010). The method is useful as a screening tool for assessing soil nitrogen status, guiding fertilizer decisions, and identifying spatial patterns in nitrate availability across a site (Magdoff et al., 1984). It is not a substitute for the quantitative KCl extraction and colorimetric analysis described in SOP-L403 (Mulvaney, 1996), but provides rapid, low-cost results that can be obtained in the field or with minimal laboratory equipment.

2. Scope

- **Applies to:** Rapid estimation of soil nitrate in field-moist or air-dried mineral soils from all EA Program sites; on-site field screening during sampling campaigns
- **Does not apply to:** Quantitative reporting of soil nitrate for publication (use SOP-L403); ammonium determination (test strips do not measure NH_4^+); water sample nitrate (use SOP-L303 Hach Colorimetric)
- **Location:** Field sites or EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Field Lead	Coordinate field testing logistics
Trained Personnel	Perform tests, record and report results
New Students	Complete training before independent use

4. Definitions

- **Calcium chloride ($CaCl_2$) extractant:** A dilute salt solution (approximately 0.01 M) used in the quick test to displace some exchangeable NO_3^- from soil without requiring the high-ionic-strength KCl used in quantitative extractions; minimizes matrix effects on the test strip reaction.
- **Colorimetric test strip:** A plastic strip impregnated with reagents that react with nitrate (or another target) to produce a visible color change; read visually against a printed color chart or with a reflectometer; semi-quantitative.
- **Detection range:** The span of concentrations over which a test strip or method produces a distinguishable response; for most nitrate strips, approximately 0–500 mg NO_3^--N L^{-1} in the extract; values outside this range require dilution or a quantitative method.
- **Matrix effect:** Interference from sample components (chloride, organic matter, pH) that alter the test strip reaction; minimized by using $CaCl_2$ extractant and diluting dark or strongly colored samples.

- **Semi-quantitative:** A measurement that provides an approximate concentration within broad categories (e.g., 0, 10, 25, 50, 100 mg/L) rather than a precise numerical value; appropriate for screening but not for publication or regulatory reporting.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Calcium chloride ($CaCl_2$) is a mild irritant to skin and eyes. The extracting solution is dilute (approximately 0.01 M) and poses minimal risk.
- **Biological:** Soil may contain pathogens. Wash hands after handling.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Closed-toed shoes (if testing in the field)

5.2. Emergency Procedures

For $CaCl_2$ solution contact with eyes, flush with water for 15 minutes. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Graduated centrifuge tubes (50 mL, e.g., Falcon tubes)	With volume markings at 30 and 40 mL
1-gallon jug or carboy	For preparing $CaCl_2$ extracting solution
Soil probe or auger	For field sampling
Bucket	For compositing and mixing field samples
Timer or watch	For tracking settling and reading times
Field notebook	For recording results

6.2. Consumables

Item	Notes
Nitrate test strips	Semi-quantitative colorimetric strips (e.g., Hach, EM Quant, or equivalent)
Calcium chloride ($CaCl_2$), anhydrous or dihydrate	For preparing extracting solution
Distilled or deionized water	For preparing extracting solution
Color comparison chart	Provided with test strips

7. Procedure

7.1. Preparing the Extracting Solution

1. Add 6 g (approximately 1 teaspoon) of anhydrous $CaCl_2$ to 1 gallon (3.78 L) of distilled water
2. Cap and shake until dissolved
3. This produces approximately 0.014 M $CaCl_2$, sufficient for approximately 125 tests
4. Label the container with the reagent name, concentration, and preparation date
5. Store at room temperature. The solution is stable indefinitely.

> **Note**
>
> $CaCl_2$ is used instead of plain water because the calcium ions displace nitrate from soil exchange sites, giving a more complete extraction (Gelderman & Beegle, 2015; Houba et al., 1986). The low concentration avoids interference with the test strip chemistry.

7.2. Collecting the Soil Sample

1. Collect soil from the target depth (typically 0–15 cm or 0–30 cm) using a probe or auger per SOP-F400 Soil Sampling
2. Composite multiple subsamples if a plot-level estimate is desired
3. Homogenize the soil in a bucket by hand-mixing
4. Remove visible roots and stones

7.3. Performing the Quick Test

1. Fill a graduated centrifuge tube with 30 mL of the $CaCl_2$ extracting solution
2. Add soil to the tube until the total volume reaches the 40 mL mark (approximately 10 mL of soil displaced)
3. Cap the tube securely
4. Shake vigorously until all soil is well mixed (approximately 30 seconds)
5. Allow the tube to stand upright for the soil particles to settle. Wait at least 10 minutes but no more than 1 hour.
6. Dip a nitrate test strip into the clearer supernatant near the top of the tube for 1 second. Do not stir up settled soil.

7. Remove the strip and hold horizontally
8. Compare the strip to the manufacturer's color chart at both 30 seconds and 60 seconds after dipping
9. Record the nitrate reading (ppm NO_3^--N or ppm NO_3^-, depending on the strip brand) and the reading time used

> **!** Important
>
> Read the strip at the times specified by the manufacturer. Reading too early or too late gives inaccurate results. If the strip brand reports in ppm NO_3^-, divide by 4.43 to convert to ppm NO_3^--N.

7.4. Applying the Soil Texture Correction Factor

The test strip reads the nitrate concentration in the extract solution, not in the soil. To estimate the nitrate concentration in the soil itself, apply a correction factor based on soil texture and moisture status (Gelderman & Beegle, 2015):

Texture	Moist Soil Factor	Dry Soil Factor
Sand	2.3	2.6
Loam	2.0	2.5
Clay	1.7	2.2

$$\text{Soil } NO_3^-\text{-N (ppm)} = \text{Strip reading (ppm)} \times \text{Correction factor}$$

Determine the soil texture using SOP-F401 Texture by Feel or SOP-L401 Hydrometer.

7.5. Recording Results

Record the following in the field notebook or data sheet for each test:

- Date, time, site, plot ID
- Sampling depth (cm)
- Soil condition (moist or dry)
- Estimated texture (sand, loam, or clay)
- Strip reading (ppm) and reading time (30 s or 60 s)
- Correction factor applied
- Corrected soil NO_3^--N (ppm)

8. Quality Control and Acceptance Criteria

8.1. Limitations

The test strip method is semi-quantitative. Results should be interpreted as approximate categories (low, moderate, high) rather than precise concentrations (Hartz, 2010; Schmidhalter, 2005). Key limitations include:

- Strip resolution is typically 10–25 ppm increments; fine differences are not detectable
- Color matching is subjective (ambient lighting affects perception)
- The method does not measure ammonium (NH_4^+); total inorganic N requires SOP-L403
- Soil moisture, organic matter, and temperature all influence extraction efficiency

8.2. Interpretation Guide

Corrected Soil NO_3^--N (ppm)	Interpretation	Agronomic Implication
Less than 10	Low	N likely limiting; fertilization may be warranted
10 – 25	Moderate	Adequate for many crops
25 – 50	High	Sufficient N; reduce or defer applications
Greater than 50	Very high	Excess N; potential leaching risk

These thresholds are general guidelines adapted from Magdoff et al. (1984) and Hartz (2010). Consult crop-specific guidelines and local extension recommendations.

8.3. QC Requirements

- Run a blank ($CaCl_2$ solution only, no soil) at the start of each testing session. The strip should read 0 ppm or below the lowest detection increment.
- Run a duplicate test on at least 1 in 10 samples. Duplicates should agree within one color-chart increment.
- Verify test strip expiration date before use. Expired strips may give unreliable color development.
- For critical decisions, confirm quick-test results with the quantitative KCl extraction method (SOP-L403)

9. Waste Disposal

Waste Type	Disposal Method
Soil slurry waste	Return to field site or standard trash
$CaCl_2$ solution	Drain (non-hazardous at this concentration)

Waste Type	Disposal Method
Used test strips	Standard trash
Used centrifuge tubes	Rinse and reuse, or standard trash

10. References

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11. Training Record

Before performing the nitrate quick test independently, personnel must:

1. Read this SOP in full
2. Complete SOP-F001 Field Safety training (if testing in the field) or SOP-L002 Laboratory Safety (if in the lab)
3. Prepare the $CaCl_2$ extracting solution under supervision
4. Perform at least three practice tests with acceptable blank and duplicate results
5. Demonstrate correct use of the color chart and correction factor
6. Be signed off by the PI, field lead, or lab manager

12. Appendix A: Example Calculation

```
# Nitrate quick test correction example

strip_reading_ppm <- 15       # ppm NO3-N from test strip
soil_texture <- "loam"
soil_condition <- "moist"     # "moist" or "dry"

# Correction factors
factors <- data.frame(
  texture = c("sand", "loam", "clay"),
  moist = c(2.3, 2.0, 1.7),
  dry = c(2.6, 2.5, 2.2)
)

cf <- factors[factors$texture == soil_texture, soil_condition]

corrected_ppm <- strip_reading_ppm * cf

cat(sprintf("Strip reading:     %d ppm NO3-N\n", strip_reading_ppm))
cat(sprintf("Texture:           %s\n", soil_texture))
cat(sprintf("Condition:         %s\n", soil_condition))
cat(sprintf("Correction factor: %.1f\n", cf))
cat(sprintf("Corrected value:   %.0f ppm NO3-N\n", corrected_ppm))
```

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L405

Soil Extractable Phosphorus

Standard Operating Procedure

SOP Number	SOP-L405
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the extraction and colorimetric determination of plant-available (extractable) phosphorus in soils. Two extraction methods are presented: the Olsen method (0.5 M $NaHCO_3$ at pH 8.5) for neutral to alkaline soils, and the Bray-1 method (0.03 M NH_4F + 0.025 M HCl) for acidic soils (Bray & Kurtz, 1945; Olsen et al., 1954). Extractable P is quantified colorimetrically using the ascorbic acid/molybdate blue method (Kuo, 1996; Murphy & Riley, 1962).

Soil phosphorus availability is a key factor controlling plant productivity, and excess P in runoff is a primary driver of eutrophication in freshwater systems. Routine soil P testing guides fertilizer management and helps assess the environmental risk of P loss from agricultural and urban soils (Sharpley et al., 2003).

2. Scope

- **Applies to:** Air-dried, sieved (less than 2 mm) mineral soils from all EA Program field sites. The Olsen method is the default for southern California soils, which are typically neutral to alkaline. Use Bray-1 for soils with pH below 6.5.
- **Does not apply to:** Total phosphorus determination (requires acid digestion); dissolved reactive phosphorus in water samples (see SOP-L303 Hach Colorimetric); organic phosphorus fractionation
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Prepare and standardize reagents, maintain spectrophotometer
Trained Personnel	Perform extractions and analyses, report results
New Students	Complete training before independent use

4. Definitions

- **Available phosphorus:** Phosphorus in soil solution and loosely sorbed fractions that can be taken up by plant roots; estimated by extraction with reagents that mimic root chemistry (Olsen bicarbonate in alkaline soils; Mehlich-3 or Bray in acid soils).
- **Bray P1 extraction:** An extraction using 0.025 M HCl + 0.03 M NH_4F; removes acid-soluble (Al- and Fe-bound) phosphorus; appropriate for acid and neutral soils (pH < 7.4) but not for calcareous soils.
- **Mehlich-3 extraction:** A multi-element extractant (0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.015 M NH_4F + 0.013 M HNO_3 + 0.001 M EDTA); extracts plant-available P, K, Ca, Mg, and several micronutrients simultaneously; widely used in the eastern US and California.
- **Murphy-Riley method:** The standard colorimetric procedure for measuring dissolved reactive phosphorus; reacts phosphate with ammonium molybdate and ascorbic acid to form a blue phosphomolybdenum complex measured at 880 nm (Murphy & Riley, 1962).
- **Olsen extraction:** An extraction using 0.5 M $NaHCO_3$ (pH 8.5); removes Ca-phosphate forms; the standard method for calcareous and alkaline soils (pH > 7.0); results expressed as mg P kg^{-1}.
- **Sorption:** The attachment of phosphate ions to soil mineral surfaces (primarily Fe and Al oxides in acid soils; $CaCO_3$ in alkaline soils); limits plant availability and affects the choice of extraction method.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Ascorbic acid, ammonium molybdate, and antimony potassium tartrate are mild irritants. Sulfuric acid (used in the color reagent) is corrosive at concentrated strengths. Ammonium fluoride (NH_4F, Bray-1 extractant) is toxic and corrosive; handle in the fume hood. Sodium hydroxide (NaOH, for pH adjustment of Olsen reagent) is corrosive.
- **Physical:** Standard glassware handling risks.

5.1. Required PPE

☐ Safety glasses (splash goggles when handling H_2SO_4 or NH_4F)
☐ Lab coat
☐ Nitrile gloves

☐ Work in fume hood when handling NH_4F

5.2. Emergency Procedures

For acid or NH_4F splashes, flush with water for at least 15 minutes. For NH_4F skin contact, flush with water and apply 2.5% calcium gluconate gel if available. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Reciprocating or end-over-end shaker	For extraction
Spectrophotometer or colorimeter	880 nm wavelength; Hach DR3900 (SOP-L303) or equivalent
Analytical balance (0.01 g)	See SOP-L102
pH meter	For verifying extractant pH; see SOP-L300
Centrifuge or vacuum filtration apparatus	For clarifying extracts
Volumetric flasks (100 mL, 500 mL, 1 L)	Class A
Erlenmeyer flasks (125 or 250 mL)	One per sample plus blanks
Graduated cylinders (25, 50, 100 mL)	For measuring extractant volumes
Pipettes (1–10 mL)	Class A volumetric or calibrated micropipettes
Cuvettes (1 cm path length)	Glass or polystyrene, matched
Filter funnels and Whatman No. 42 filter paper	For clarifying extracts

6.2. Reagents – Olsen Extraction

Reagent	Preparation / Notes
Sodium bicarbonate ($NaHCO_3$), 0.5 M at pH 8.5	Dissolve 42.0 g $NaHCO_3$ in approximately 900 mL DI water. Adjust to pH 8.5 with 1 M NaOH. Bring to 1 L. Prepare fresh weekly.
Activated charcoal (P-free)	For decolorizing extracts; wash with 0.5 M $NaHCO_3$ before use to remove any residual P (Olsen et al., 1954)

6.3. Reagents – Bray-1 Extraction

Reagent	Preparation / Notes
Bray-1 extractant (0.03 M NH_4F + 0.025 M HCl)	Dissolve 1.11 g NH_4F in approximately 900 mL DI water. Add 2.1 mL concentrated HCl (12 M). Bring to 1 L. Prepare in fume hood. Store in polyethylene. (Bray & Kurtz, 1945)

6.4. Reagents – Ascorbic Acid Colorimetric Determination

Reagent	Preparation / Notes
Sulfuric acid (H_2SO_4), 5 N	Dilute 70 mL concentrated H_2SO_4 (18 M) into approximately 400 mL DI water; bring to 500 mL after cooling. **Add acid to water.**
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$-$4H_2O$), 4%	Dissolve 20 g in 500 mL DI water
Antimony potassium tartrate ($K(SbO)C_4H_4O_6$-$1/2H_2O$), 0.274%	Dissolve 0.274 g in 100 mL DI water
Ascorbic acid, 1.76%	Dissolve 1.76 g in 100 mL DI water. Prepare fresh daily.
Combined color reagent	Mix in order: 50 mL 5 N H_2SO_4 + 15 mL ammonium molybdate + 5 mL antimony potassium tartrate + 30 mL ascorbic acid. Prepare fresh for each batch. (Murphy & Riley, 1962)
Phosphorus stock standard (1000 mg/L P)	Dissolve 4.394 g anhydrous KH_2PO_4 (dried at 105 C) in 1 L DI water. Stable for months at 4 C.
Phosphorus working standards	Dilute stock to 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 mg/L P in the appropriate extractant matrix ($NaHCO_3$ or Bray-1)

6.5. Reagents – General

Reagent	Notes
Deionized water (Type I or II)	From *SOP-L11*

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature until constant mass
2. Pass through a 2 mm sieve per SOP-F400 Soil Sampling
3. Determine gravimetric moisture content on a subsample per SOP-L400 to express results on a dry-weight basis

7.2. Selecting the Extraction Method

Soil pH	Recommended Extractant	Reference
6.5 or above	Olsen (0.5 M $NaHCO_3$, pH 8.5)	Olsen et al. (1954)
Below 6.5	Bray-1 (0.03 M NH_4F + 0.025 M HCl)	Bray & Kurtz (1945)

Determine soil pH per SOP-L402 before selecting the extractant. If comparing across sites with widely varying pH, consider using Mehlich-3 for all samples (consult the PI).

> **Note**
>
> The Olsen extractant ($NaHCO_3$ at pH 8.5) dissolves calcium phosphate precipitates and reduces Fe^{3+} and Al^{3+} activity, releasing adsorbed P. It works across a wide pH range but is the standard for calcareous and alkaline soils. The Bray-1 extractant dissolves Al-bound and Fe-bound P via fluoride complexation and acid dissolution; it underestimates P in calcareous soils because the acid is neutralized by free $CaCO_3$ (Kuo, 1996).

7.3. Olsen Extraction

1. Weigh 2.50 g of air-dried, sieved soil into a 125 mL Erlenmeyer flask
2. Add approximately 0.25 g of P-free activated charcoal (pre-washed with 0.5 M $NaHCO_3$)
3. Add 50 mL of 0.5 M $NaHCO_3$ extractant (pH 8.5)
4. Stopper and shake on the reciprocating shaker at 180 oscillations per minute for 30 minutes
5. Filter immediately through Whatman No. 42 filter paper into a clean flask or tube. The filtrate should be clear and colorless; if yellow-brown, the charcoal was insufficient or not pre-washed.
6. Proceed to colorimetric analysis (Section 6.5) within 24 hours. Store extracts at 4 C if analysis is delayed.

7.4. Bray-1 Extraction

1. Weigh 2.00 g of air-dried, sieved soil into a 125 mL Erlenmeyer flask
2. Add 20 mL of Bray-1 extractant (1:10 soil-to-solution ratio)
3. Shake on the reciprocating shaker at 180 oscillations per minute for 5 minutes (the short extraction time is critical; extended shaking can re-adsorb released P)
4. Filter immediately through Whatman No. 42 filter paper
5. Proceed to colorimetric analysis (Section 6.5) within 24 hours

> **Important**
>
> The Bray-1 extraction is exactly 5 minutes. Timing begins when the extractant contacts the soil. Over-extraction will underestimate available P due to re-sorption, while under-extraction will miss slowly dissolving forms.

7.5. Colorimetric Determination (Ascorbic Acid/Molybdate Blue)

This procedure follows Murphy & Riley (1962) as adapted by Kuo (1996).

1. Prepare the combined color reagent fresh (see Section 5, Reagents)
2. Pipette a 5 mL aliquot of the filtered extract into a clean test tube or cuvette
3. For Olsen extracts: acidify the aliquot with 1 drop (approximately 0.05 mL) of concentrated H_2SO_4 and swirl to expel CO_2 (the $NaHCO_3$ matrix effervesces; wait until bubbling stops before adding color reagent)
4. Add 0.8 mL of the combined color reagent to each tube
5. Mix by vortexing or inversion
6. Allow the color to develop for 15 minutes at room temperature. The solution turns blue in proportion to the phosphorus concentration.
7. Read the absorbance at 880 nm (or 882 nm) against a DI water blank within 30 minutes of color development
8. Determine the P concentration from the calibration curve (Section 6.6)

Alternative: Hach DR3900 PhosVer 3 method. If using the Hach spectrophotometer, follow the PhosVer 3 (ascorbic acid) method per SOP-L303, Program 490. Prepare standards in the same extractant matrix as the samples.

7.6. Calibration

1. Prepare a minimum of five working standards (e.g., 0, 0.1, 0.5, 1.0, 2.0, 5.0 mg/L P) by diluting the stock standard with the same extractant used for the samples
2. Treat each standard exactly as the samples (acidification for Olsen, color reagent addition, timing)
3. Read absorbance at 880 nm
4. Plot absorbance versus concentration and fit a linear regression. Acceptable r^2 is 0.995 or higher.

7.7. Calculations

Extractable P in soil (mg P / kg dry soil):

$$P_{\text{soil}} = \frac{C \times V}{M \times \left(\frac{100}{100+\theta_H}\right)}$$

where C = P concentration in the extract (mg/L), V = volume of extractant (mL), M = mass of air-dried soil (g), and θ_H = hygroscopic moisture (%, determined per SOP-L400). The term $\frac{100}{100+\theta_H}$ converts the air-dried mass to an oven-dry basis.

For the Olsen method with the standard parameters (2.50 g soil, 50 mL extractant):

$$P_{\text{soil}}\ (\text{mg/kg}) = C \times \frac{50}{2.50} \times \frac{100+\theta_H}{100} = C \times 20 \times \frac{100+\theta_H}{100}$$

For Bray-1 (2.00 g soil, 20 mL extractant):

$$P_{\text{soil}}\ (\text{mg/kg}) = C \times \frac{20}{2.00} \times \frac{100 + \theta_H}{100} = C \times 10 \times \frac{100 + \theta_H}{100}$$

8. Quality Control and Acceptance Criteria

8.1. Interpretation of Olsen P

Olsen P (mg/kg)	Classification	Agronomic Interpretation
Less than 5	Very low	P deficiency likely; fertilize
5 – 10	Low	P may limit yield; fertilize
10 – 20	Medium	Adequate for most crops
20 – 40	High	No response to added P expected
Greater than 40	Very high	Excess P; environmental risk of runoff losses

Thresholds are approximate and crop-dependent (Olsen et al., 1954; Sharpley et al., 2003). Consult local extension guidelines.

8.2. Interpretation of Bray-1 P

Bray-1 P (mg/kg)	Classification	Agronomic Interpretation
Less than 10	Very low	P deficiency likely
10 – 20	Low	Fertilize to build P levels
20 – 40	Medium	Adequate for most crops
40 – 80	High	No additional P needed
Greater than 80	Very high	Environmental risk

8.3. QC Requirements

- Prepare all standards in the same extractant matrix as the samples (matrix-matched calibration) to account for background color and ionic strength effects
- Run a reagent blank (extractant only, no soil) with each batch. The blank absorbance should be near zero at 880 nm.
- Run a laboratory reference soil with known extractable P in each batch. Recovery should be within +/- 15% of the established value.
- Run duplicate extractions on at least 10% of samples. Acceptable precision: relative percent difference (RPD) less than 15%.
- Calibration curve r^2 must be 0.995 or higher
- Check the mid-range standard after every 20 samples. Recalibrate if the check standard deviates by more than 10%.
- For Olsen extracts, verify the $NaHCO_3$ pH is 8.5 +/- 0.1 before each batch; pH drift changes extraction efficiency substantially.

9. Waste Disposal

Waste Type	Disposal Method
Olsen extracts ($NaHCO_3$)	Neutralize to pH 6–8 and drain, or collect for EH&S
Bray-1 extracts (NH_4F/HCl)	Collect in fluoride waste carboy; EH&S pickup. Do not drain.
Color reagent waste (molybdate/H_2SO_4)	Collect in inorganic waste; EH&S pickup
Used filter paper and soil	Standard trash (if non-hazardous soil)
Spent charcoal	Standard trash

Fluoride Waste

Bray-1 extracts contain ammonium fluoride. **Do not pour down the drain.** Collect all fluoride-containing waste in a labeled polyethylene carboy for EH&S hazardous waste pickup.

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11. Training Record

Before performing soil extractable P analyses independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Review the SDS for ammonium fluoride (if using Bray-1)
4. Demonstrate preparation of the extractant, color reagent, and calibration standards under supervision
5. Run a practice batch (at least 5 samples + blank + reference soil + duplicate) with acceptable QC results
6. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation in R

```r
library(tidyverse)

# --- Calibration curve ---
standards <- tibble(
  P_mg_L = c(0, 0.1, 0.5, 1.0, 2.0, 5.0),
  abs_880 = c(0.002, 0.018, 0.075, 0.148, 0.295, 0.740)
)

cal_model <- lm(abs_880 ~ P_mg_L, data = standards)
cat(sprintf("Calibration R-squared: %.5f\n", summary(cal_model)$r.squared))

# --- Sample calculation (Olsen method) ---
sample_abs <- 0.210            # Absorbance at 880 nm
soil_mass_g <- 2.50            # Air-dried soil (g)
extract_vol_mL <- 50           # Extractant volume (mL)
hygroscopic_moisture_pct <- 2.1 # Percent hygroscopic moisture

# Determine C from calibration
C_mg_L <- (sample_abs - coef(cal_model)[1]) / coef(cal_model)[2]

# Calculate soil extractable P (mg/kg dry soil)
P_soil_mg_kg <- C_mg_L * (extract_vol_mL / soil_mass_g) *
                ((100 + hygroscopic_moisture_pct) / 100)

cat(sprintf("Extract P concentration: %.3f mg/L\n", C_mg_L))
```

```
cat(sprintf("Olsen P:                %.1f mg/kg dry soil\n", P_soil_mg_kg))

# Interpret
interpret <- case_when(
  P_soil_mg_kg < 5  ~ "Very low",
  P_soil_mg_kg < 10 ~ "Low",
  P_soil_mg_kg < 20 ~ "Medium",
  P_soil_mg_kg < 40 ~ "High",
  TRUE              ~ "Very high"
)
cat(sprintf("Classification:         %s\n", interpret))
```

13. Appendix B: Reagent Preparation Quick Reference

13.1. Olsen Extractant (0.5 M $NaHCO_3$, pH 8.5)

1. Dissolve 42.0 g $NaHCO_3$ in approximately 900 mL DI water
2. Adjust pH to 8.5 with 1 M NaOH (dropwise, with stirring)
3. Bring to 1 L with DI water
4. Check pH before each batch; re-adjust if needed
5. Prepare fresh weekly

13.2. Bray-1 Extractant (0.03 M NH_4F + 0.025 M HCl)

1. In a fume hood, dissolve 1.11 g NH_4F in approximately 900 mL DI water
2. Add 2.1 mL concentrated HCl (12 M)
3. Bring to 1 L with DI water
4. Store in polyethylene at room temperature. Stable for several weeks.

13.3. Combined Color Reagent (Murphy-Riley)

Mix in order, swirling between additions:

1. 50 mL of 5 N H_2SO_4
2. 15 mL of 4% ammonium molybdate
3. 5 mL of 0.274% antimony potassium tartrate
4. 30 mL of 1.76% ascorbic acid (freshly prepared)

Total volume: 100 mL. Prepare fresh for each analytical batch.

13.4. P Stock Standard (1000 mg/L P)

1. Dry KH_2PO_4 at 105 C for 2 hours; cool in a desiccator
2. Dissolve 4.394 g in DI water in a 1 L volumetric flask
3. Bring to volume. Store at 4 C. Stable for months.

14. Appendix C: BibTeX Entries

Append these to the project `references.bib`:

15. Appendix D: Troubleshooting

Symptom	Probable Cause	Corrective Action
Yellow-brown Olsen extract	Insufficient charcoal; charcoal not pre-washed	Increase charcoal; pre-wash with 0.5 M $NaHCO_3$
Calibration curve non-linear above 5 mg/L	Beer's Law limit exceeded	Dilute high samples; add lower standards
Bray-1 P unexpectedly low on calcareous soil	Acid neutralized by $CaCO_3$	Use Olsen method for soils with pH above 6.5
Blank reads above 0.01 absorbance	Contaminated glassware or reagent	Acid-wash glassware (SOP-L101); prepare fresh reagents
Color fades after 30 min	Ascorbic acid degradation	Read within 30 min; prepare ascorbic acid solution fresh daily
Duplicates disagree by more than 15% RPD	Soil heterogeneity; inconsistent extraction timing	Homogenize soil more thoroughly; use a timer for extraction
Reference soil out of range	Extractant pH drift (Olsen); reagent degradation	Verify $NaHCO_3$ pH; remake color reagent

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L406

Soil Organic Matter – Loss on Ignition

Standard Operating Procedure

SOP Number	SOP-L406
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the estimation of soil organic matter (SOM) content using the loss-on-ignition (LOI) method. SOM is quantified by measuring the mass lost when oven-dried soil is combusted at 550 °C in a muffle furnace (Heiri et al., 2001; Nelson & Sommers, 1996).

2. Scope

- **Applies to:** Air-dried, sieved soil samples collected per SOP-F400 Soil Sampling and Processing.
- **Does not apply to:** Soils with high calcium carbonate content ($CaCO_3$ decomposes above ~450 °C and may inflate LOI values; consider an alternative method such as elemental analysis for calcareous soils (Heiri et al., 2001)).
- **Location:** EA Program laboratory, SGM 133.

3. Definitions

- **Ash-free dry mass (AFDM):** The mass remaining after removing water (drying at 105 °C) and organic matter (igniting at 550 °C); equivalent to the inorganic mineral mass of the sample.
- **Crucible:** A heat-resistant porcelain or ceramic container used to hold soil samples during furnace ignition; must be pre-ignited and weighed to constant mass before use.
- **Inorganic carbon (IC):** Carbon associated with carbonates ($CaCO_3$, $MgCO_3$); released as CO_2 during ignition above ~550 °C; can inflate LOI values in calcareous soils if ignition temperature is not controlled or if acid pre-treatment is omitted (Heiri et al., 2001).
- **Loss on ignition (LOI):** The percentage mass loss when an oven-dried soil is ignited at 550 °C for 4 hours: $LOI = (M_{105} - M_{550})/M_{105} \times 100$; used as a proxy for SOM content (Heiri et al., 2001).
- **Soil organic matter (SOM):** The organic fraction of soil, including plant and animal residues at various stages of decomposition, microbial biomass, and stabilized humus; estimated by LOI as approximately SOM $\approx 1.72 \times$ LOI (the Van Bemmelen factor) when organic carbon is the target (Nelson & Sommers, 1996).
- **Tare mass:** The pre-weighed mass of the empty, pre-ignited crucible; subtracted from all subsequent measurements to obtain sample mass.
- **Van Bemmelen factor:** The empirical conversion factor (1.724) relating organic carbon content to SOM by assuming that SOM contains 58% carbon by mass; use with caution for soils with unusual organic matter composition (Nelson & Sommers, 1996).

4. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Trained Personnel	Perform analysis, record and report results
New Students	Complete training before independent use

5. Safety and Hazards

⚠ Hazards

- **Thermal:** Muffle furnace operates at 550 °C; severe burn risk. Drying oven operates at 105 °C. Ceramic crucibles remain extremely hot for many minutes after removal from the furnace.
- **Physical:** Crucible tongs can slip; always use two hands when moving hot crucibles.

5.1. Required PPE

☐ Safety glasses
☐ Laboratory coat
☐ Heat-resistant gloves (mandatory when handling furnace-hot crucibles)

5.2. Emergency Procedures

Refer to SOP-L002 Laboratory Safety and SOP-L111 Muffle Furnace. For burns, cool with running water for at least 10 minutes and seek medical attention.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Muffle furnace	Capable of 550 ±10 °C; programmable ramp preferred
Drying oven	Regulated at 105 ±2 °C
Analytical balance	Resolution 0.0001 g (0.1 mg)
Desiccator	With active color-indicating silica gel
Crucible tongs	Heat-resistant; long-handled

6.2. Consumables

Item	Notes
Porcelain or ceramic crucibles	Pre-ignited to constant mass
Weighing paper or spatulas	For soil transfer

7. Procedure

7.1. Crucible Preparation

1. Pre-ignite crucibles in the muffle furnace at 550 °C for at least 1 hour to remove any residual organic matter.
2. Transfer to a desiccator and cool to room temperature.
3. Weigh each crucible to the nearest 0.0001 g and record as the tare mass (W_{crucible}).

7.2. Oven Drying at 105 °C

1. Using a spatula, transfer 5–10 g of air-dried, sieved soil into a pre-weighed crucible.
2. Record the mass of crucible plus moist or air-dry soil.
3. Place uncovered crucibles in the drying oven at 105 °C for at least 24 hours.
4. Transfer crucibles to a desiccator and cool to room temperature (approximately 20–30 minutes).
5. Weigh to the nearest 0.0001 g and record as W_{105} (mass of oven-dried soil only, i.e., crucible + dry soil mass minus crucible tare).

7.3. Combustion at 550 °C

1. Transfer the crucibles containing oven-dried soil directly into the muffle furnace (Heiri et al., 2001).
2. Ramp the furnace to 550 °C (if programmable, use a ramp rate of approximately 10 °C per minute to prevent crucible cracking and reduce convective loss of fine particles).
3. Hold at 550 °C for 4 hours (Heiri et al., 2001).
4. Turn off the furnace and allow crucibles to cool inside the furnace until they can be safely transferred (below approximately 200 °C).
5. Transfer crucibles to a desiccator and cool to room temperature.
6. Weigh to the nearest 0.0001 g and record as W_{550} (mass of combusted soil, excluding crucible).

7.4. Calculation

Soil organic matter content is calculated as (Heiri et al., 2001):

$$\text{SOM (\%)} = \left(\frac{W_{105} - W_{550}}{W_{105}} \right) \times 100$$

where:

- W_{105} = mass of oven-dried soil (g)
- W_{550} = mass of soil after combustion at 550 °C (g)

To convert LOI (%) to approximate organic carbon (%OC), divide by 1.724 (the Van Bemmelen factor); note that this conversion assumes constant elemental composition and is less accurate for samples with unusual organic matter (Nelson & Sommers, 1996).

8. Quality Control

- Run analytical duplicates for at least 10% of samples; coefficient of variation between duplicates should be less than 5% (Heiri et al., 2001).
- Include an empty crucible blank in each furnace run to verify no mass gain from ambient contamination.
- Include a laboratory reference soil with known SOM content in each batch.
- Verify oven (105 °C) and furnace (550 °C) temperatures using a calibrated thermocouple at regular intervals.

9. Waste Disposal

Waste Type	Disposal Method
Combusted soil residue	Standard trash (inert mineral ash)
Crucibles	Clean and reuse

10. References

- Heiri et al. (2001)
- Nelson & Sommers (1996)
- SOP-L002 Laboratory Safety
- SOP-L111 Muffle Furnace

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology*, *25*(1), 101–110. https://doi.org/10.1023/A:1008119611481

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In D. L. Sparks (Ed.), *Methods of soil analysis, part 3: Chemical methods* (pp. 961–1010). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c34

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L111	Muffle Furnace	Instrument operation
L400	Soil Moisture	Companion moisture determination
L407	Total Carbon	Direct combustion alternative

12. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L111 Muffle Furnace training
3. Demonstrate safe operation of the muffle furnace and drying oven
4. Demonstrate proper use of the analytical balance and desiccator
5. Perform the procedure under supervision with acceptable duplicate precision
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L407

Total Carbon – Shimadzu TOC-L with SSM-5000A

Standard Operating Procedure

SOP Number	SOP-L407
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the operation of the Shimadzu TOC-L total organic carbon analyzer and the SSM-5000A Solid Sample Module for quantifying dissolved organic carbon (DOC) in soil extracts and total carbon (TC) or total organic carbon (TOC) in solid soil samples (Shimadzu Corporation, 2016, 2019).

2. Scope

- **Applies to:** K_2SO_4 extracts from the chloroform fumigation-extraction method (SOP-L409); water samples; and dried, ground solid soil samples.
- **Does not apply to:** Samples with extremely high salt concentrations that exceed the instrument's tolerance (consult the instrument manual); sea water or marine sediments with high salt content.
- **Location:** EA Program analytical laboratory, SGM 133.

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, authorize instrument access
Instrument Manager	Maintain instrument, replace catalyst and consumables
Trained Personnel	Operate instrument, prepare standards and samples
New Students	Complete training before independent use

4. Definitions

- **Calibration verification standard (CVS):** A known-concentration standard analyzed at regular intervals during a run to confirm that instrument response has not drifted since initial calibration; acceptance criterion is within 10% of the certified value (Shimadzu Corporation, 2019).
- **Dissolved organic carbon (DOC):** Organic carbon in a liquid sample (water or soil extract) that passes through a 0.45 µm filter; quantified by the NPOC method on the Shimadzu TOC-L.
- **Inorganic carbon (IC):** Carbon present as carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and dissolved CO_2; removed from liquid samples by acidification and sparging; measured directly in soils at higher temperatures on the SSM-5000A.
- **NDIR detector:** Non-dispersive infrared detector; measures CO_2 evolved from sample combustion by absorption at 4.26 µm; the primary detection element of the TOC-L and SSM-5000A (Shimadzu Corporation, 2019).
- **NPOC (non-purgeable organic carbon):** The organic carbon fraction remaining after acidification and sparging to remove IC and volatile organic carbon; the operationally defined DOC fraction measured by the TOC-L for liquid samples.

- **SSM-5000A:** The Shimadzu Solid Sample Module that accepts dried, ground solid samples loaded in ceramic boats and combusts them at up to 900 °C to determine total carbon directly (Shimadzu Corporation, 2016).
- **TC (total carbon):** Sum of all carbon forms (organic and inorganic) in a sample; measured by direct high-temperature combustion of solid samples or by TC mode for liquid samples (Nelson & Sommers, 1996).
- **TOC (total organic carbon):** TC minus IC; in liquid samples, determined as NPOC; in solid soil samples, determined as TC minus IC or after acid treatment to remove carbonates before combustion (Nelson & Sommers, 1996).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Hydrochloric acid (HCl) is used to acidify liquid samples for IC removal; handle in a fume hood with appropriate PPE.
- **Thermal:** The combustion furnace operates at 680 °C (liquid mode) or up to 900 °C (SSM mode); do not contact the furnace chamber.
- **Pressurized gas:** Carrier gas (high-purity air or O_2) is supplied under pressure; inspect fittings for leaks.

5.1. Required PPE

☐ Safety glasses
☐ Laboratory coat
☐ Nitrile gloves

5.2. Emergency Procedures

Refer to SOP-L002 Laboratory Safety and the Shimadzu TOC-L instrument manual for instrument-specific emergency shutdowns.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Shimadzu TOC-L	With ASI-L autosampler; SGM 133
SSM-5000A	Solid Sample Module; SGM 133
Analytical balance	0.01 mg resolution for solid sample weighing
0.45 µm syringe filters	For liquid sample pre-filtration
Autosampler vials	Acid-washed glass or certified TOC-clean

6.2. Reagents and Consumables

Item	Notes
Potassium hydrogen phthalate (KHP)	Primary standard for C calibration
Sodium bicarbonate / carbonate	IC calibration standards
2 M HCl	For carbonate removal from solid samples
High-purity air or O_2	Carrier gas for SSM-5000A combustion
Pre-combusted ceramic boats	900 °C prior to use; one per sample
0.5 M K_2SO_4	Method blank for extract analyses

7. Procedure

7.1. Startup

1. Verify carrier gas supply pressure (typically 200 kPa or per instrument manual (Shimadzu Corporation, 2019)).
2. Turn on the TOC-L main unit and allow the furnace to reach operating temperature (680 °C for liquid mode).
3. Launch LabSolutions TOC software on the connected computer.
4. Run a system blank check using deionized water to verify baseline stability; the blank value should be below 0.1 mg/L C.

7.2. Calibration

1. Prepare a multi-point calibration curve using KHP dissolved in deionized water.
2. Typical calibration range: 0, 1, 5, 10, 25, 50, 100 mg/L C (adjust to expected sample range).
3. For IC calibration (if measuring TC - IC = TOC), prepare sodium bicarbonate/carbonate standards.
4. Run calibration standards and verify that the correlation coefficient (r^2) is at least 0.999 (Shimadzu Corporation, 2019).
5. Save the calibration curve in the software.

7.3. Liquid Sample Analysis (DOC in Soil Extracts)

1. Filter all liquid samples through 0.45 µm syringe filters or pre-combusted glass fiber filters into clean autosampler vials.
2. For K_2SO_4 extracts, include 0.5 M K_2SO_4 blanks in the run.
3. Select the appropriate measurement mode in the software:
   - **NPOC mode** (recommended for most soil extracts): The instrument acidifies the sample with HCl, sparges with carrier gas to remove IC as CO_2, then combusts the remaining sample at 680 °C. The evolved CO_2 is measured by the NDIR detector.
   - **TC-IC mode** (if both total and inorganic carbon are needed): TC is measured by direct combustion; IC is measured by acidification only. TOC = TC - IC.

4. Load vials into the autosampler and start the run.
5. The instrument performs replicate injections (typically 3) and reports the mean and coefficient of variation.

> **High-Salt Extracts**
>
> If K_2SO_4 extracts have high salt content, use the instrument's automatic dilution function to extend catalyst and combustion tube life. Dilution factors are applied automatically to the reported concentrations (Shimadzu Corporation, 2019).

7.4. Solid Sample Analysis (SSM-5000A)

1. Switch the instrument to SSM mode in the LabSolutions software (Shimadzu Corporation, 2016).
2. Pre-combust ceramic boats at 900 °C to remove any residual carbon.
3. Weigh 10–50 mg of dried, finely ground soil into a clean ceramic boat using the analytical balance (record mass to 0.01 mg).
4. For TOC measurement (inorganic carbon removal): pre-treat the sample by adding a few drops of 2 M HCl to the boat, allow to react until effervescence stops, and dry at 105 °C before analysis (Nelson & Sommers, 1996).
5. For TC measurement (no acid pre-treatment): proceed directly.
6. Place the ceramic boat into the SSM sample port.
7. The SSM combusts the sample in an oxygen-rich environment at high temperature; the evolved CO_2 is carried to the NDIR detector.
8. The software reports total carbon content in mg C or as a percentage of sample mass.

7.5. Instrument Shutdown

1. After the final sample, run 3–5 deionized water blanks to flush the system.
2. Review all data in the software for outliers or failed injections.
3. Export data and save the run file.
4. If the instrument will not be used for several days, turn off the furnace and carrier gas per the instrument manual (Shimadzu Corporation, 2019).

8. Quality Control

- Run a calibration verification standard (mid-range) every 10–15 samples; acceptance criterion is within 10% of the known value.
- Include instrument blanks (deionized water) at the beginning, middle, and end of each run.
- Include method blanks (0.5 M K_2SO_4 for extract analyses) in each batch.
- Run sample duplicates at a rate of at least 1 per 10 samples.
- Monitor the coefficient of variation among replicate injections; re-run if CV exceeds 2% for liquid samples.
- Log catalyst replacement, combustion tube changes, and calibration dates in the instrument logbook (Shimadzu Corporation, 2019).

9. Maintenance

Task	Frequency
System blank check	Before each analytical run
Calibration verification	Each run (mid-range check standard)
Combustion tube inspection	Monthly or after approximately 500 injections
Catalyst replacement	As needed (when blank values increase or sensitivity decreases)
Drain trap and humidifier check	Weekly
Carrier gas cylinder replacement	As needed (monitor pressure gauge)

10. Waste Disposal

Waste Type	Disposal Method
Acidified liquid waste	Neutralize and dispose per EH&S guidelines
Spent catalyst	Collect for EH&S hazardous waste pickup
Used ceramic boats	Clean and reuse, or dispose in standard trash
K_2SO_4 extract waste	Aqueous waste; drain if approved, or collect

11. References

- Shimadzu Corporation (2019)
- Shimadzu Corporation (2016)
- Nelson & Sommers (1996)
- SOP-L002 Laboratory Safety
- SOP-L204 Shimadzu TOC-L (Instrument)
- SOP-L409 Microbial Biomass Carbon

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In D. L. Sparks (Ed.), *Methods of soil analysis, part 3: Chemical methods* (pp. 961–1010). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c34

Shimadzu Corporation. (2016). *SSM-5000A solid sample combustion unit: User manual.*

Shimadzu Corporation. (2019). *TOC-L series total organic carbon analyzer: User manual.*

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L204	Shimadzu TOC-L Instrument	Instrument startup/shutdown SOP

SOP	Title	Relationship
L406	Loss on Ignition	Complementary SOM method
L409	Microbial Biomass C	Source of K_2SO_4 extracts
L418	Water-Extractable DOC	Water extraction extracts

13. Training Record

Before operating this instrument independently, personnel must:

1. Read this SOP and the relevant sections of the Shimadzu TOC-L and SSM-5000A user manuals (Shimadzu Corporation, 2016, 2019)
2. Complete SOP-L003 Hazardous Materials Handling training
3. Observe a trained operator perform startup, calibration, sample analysis, and shutdown
4. Demonstrate competence with calibration standard preparation
5. Perform a supervised analytical run with acceptable QC results
6. Be signed off by the instrument manager or PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L408

Soil Respiration – Solvita CO_2 Burst Test

Standard Operating Procedure

SOP Number	SOP-L408
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the measurement of soil microbial respiration using the Solvita CO_2 burst test. The method quantifies CO_2 evolution from rewetted, air-dried soil as an index of microbial activity and labile carbon availability (Haney et al., 2008a; Haney et al., 2008b).

The CO_2 burst exploits the "Birch effect," the well-documented pulse of microbial respiration that occurs when dry soil is rapidly rewetted (Birch, 1958; Fierer & Schimel, 2003). Drying and rewetting disrupts soil aggregates, lyses a fraction of the microbial biomass, and releases intracellular osmolytes, all of which provide readily mineralizable substrate that surviving microbes respire as CO_2 (Fierer & Schimel, 2002; Xiang et al., 2008). The magnitude of the burst integrates microbial biomass size, community physiological status, and the pool of labile organic carbon, making it a useful single-number indicator of biological soil fertility (Haney et al., 2008b).

2. Scope

- **Applies to:** Air-dried, sieved soil samples collected per SOP-F400 for microbial activity assessment
- **Does not apply to:** Compost maturity testing (see SOP-L413); field-moist basal respiration; long-term (7–28 day) incubation-based respirometry; or chamber-based field CO_2 flux measurements (see SOP-F406)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Lab Manager	Maintain Solvita kit inventory, verify probe expiration dates
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Basal respiration:** The rate of CO_2 production from soil under controlled, standardized conditions (specific moisture, temperature, incubation time) without additional substrate; reflects the size and activity of the soil microbial community.
- **CO_2 burst:** The pulse of CO_2 released from dry soil immediately upon rewetting; caused by osmotic stress on microorganisms, physical disruption of aggregates, and rapid oxidation of previously protected organic matter; the basis of the Solvita CO_2 Burst test.
- **Colorimetric gel pad:** The CO_2-sensitive indicator element in the Solvita kit; changes color in proportion to the CO_2 concentration in the jar headspace; read with a Solvita paddle reader or digitally.

- **Digital Color Reader (DCR):** The Solvita handheld device that reads the color of the gel pad and converts it to a numerical Solvita Index value.
- **Solvita Index:** A unitless scale (1–8) reported by the Solvita CO_2 Burst test; higher values indicate greater biological activity and organic matter availability; values correlate with potentially mineralizable nitrogen.
- **Soil microbial biomass:** The mass of living microbial cells (bacteria, fungi, protozoa) in soil; the primary driver of organic matter decomposition and nutrient cycling; estimated indirectly by CO_2 respiration or directly by fumigation-extraction (see SOP-L409).
- **Water-holding capacity (WHC):** The maximum amount of water a soil can hold after free drainage; expressed as a percentage of dry soil mass; the Solvita protocol adjusts sample moisture to a defined fraction of WHC for standardization.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Minor risk of glass breakage from incubation jars. Handle jars carefully; clean up broken glass with a brush and dustpan, not bare hands.
- **Biological:** Soil samples may contain soil-borne pathogens. Wash hands thoroughly after handling soil. Do not touch your face during the procedure.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Lab coat

6. Equipment and Materials

Item	Notes
Solvita CO_2 burst test kit	Should include enough probes for all samples, duplicates and controls.
Solvita Digital Color Reader (DCR)	For quantifying gel color change; calibrate before each batch
Incubation jars (475 mL)	Glass jars with screw-top lids
30 cc soil scoop	Provided with kit
Small beakers	Can be plastic or glass. Must fit inside incubation jar
Analytical balance	Capable of 0.1 g resolution
10ml pipette + pipette pump	For adding deionized water
Deionized water	From Milli-Q system or equivalent
Incubator or controlled room	Set to 25 +/- 1 C
Timer	24-hour countdown
Lab notebook or data sheet	For recording results

7. Procedure

7.1. Sample Preparation

1. Use soil that has been collected per SOP-F400.
2. If samples were stored at 4 C after sieving, allow them to equilibrate to room temperature (20–25 C) for at least 30 minutes before beginning the assay.
3. Sieve soils through a 2mm sieve. Use a mortar and pestle to break up soil clumps that did not pass through the sieve initially and resieve it. Discard any rocks or other materials that do not pass through the sieve.

> **Note**
>
> The CO_2 burst test is designed for air-dried soil. Drying standardizes the starting moisture across samples and maximizes the rewetting response. Results from field-moist soil are not comparable to published Solvita norms (Woods End Laboratories, 2019).

7.2. Incubation Setup

1. Measure 30ccs of the dried and sieved soils using a 30cc volume scoop and add it to a small beaker. Weight the soil (do not include weight of beaker) and record the exact mass to 0.1 g. Shake it gently to level the surface.
2. Gently sprinkle 9ccs of deionized water onto the surface of the soil using a pipette. The rewetting triggers the microbial CO_2 burst (Fierer & Schimel, 2003).
3. Remove a fresh CO_2-sensitive gel probe from its sealed foil packet. Inspect the gel: it should be attached to the probe, uniform in color with no cracks, discoloration, or signs of prior exposure. Discard compromised probes. Make sure not to touch the gel while handling the probe.
4. Insert the probe into the jar so that the gel face is exposed to the headspace above the soil surface. Do not bury the probe in soil.
5. Seal the jar immediately and securely.
6. Record the jar number, sample ID, soil mass, water volume added, and start time.

7.3. Incubation

1. Place sealed jars in the incubator set to 25 +/- 1 C
2. Incubate undisturbed for 24 hours
3. Do not open or move jars during incubation

7.4. Reading Results

1. After exactly 24 hours (+/- 15 minutes), remove the gel probe from the jar
2. Immediately insert the probe into the Solvita Digital Color Reader (DCR) and record the numerical reading

3. Record the color number and the corresponding CO_2-C concentration (mg CO_2-C kg^{-1} soil) from the DCR or the Solvita conversion table

> **Tip**
>
> Read probes promptly after removing them from the jar. Prolonged exposure to ambient air will alter the gel color and compromise the reading. The manufacturer recommends reading within 2 minutes of opening the jar.

7.5. Calculating Results

Express results on a dry-soil basis:

$$\text{CO}_2\text{-C (mg kg}^{-1}) = \frac{\text{DCR reading (mg CO}_2\text{-C)}}{\text{oven-dry soil mass (kg)}}$$

If the oven-dry mass is not known, calculate it from the air-dried mass and the gravimetric moisture content of a companion subsample determined by SOP-L400.

7.6. Cleanup

1. Dispose of used soil in the designated waste container (return to field site if uncontaminated, or standard trash)
2. Wash incubation jars thoroughly with soap and water; rinse with deionized water; dry completely before reuse
3. Dispose of used gel probes in standard trash

8. Quality Control

8.1. Routine QC

QC Element	Requirement
Duplicates	At least 1 per 10 samples; duplicates should agree within 15% relative percent difference (RPD)
Control	One empty, sealed jar with gel probe per batch; probe should read at or near baseline
Incubator temperature	Verify and record at start and end of each 24 h period; must be 25 +/- 1 C
DCR calibration	Calibrate per manufacturer instructions before each batch
Gel probe storage	Store sealed in foil packets at room temperature, away from light; check expiration dates before use

8.2. Corrective Actions

- If the blank reading exceeds the baseline by more than one color increment, check for CO_2 contamination sources (e.g., proximity to gas burners, poor jar seals) and repeat the batch
- If duplicate RPD exceeds 15%, re-run the sample pair. Persistent poor precision may indicate sample heterogeneity; increase the number of subsamples

9. Data Interpretation

The CO_2 burst is proportional to microbial biomass and labile carbon and is correlated with potential carbon, nitrogen, and phosphorus mineralization (Haney et al., 2008b). Typical ranges for the Solvita CO_2 burst test on agricultural and wildland soils are given below, adapted from Haney et al. (2008a) and the Solvita technical literature (Woods End Laboratories, 2019):

CO_2-C (mg kg^{-1})	Interpretation	Implication
< 20	Very low	Severely depleted microbial activity; very low labile C
20–50	Low	Limited biological activity; management intervention may be needed
50–100	Moderate	Adequate microbial activity for many soils
100–150	High	Active microbial community; good labile C supply
> 150	Very high	Highly active soil; common in recently amended or high-organic soils

! Important

These thresholds are general guidelines. Interpret results in the context of soil type, land use, climate, and management history. Comparisons are most meaningful when made across treatments within a single study using the same sampling and analytical protocol.

10. Waste Disposal

Waste Type	Disposal Method
Used soil	Return to field site or standard trash
Used gel probes	Standard trash
Deionized water rinse	Drain
Incubation jars	Wash, rinse, and reuse

11. References

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*(1), 9–31. https://doi.org/10.1007/BF01343734

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Fierer, N., & Schimel, J. P. (2003). A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal*, *67*(3), 798–805. https://doi.org/10.2136/sssaj2003.7980

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12. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	Sample collection and preparation
F406	Soil Respiration Chambers	Field respiration complement
L002	Laboratory Safety	Required safety training
L400	Soil Moisture (Gravimetric)	Moisture correction for dry-mass basis
L406	Loss on Ignition (SOM)	Complementary organic matter index
L409	Microbial Biomass Carbon (CFE)	Direct biomass measurement
L413	Solvita Compost Maturity	Related Solvita protocol for compost

13. Training Record

Before performing this assay independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training

3. Complete SOP-F400 Soil Sampling training (for sample preparation context)
4. Review the Solvita CO_2 Burst protocol sheet included in the kit
5. Observe a trained operator perform the full procedure (setup through DCR reading)
6. Perform the assay under supervision with acceptable QC results (duplicate RPD within 15%; blank within acceptable range)
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L409

Microbial Biomass Carbon – Chloroform Fumigation-Extraction

Standard Operating Procedure

SOP Number	SOP-L409
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of soil microbial biomass carbon (MBC) using the chloroform fumigation-extraction (CFE) method. Chloroform vapor lyses microbial cells, releasing intracellular carbon that is then extracted with 0.5 M K_2SO_4 and quantified as dissolved organic carbon (DOC) (Joergensen, 1996; Vance et al., 1987).

2. Scope

- **Applies to:** Field-moist soil samples collected per SOP-F400 Soil Sampling and Processing for microbial biomass assessment.
- **Does not apply to:** Air-dried or frozen soils (unless pre-validated for this method); microbial biomass nitrogen (requires additional analytical steps).
- **Location:** EA Program laboratory, SGM 133.

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure safety compliance
Trained Personnel	Perform fumigation and extraction, report results
New Students	Complete training and safety orientation before use

4. Definitions

- **Chloroform fumigation-extraction (CFE):** The standard method for measuring soil microbial biomass carbon; soil is fumigated with ethanol-free chloroform to lyse microbial cells, then extracted with K_2SO_4; MBC is calculated as the difference in extractable C between fumigated and unfumigated soils divided by the k_{EC} factor (Vance et al., 1987).
- **Extractable organic carbon (EOC):** The organic carbon recovered from soil in K_2SO_4 extract; includes a background pool of soluble organic carbon from non-microbial sources as well as carbon released from lysed microbial cells.
- k_{EC} **factor:** The extraction efficiency correction factor for microbial biomass carbon; commonly 0.45, meaning 45% of lysed microbial carbon is recovered in the extract; must be stated when reporting results (Joergensen, 1996; Vance et al., 1987).
- **Microbial biomass carbon (MBC):** The total carbon contained in the living soil microbial community (bacteria, fungi, protozoa, nematodes); expressed as mg C kg^{-1} oven-dry soil; a key indicator of soil biological health and nutrient cycling potential (Robertson et al., 1999).
- **Potentially mineralizable nitrogen (PMN):** The nitrogen that can be released from soil organic matter by microbial activity under aerobic conditions; correlated with microbial biomass and organic matter quality.
- **Unfumigated control:** Soil extracted with K_2SO_4 without prior chloroform fumigation; provides the background extractable carbon value subtracted from the fumigated extract to calculate MBC (Vance et al., 1987).

5. Safety and Hazards

5.1. Critical Safety Warning

Chloroform ($CHCl_3$) is a volatile, toxic solvent and a suspected carcinogen. All fumigation steps must be performed inside a fume hood. Do not inhale chloroform vapors. Use ethanol-free chloroform only (Food and Agriculture Organization of the United Nations, 2024).

5.2. Hazards

- **Chemical:** Chloroform (toxic, volatile, suspected carcinogen); 0.5 M K_2SO_4 (mild irritant).
- **Physical:** Vacuum desiccator under reduced pressure; risk of implosion if cracked.

5.3. Required PPE

☐ Nitrile gloves (double-gloving recommended when handling chloroform)
☐ Safety glasses or goggles
☐ Laboratory coat
☐ Work inside a fume hood at all times during fumigation

5.4. Emergency Procedures

In case of chloroform spill, refer to the SDS and SOP-L003 Hazardous Materials Handling. Evacuate the area if the spill is large; ventilate the space and notify the supervisor.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Vacuum desiccator	Glass, with stopcock and vacuum line
Vacuum pump	Oil-free preferred
Fume hood	Required for all chloroform handling
Orbital or end-over-end shaker	For extraction step
Centrifuge	Capable of 4,000 × g (optional)
Filtration apparatus	With 0.45 µm glass fiber filters
Analytical balance	0.01 g resolution
Incubator or dark cabinet	For 24-hour fumigation at room temperature
TOC analyzer	Shimadzu TOC-L; see SOP-L407

6.2. Reagents and Consumables

Item	Notes
Ethanol-free chloroform	ACS grade; stored in fume hood
0.5 M K_2SO_4 solution	Prepare fresh or within 1 week
Glass fiber filters (0.45 µm)	Pre-combusted at 450 °C for 4 hours
Boiling chips or glass beads	For chloroform evaporation under vacuum
Extraction bottles (100 mL)	Screw-top, HDPE or glass
Filter funnels and flasks	Clean, acid-washed
Sample vials	Acid-washed, for filtered extracts

7. Procedure

7.1. Preparation

1. Prepare 0.5 M K_2SO_4 by dissolving 87.1 g K_2SO_4 per liter of deionized water. The 1:4 soil-to-extractant ratio is standard for CFE (Vance et al., 1987).
2. Pre-combust glass fiber filters at 450 °C for 4 hours and store in a clean container.
3. Determine the gravimetric moisture content of a separate subsample (dry at 105 °C for 24 hours per SOP-L400) so results can be expressed on an oven-dry-weight basis.

7.2. Fumigation

1. Weigh two paired subsamples of 15 g field-moist soil into labeled extraction bottles.
2. Place one set (fumigated) into the vacuum desiccator inside the fume hood.
3. Place a beaker containing approximately 30 mL of ethanol-free chloroform and a few boiling chips in the center of the desiccator.
4. Evacuate the desiccator using the vacuum pump until the chloroform begins to boil vigorously (approximately 2 minutes) (Vance et al., 1987).
5. Close the stopcock and incubate in the dark at room temperature (approximately 25 °C) for 24 hours (Food and Agriculture Organization of the United Nations, 2024; Vance et al., 1987).
6. The second set (unfumigated controls) is extracted immediately (proceed to §6.3).

7.3. Extraction

1. After 24 hours, release the vacuum inside the fume hood and remove the chloroform beaker.
2. Evacuate the desiccator repeatedly (3–5 times) to remove residual chloroform vapor from the soil.
3. Add 60 mL of 0.5 M K_2SO_4 to each bottle (both fumigated and unfumigated), achieving a 1:4 soil-to-extractant ratio (Vance et al., 1987).
4. Cap bottles and shake on an orbital or end-over-end shaker for 1 hour.
5. Allow the suspension to settle briefly, or centrifuge at 4,000 × g for 10 minutes.
6. Filter the supernatant through a pre-combusted 0.45 µm glass fiber filter into a clean sample vial.
7. Store filtered extracts at 4 °C if not analyzed immediately; analyze within 48 hours.

7.4. DOC Analysis

1. Analyze filtered extracts for dissolved organic carbon using the Shimadzu TOC-L per SOP-L407 Total Carbon.
2. Run 0.5 M K_2SO_4 blanks alongside samples.

7.5. Calculation

Microbial biomass carbon is calculated as (Joergensen, 1996; Vance et al., 1987):

$$\text{MBC} = \frac{C_{\text{fumigated}} - C_{\text{unfumigated}}}{k_{EC}}$$

where:

- $C_{\text{fumigated}}$ = DOC in the fumigated extract (mg C kg^{-1} oven-dry soil)
- $C_{\text{unfumigated}}$ = DOC in the unfumigated extract (mg C kg^{-1} oven-dry soil)
- $k_{EC} = 0.45$, the extraction efficiency coefficient (Joergensen, 1996; Vance et al., 1987)

> **Note**
>
> The unfumigated extract DOC value represents the background dissolved organic carbon pool and is often reported separately as an indicator of labile soil carbon (Robertson et al., 1999).

8. Quality Control

- Run analytical triplicates for at least 10% of samples; the coefficient of variation among replicates should be less than 15% (Food and Agriculture Organization of the United Nations, 2024).
- Include a reagent blank (0.5 M K_2SO_4 only, no soil) in each batch.
- Include a laboratory reference soil in each batch to monitor method consistency.
- Verify that the TOC calibration $r^2 \geq 0.999$ before reporting results.

9. Waste Disposal

Waste Type	Container	Disposal Method
Chloroform waste	Halogenated waste jug	EH&S hazardous waste pickup
K_2SO_4 extracts	Aqueous waste container	Drain with copious water (if approved) or collect for EH&S
Used soil	Solid waste bag	Standard trash
Used glass fiber filters	Standard trash	Standard trash

10. References

- Vance et al. (1987)
- Joergensen (1996)
- Food and Agriculture Organization of the United Nations (2024)
- Robertson et al. (1999)
- SOP-L003 Hazardous Materials Handling
- SOP-L400 Soil Moisture
- SOP-L407 Total Carbon

Food and Agriculture Organization of the United Nations. (2024). *Standard operating procedure for soil microbial biomass (Carbon): Chloroform fumigation-extraction method*. FAO. https://www.fao.org/soils-portal/soil-survey/soil-analysis/en/

Joergensen, R. G. (1996). The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_C value. *Soil Biology and Biochemistry*, *28*(1), 25–31. https://doi.org/10.1016/0038-0717(95)00102-6

Robertson, G. P., Coleman, D. C., Bledsoe, C. S., & Sollins, P. (1999). *Standard soil methods for long-term ecological research*. Oxford University Press.

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

11. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials	Chloroform handling
L400	Soil Moisture	Required companion analysis
L407	Total Carbon	DOC analysis of extracts
L408	Solvita CO_2 Burst	Complementary respiration index

12. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for chloroform
3. Observe a trained operator perform the full fumigation-extraction procedure
4. Demonstrate competence with vacuum desiccator operation
5. Perform the procedure under supervision
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L410

Hot-Plate Acid Digestion of Soils for Trace Metal Analysis

Standard Operating Procedure

SOP Number	SOP-L410
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the open-vessel, hot-plate acid digestion of soil, sediment, and sludge samples for near-total extraction of lead (Pb) and other trace metals, following US EPA Method 3050B (U.S. Environmental Protection Agency, 1996). The digested extract can be analyzed by ICP-OES, ICP-MS, or flame/ graphite furnace AAS. This is a near-total-dissolution method; silicates and some refractory oxides are not fully dissolved, but environmentally available metals are recovered.

For microwave-assisted digestion using the MARS 5 system, see SOP-L411. The microwave method is faster but processes fewer samples per batch; the hot-plate method described here is slower but requires only standard fume-hood equipment.

2. Scope

- **Applies to:** Soils, sediments, and sludge samples requiring trace metal analysis (Pb, Cd, Cu, Zn, Cr, As, Ni, Fe, Mn, and others), including urban soil lead surveys and photovoltaic panel site characterizations
- **Does not apply to:** Water samples (direct acidification or separate digestion); samples requiring total silicate dissolution (HF-based methods); microwave digestion (see SOP-L411)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain equipment, stock reagents
Trained Personnel	Perform digestion per this SOP
New Students	Complete training before independent use; supervised for first digestion

4. Definitions

- **Aqua regia:** A 3:1 (v/v) mixture of HCl and HNO_3; dissolves noble metals (gold, platinum) and many sulfide minerals not attacked by HNO_3 alone; more aggressive than single-acid digestion but rarely necessary for soil and sediment.

- **Certified reference material (CRM):** A soil or sediment with certified elemental concentrations from an accredited producer (NIST, USGS); run alongside every digestion batch to verify accuracy.
- **Digestion blank (method blank):** A vessel containing only acid reagents and no soil, processed through the entire digestion procedure; detects contamination from acids, vessels, or the hot plate environment.
- **EPA Method 3050B:** The US EPA hot-plate acid digestion procedure for soils, sediments, and sludges; uses repeated refluxes with HNO_3 and H_2O_2 to dissolve environmentally available trace metals; the reference method for this SOP.
- **Matrix spike:** A sample to which a known concentration of analyte has been added before digestion; used to assess matrix-specific recovery and method accuracy.
- **Partial digestion:** Dissolution of the environmentally available or acid-soluble metal fraction of a sample (EPA 3050B); does not dissolve silicate minerals; appropriate for assessing bioavailability and regulatory compliance; distinguished from total digestion (which requires HF).
- **Reflux:** Heating an acid-sample mixture such that acid vapors condense and return to the vessel; increases digestion efficiency for resistant matrices.

5. Safety and Hazards

5.1. Critical Safety Warning

This procedure uses concentrated mineral acids (HNO_3, HCl) and hydrogen peroxide (H_2O_2) at elevated temperatures in open vessels. All acid handling and digestion must be performed in a certified chemical fume hood.

5.2. Hazards

- **Chemical:** Concentrated HNO_3 (15.8 M) is a severe oxidizer and corrosive. Concentrated HCl (12 M) is severely corrosive. 30% H_2O_2 is a reactive oxidizer that can cause exothermic reactions with organic matter.
- **Physical:** Hot plate operates at approximately 95 C; digestion vessels and watch glasses are hot. Burns from contact with heated glassware or splashing acid.
- **Inhalation:** HNO_3 digestion produces toxic NO_x fumes. HCl produces irritating HCl vapor. All heating must occur in the fume hood.

5.3. Required PPE

☐ Chemical splash goggles or face shield
☐ Lab coat
☐ Acid-resistant gloves (neoprene or heavy-duty nitrile, long-cuffed)
☐ Closed-toed shoes

5.4. Emergency Procedures

For acid splashes to skin or eyes, flush immediately at the eyewash station or safety shower for at least 15 minutes. For inhalation of fumes, move to fresh air. For acid spills, neutralize small spills with sodium bicarbonate; for large spills, evacuate and use the spill kit. Refer to SOP-L003 Hazardous Materials Handling.

> **!** Important
>
> Never add water to concentrated acid. Add acid slowly to samples. Never allow digestion vessels to go to dryness; this can volatilize analytes and damage vessels.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Hot plate with temperature control	Capable of maintaining 95 C; placed inside fume hood
Digestion vessels	Teflon (PFA) beakers, glass beakers, or borosilicate digestion tubes
Watch glasses	Sized to cover the digestion vessels
Analytical balance (0.001 g)	See SOP-L102
Fume hood	Required for all steps involving acid and heating
Volumetric flasks (50 mL, Class A)	For final dilution
Graduated pipettes or dispensers	For acid delivery
Filter funnels and Whatman No. 41 filter paper	For clarifying digests
Tongs or heat-resistant clamps	For handling hot vessels

6.2. Reagents

Reagent	Grade / Notes
Nitric acid (HNO_3), concentrated (15.8 M)	Trace Metal grade
Hydrochloric acid (HCl), concentrated (12 M)	Trace Metal grade
Hydrogen peroxide (H_2O_2), approximately 30%	ACS reagent grade
Deionized water (Type I)	From *SOP-L11*

> **Note**
>
> All reagents must be Trace Metal grade or equivalent. Analyze a reagent blank before each batch; the blank must read below the method detection limit (MDL) for all target analytes.

7. Procedure

7.1. Sample Preparation

1. Air-dry the soil at room temperature or at 60 C until constant mass
2. Pass through a No. 10 sieve (2 mm) per SOP-F400 Soil Sampling
3. Homogenize thoroughly using a mortar and pestle
4. Determine gravimetric moisture on a companion subsample per SOP-L400

7.2. Glassware Cleaning

Acid-wash all vessels, volumetric flasks, and watch glasses per SOP-L101 Glassware Cleaning, Section 6.3 (10% HNO_3 acid bath). Rinse with reagent water and dry in a clean environment.

7.3. Weighing

1. Weigh 0.50–1.00 g of prepared soil into a clean digestion vessel
2. Record the exact mass to the nearest 0.001 g in the lab notebook

7.4. Initial Nitric Acid Digestion

1. Working in the fume hood, add approximately 10 mL of concentrated HNO_3 to the vessel
2. Cover with a watch glass
3. Place on the hot plate set to approximately 95 C. Do not allow the solution to boil.
4. Reflux gently until the initial vigorous reaction subsides and the volume begins to reduce. Do not allow the sample to go to dryness.
5. Continue heating for approximately 30–60 minutes, periodically lifting the watch glass to rinse the underside with DI water back into the vessel

7.5. Oxidation with H_2O_2

1. Remove the vessel from the hot plate and allow to cool slightly
2. Add 1–2 mL of 30% H_2O_2 dropwise. Wait for effervescence to subside between drops.
3. Return to the hot plate and resume heating at 95 C
4. Repeat the H_2O_2 additions until the reaction is minimal (typically 2–4 additions) and the digest appears light-colored

> ⚠ Warning
>
> Add H_2O_2 very slowly. The reaction with organic matter is exothermic and can cause violent spattering if added too quickly, especially to hot solutions. Always cool the vessel first.

7.6. HCl Treatment

1. Add approximately 5 mL of concentrated HCl to the digest
2. Cover and heat at approximately 95 C for 15–30 minutes
3. This step dissolves residual metal precipitates and improves recovery of metals such as Fe, Al, and Pb

7.7. Volume Reduction

1. Remove the watch glass and continue heating at approximately 95 C
2. Reduce the volume to approximately 5 mL
3. Monitor closely and do not allow the sample to go to dryness

> ! Important
>
> Evaporation to dryness will cause loss of volatile analytes (As, Se, Hg) and can bake residues onto the vessel walls, making quantitative recovery impossible.

7.8. Dilution and Filtration

1. Remove from the hot plate and allow to cool to room temperature
2. Rinse the vessel walls and watch glass with small portions of DI water
3. If undissolved material is present, filter through Whatman No. 41 filter paper into a clean 50 mL volumetric flask, rinsing the filter and vessel quantitatively
4. If the solution is clear, transfer directly to the volumetric flask
5. Bring to 50.0 mL with DI water
6. Mix thoroughly
7. Transfer to a clean, labeled polyethylene or polypropylene storage bottle. Store at 4 C until analysis.

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

- **Method blank:** Include one vessel containing reagents only (no soil) in each digestion batch. The blank must read below the MDL for all target analytes.
- **Certified reference material (CRM):** Digest a CRM with known metal concentrations in each batch. Recovery: 80–120%.

- **Matrix spike:** Spike a duplicate sample with a known concentration of the target analyte(s). Recovery: 75–125%.
- **Duplicate digestions:** At least 10% of samples. Relative percent difference (RPD) less than 20%.
- **Calibration verification:** Verify with a mid-range check standard every 10 samples during instrumental analysis.

8.2. Calculations

Metal concentration in soil (mg/kg dry weight):

$$C_{\text{soil}} = \frac{C_{\text{digest}} \times V_{\text{final}}}{M_{\text{soil}}}$$

where C_{digest} = concentration in the digest (mg/L), V_{final} = final volume (mL, typically 50), and M_{soil} = mass of dried soil (g).

For soil dried at 60 C, apply a moisture correction to report on a 105 C oven-dry basis (see SOP-L400):

$$C_{\text{soil, OD}} = C_{\text{soil}} \times \frac{100 + \theta_{60}}{100 + \theta_{105}}$$

9. Waste Disposal

Waste Type	Disposal Method
Acid digest waste (all liquids from digestion)	Collect in labeled acid waste carboy; EH&S hazardous waste pickup
Used filter paper	Acid waste stream (contaminated with metals and acid)
Digested soil residue	Acid waste carboy
Spent H_2O_2	Small volumes may go into acid waste; large volumes, consult EH&S

Acid Waste

All waste from this procedure contains concentrated mineral acids and dissolved metals. **Do not pour down the drain.** Collect in a labeled polyethylene carboy for EH&S hazardous waste pickup.

10. References

- USEPA. (1996). Method 3050B: Acid digestion of sediments, sludges, and soils. Revision 2. In: *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods* (SW-846). U.S. Environmental Protection Agency, Washington, DC.

- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

U.S. Environmental Protection Agency. (1996). *Method 3050B: Acid digestion of sediments, sludges, and soils* (SW-846, Revision 2). U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

11. Training Record

Before performing hot-plate acid digestion independently, personnel must:

1. Read this SOP, SOP-L002 Laboratory Safety, and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for concentrated HNO_3, HCl, and 30% H_2O_2
3. Observe a trained operator perform a complete digestion cycle
4. Perform at least one complete digestion batch under direct supervision, including blank and CRM, with acceptable QC results
5. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation

```
# Trace metal concentration from hot-plate digest
# EPA 3050B

C_digest_mg_L <- 1.24      # Pb concentration from ICP-MS (mg/L)
V_final_mL <- 50.0         # Final dilution volume (mL)
M_soil_g <- 0.752           # Mass of dried soil digested (g)

C_soil_mg_kg <- (C_digest_mg_L * V_final_mL) / M_soil_g

cat(sprintf("Digest concentration: %.3f mg/L Pb\n", C_digest_mg_L))
cat(sprintf("Soil mass:           %.3f g\n", M_soil_g))
cat(sprintf("Final volume:        %.1f mL\n", V_final_mL))
cat(sprintf("Soil Pb:             %.1f mg/kg\n", C_soil_mg_kg))

# Screening levels
cat(sprintf("\nCA DTSC residential screening level: 80 mg/kg\n"))
cat(sprintf("EPA RSL residential: 400 mg/kg\n"))
```

13. Appendix B: Method Comparison – Hot Plate vs. Microwave

Feature	Hot Plate (EPA 3050B, this SOP)	Microwave (EPA 3051A, SOP-L411)
Vessel type	Open (reflux under watch glass)	Sealed (pressurized)
Temperature	Approximately 95 C	175 C
Digestion time	2–4 hours (hands-on)	10 minutes (automated)
Sample throughput	Limited by hot plate space	Up to 24 vessels per run
Equipment required	Hot plate + fume hood	MARS 5 microwave system
Acid volume per sample	Approximately 15 mL total	10 mL
Organic matter handling	H_2O_2 oxidation step	Sealed vessel; pre-digest if vigorous
Metal recovery	Comparable for most elements	Comparable; slightly higher for volatile elements due to sealed system
Best for	Small batches; no access to microwave	Routine batches; time-sensitive work

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L411

Microwave-Assisted Acid Digestion of Soils for Trace Metal Analysis

Standard Operating Procedure

SOP Number	SOP-L411
Revision	0.2
Author(s)	Marc Los Huertos, Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the microwave-assisted acid digestion of soil, sediment, and sludge samples for trace metal analysis, following US EPA Method 3051A (U.S. Environmental Protection Agency, 2007). The digested extract can be analyzed by flame atomic absorption spectrometry (FAAS), inductively coupled plasma mass spectrometry (ICP-MS), or other suitable methods. This is a near-total-dissolution method; silicates and some refractory oxides (SiO_2, TiO_2) may not fully dissolve but environmentally available metals are recovered.

For non-microwave hot-plate acid digestion, see *SOP-L65*.

2. Scope

- **Applies to:** Soils, sediments, and sludge samples requiring trace metal analysis (Pb, Cd, Cu, Zn, Cr, As, Ni, Fe, Mn, and others) at all EA Program sites, including urban soil lead surveys and photovoltaic panel site characterizations
- **Does not apply to:** Water samples (direct acidification or separate digestion per EPA 3005A); samples requiring complete silicate dissolution (use HF-based methods); organic tissue digestion
- **Location:** Advanced Laboratory, Seaver North (MARS 5 microwave); EA Program laboratory, SGM 133 (sample preparation and filtration)

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain MARS 5 microwave, stock reagents
Trained Personnel	Perform digestion and filtration per this SOP
New Students	Complete training before independent use; must be supervised for first digestion run

4. Definitions

- **Closed-vessel digestion:** Digestion performed in sealed vessels under pressure; higher temperatures are achievable (up to 240 °C) compared to open-vessel hot-plate digestion; reduces loss of volatile elements and acid consumption.

- **EPA Method 3051A:** The US EPA microwave-assisted acid digestion method for soils, sediments, and sludges; uses HNO_3 (with optional HCl) in closed PTFE vessels at controlled temperature and pressure; the reference method for this SOP.
- **MARS system (Microwave Accelerated Reaction System):** The CEM Corporation microwave digestion system used in the EA Program lab; controls temperature and pressure in each vessel during digestion.
- **Maximum allowable working pressure (MAWP):** The pressure limit of the digestion vessel specified by the manufacturer; exceeding this limit risks vessel rupture; monitored by the MARS control software.
- **PTFE (polytetrafluoroethylene):** A chemically inert fluoropolymer used to fabricate digestion vessels; resistant to concentrated acids and temperatures up to ~260 °C; must be pre-cleaned with HNO_3 before use to remove trace metal contamination.
- **Ramp time:** The programmed time over which the microwave system increases temperature from ambient to the target; too-rapid ramping can generate excessive pressure and risk vessel failure.

5. Safety and Hazards

5.1. Critical Safety Warning

This procedure uses concentrated nitric acid (HNO_3) under high temperature and pressure in sealed vessels. Improper handling can cause severe acid burns, toxic fume exposure, or vessel rupture. Read the entire procedure and the MARS 5 operator's manual before beginning.

5.2. Hazards

- **Chemical:** Concentrated HNO_3 (15.8 M) is severely corrosive and produces toxic NO_x fumes. Optional HCl (12 M) is also severely corrosive. Both cause severe burns on skin and eye contact.
- **Physical:** Microwave vessels reach 175 C and develop significant internal pressure. Residual pressure and acid vapor remain in the headspace after digestion. Hot vessels can cause burns.
- **Inhalation:** NO_x and HCl fumes are toxic. All acid handling and vessel venting must occur in the fume hood.

5.3. Required PPE

☐ Chemical splash goggles (not safety glasses)
☐ Face shield (when opening hot vessels)
☐ Lab coat
☐ Neoprene or heavy-duty acid-resistant gloves (long-cuffed)
☐ Closed-toed shoes

5.4. Emergency Procedures

For acid splashes to skin or eyes, flush immediately at the eyewash station or safety shower for at least 15 minutes. For inhalation of fumes, move to fresh air and seek medical attention if

symptoms persist. For vessel rupture or spill, evacuate the area and notify the lab manager. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
MARS 5 Microwave Accelerated Reaction System	CEM Corporation; with pre-programmed EPA 3051A method
Microwave digestion vessels with carbon fiber sleeves	Must be compatible with the MARS 5; pressure-relief mechanism required
Temperature and pressure sensors (if applicable)	Consult instrument supervisor
Fume hood	Required for all acid handling and vessel venting
Analytical balance (0.001 g)	See SOP-L102
Centrifuge	Capable of 2,000–3,000 rpm; see *SOP-L109* or *SOP-L108*
Mortar and pestle	For grinding dried soil
No. 10 sieve (2 mm mesh)	For sieving ground soil
Drying oven	Set to 60 C; see SOP-L105

6.2. Consumables

Item	Cat. No. / Notes
50 mL graduated centrifuge tubes	For dilution and centrifugation
15 mL centrifuge tubes	For filtered digest storage
Disposable syringes (10 mL)	Acid-resistant polypropylene
Syringe filters, 0.45 um, 25 mm	Fisherbrand Cat. No. 09-719D
100 mL beakers or aluminum crinkle dishes	For drying samples

6.3. Reagents

Reagent	Grade / Notes
Nitric acid (HNO_3), concentrated (15.8 M)	Trace Metal grade; Fisher A509P500 or equivalent
Hydrochloric acid (HCl), concentrated (12 M) (optional)	Trace Metal grade; for improved recovery of Al, Sb, Ba, Be, Cr, Fe, Mg, Ag, V (U.S. Environmental Protection Agency, 2007)

Reagent	Grade / Notes
Reagent water (Type I)	From *SOP-L11*; must be interference-free at target MDLs

> **Note**
>
> All reagents must be Trace Metal grade or equivalent. Analyze a reagent blank before use; the blank must read below the method detection limit (MDL) for all target analytes.

7. Procedure

7.1. Glassware and Vessel Cleaning

1. Acid-wash all vessels, centrifuge tubes, and volumetric ware per SOP-L101 Glassware Cleaning, Section 6.3 (10% HNO_3 acid bath)
2. Rinse with reagent water and dry in a clean environment
3. For microwave vessels, follow the manufacturer's cleaning protocol (CEM recommends a cleaning cycle with dilute HNO_3 between batches)

7.2. Sample Preparation

1. Weigh approximately 3–5 g of soil into a clean beaker or aluminum dish. Avoid obviously non-soil material (large rocks, twigs, leaves).
2. Dry at 60 C overnight in the drying oven. Do not exceed 60 C; higher temperatures can volatilize mercury and other semi-volatile metals.
3. Allow to cool. Using a clean mortar and pestle, grind the dried soil and pass through a No. 10 sieve (2 mm).

7.3. Microwave Digestion

1. Weigh 0.500 g (+/- 0.001 g) of the sieved, dried soil into a clean microwave vessel. For oil-contaminated or high-organic samples, use no more than 0.250 g. Record the exact mass.
2. Working in the fume hood, add 10.0 mL (+/- 0.1 mL) of concentrated HNO_3 (Trace Metal grade) to the vessel.

> **Vigorous Reaction**
>
> Adding acid to samples with high organic matter may cause immediate effervescence. If a vigorous reaction occurs, leave the vessel uncapped in the fume hood for 1–2 hours to allow pre-digestion before sealing.

3. *Optional:* For improved recovery of Al, Sb, Ba, Be, Cr, Fe, Mg, Ag, and V, substitute 9 mL HNO_3 + 3 mL concentrated HCl (aqua regia variant). Note: HCl can increase interferences with some ICP-MS analyses; consult the analyst.

4. Seal the vessels according to the manufacturer's instructions (CEM Corporation, 2015)
5. Place each sealed vessel into a slot on the vessel rack **with a carbon fiber sleeve in place**

7.4. Critical

Never place a vessel into a rack slot without its carbon fiber sleeve. The sleeve provides essential containment in the event of a vessel failure. Operating without the sleeve risks vessel explosion.

6. Secure the rack into the MARS 5 microwave
7. If applicable, connect the temperature and pressure sensors to the designated control vessel(s)
8. Select the pre-programmed EPA 3051A method. Verify the following parameters:

Parameter	Specification (EPA 3051A)
Ramp to 175 C	5.5 +/- 0.25 minutes
Hold at 175 +/- 5 C	4.5 minutes (remainder of 10 min total)
Maximum pressure	Per vessel rating; relief mechanism must activate before failure

9. With instructor verification that all vessels are sealed and the rack is properly installed, close the microwave door and start the program
10. After the program finishes, allow the vessels to cool inside the microwave for at least 5 minutes, then transfer to the fume hood for further cooling

7.5. Filtration and Extract Preparation

1. Working in the fume hood, carefully uncap each vessel and vent for 2–3 minutes to release residual acid vapor and pressure
2. Quantitatively transfer the digest into a clean 50 mL graduated centrifuge tube, rinsing the vessel walls with small portions of reagent water to ensure complete transfer
3. Dilute to the 25.0 mL mark with reagent water. Record the final volume. Keep all samples as close to the same volume as possible.
4. Cap and centrifuge at 2,000–3,000 rpm for 10 minutes or until the supernatant is clear

> **Note**
>
> Refractory materials (SiO_2, TiO_2, Al_2O_3) may remain undissolved and cause persistent turbidity. If the supernatant is not clear after centrifugation, allow the sample to stand overnight before filtering.

5. Using a clean 10 mL syringe, carefully withdraw 10 mL of supernatant without disturbing the pellet
6. Attach a fresh 0.45 um syringe filter (Fisherbrand 09-719D) and filter the supernatant into a clean 15 mL centrifuge tube
7. Use a new syringe and filter for each sample
8. Cap and label the filtered extract. Store at 4 C until analysis.

7.6. Disposal and Cleanup

1. Collect all acid digestion waste (vessel rinses, supernatant residues, centrifuge pellets in acid) in a labeled acid waste carboy in the fume hood. EH&S pickup.
2. Rinse and clean microwave vessels per the manufacturer's protocol
3. Dispose of used syringe filters and syringes in the acid waste stream (they are contaminated with concentrated HNO_3 digest)
4. Wipe down all bench surfaces with DI water
5. Remove and properly dispose of gloves; wash hands thoroughly

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

- **Reagent blank:** Include one vessel containing only 10 mL HNO_3 (no soil) in each digestion batch. The blank must read below the MDL for all target analytes.
- **Laboratory control sample (LCS):** Digest a certified reference material (CRM) or laboratory reference soil with known metal concentrations in each batch. Recovery should be 75–125% of the certified value.
- **Matrix spike:** Spike a duplicate sample with a known concentration of the target analyte(s). Recovery should be 75–125%.
- **Duplicate digestions:** Process at least 10% of samples in duplicate. Relative percent difference (RPD) should be less than 20%.
- **Calibration verification:** After instrument analysis, verify the calibration with a mid-range check standard every 10 samples.

8.2. Calculations

Metal concentration in soil (mg/kg dry weight):

$$C_{\text{soil}} = \frac{C_{\text{digest}} \times V_{\text{final}}}{M_{\text{soil}}}$$

where C_{digest} = concentration in the digest (mg/L from ICP-MS or FAAS), V_{final} = final digest volume (mL, typically 25), and M_{soil} = mass of dried soil (g).

Since the soil was dried at 60 C (not 105 C), apply a moisture correction if reporting on a 105 C oven-dry basis:

$$C_{\text{soil, OD}} = C_{\text{soil}} \times \frac{100 + \theta_{60}}{100 + \theta_{105}}$$

where θ_{60} and θ_{105} are the moisture contents at 60 C and 105 C respectively (determined on a companion subsample per SOP-L400).

9. Waste Disposal

Waste Type	Disposal Method
Acid digest waste (vessels, rinses, pellets)	Collect in acid waste carboy; EH&S hazardous waste pickup
Used syringe filters and syringes	Acid waste stream (contaminated with HNO_3)
Centrifuge tube residues	Acid waste carboy
Digested soil pellets	Acid waste carboy (contain concentrated acid)

> ⚠ Acid Waste
>
> All waste from this procedure contains concentrated HNO_3 and dissolved metals. **Do not pour down the drain.** Collect in a labeled polyethylene carboy for EH&S hazardous waste pickup.

10. References

CEM Corporation. (2015). *MARS 5 microwave accelerated reaction system: Operator's manual*. https://www.cem.com

U.S. Environmental Protection Agency. (2007). *Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils* (SW-846, Revision 1). U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf

11. Training Record

Before performing microwave acid digestion independently, personnel must:

1. Read this SOP, SOP-L002 Laboratory Safety, and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for concentrated HNO_3 and HCl
3. Review the MARS 5 operator's manual (vessel loading, program selection, safety interlocks)(CEM Corporation, 2015)
4. Observe a trained operator perform a complete digestion cycle (vessel loading through filtration)
5. Perform at least one complete digestion batch under direct supervision, including a blank and a CRM, with acceptable QC results
6. Be signed off by the PI or lab manager

> ! Important
>
> First-time users must be directly supervised for their entire first digestion run. Independent operation is permitted only after sign-off.

12. Appendix A: Example Calculation

```r
# Trace metal concentration from microwave digest
# EPA 3051A, analyzed by ICP-MS

C_digest_mg_L <- 0.452     # Pb concentration in filtered digest (mg/L)
V_final_mL <- 25.0         # Final volume after dilution (mL)
M_soil_g <- 0.503           # Mass of dried soil digested (g)

# Concentration in soil (mg/kg)
C_soil_mg_kg <- (C_digest_mg_L * V_final_mL) / M_soil_g

cat(sprintf("Digest concentration: %.3f mg/L Pb\n", C_digest_mg_L))
cat(sprintf("Soil mass:           %.3f g\n", M_soil_g))
cat(sprintf("Final volume:        %.1f mL\n", V_final_mL))
cat(sprintf("Soil Pb:             %.1f mg/kg\n", C_soil_mg_kg))

# Compare to screening levels
cat(sprintf("\nCalifornia DTSC residential screening level for Pb: 80 mg/kg\n"))
cat(sprintf("EPA RSL for Pb (residential): 400 mg/kg\n"))
if (C_soil_mg_kg > 400) {
  cat("WARNING: Exceeds EPA residential screening level\n")
} else if (C_soil_mg_kg > 80) {
  cat("NOTE: Exceeds CA DTSC residential screening level\n")
} else {
  cat("Below both screening levels\n")
}
```

13. Appendix B: Troubleshooting

Symptom	Probable Cause	Corrective Action
Vigorous reaction when acid added	High organic matter or carbonite content	Pre-digest uncapped in fume hood for 1–2 hours before sealing
Vessel pressure exceeds limit during run	Sample mass too large; excessive organic content	Reduce sample mass to 0.250 g; extend pre-digestion time
Supernatant not clear after centrifugation	Refractory oxides (SiO_2, TiO_2) remain undissolved	Allow to stand overnight; filter through 0.45 um; this is normal for EPA 3051A
Blank reads above MDL	Contaminated acid, vessels, or water	Use fresh Trace Metal grade acid; re-clean vessels; check DI water quality

Symptom	Probable Cause	Corrective Action
CRM recovery below 75%	Incomplete digestion; sample loss during transfer	Verify temperature reached 175 C; rinse vessels quantitatively; consider HNO_3/HCl mixture
CRM recovery above 125%	Contamination; calibration error	Check blank; recalibrate instrument; re-analyze
High RPD between duplicates	Soil heterogeneity; inconsistent weighing	Grind and sieve more thoroughly; weigh to 0.001 g precision

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L411

Nitrification Potential – Shaken Soil Slurry Method

Standard Operating Procedure

SOP Number	SOP-L411
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes a rapid method for measuring the potential rate of ammonium oxidation in soils using a shaken soil slurry. The method provides an estimate of the activity of the existing ammonium-oxidizing microbial population at the time of sampling. It is based on ISO 15685 (*Soil quality – Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation*) (International Organization for Standardization, 2012).

Ammonium oxidation is the first step in autotrophic nitrification. Soil is incubated in a buffered (pH 7.2) ammonium sulfate solution with sodium chlorate added to inhibit nitrite oxidation. The accumulation of nitrite (NO_2^-) over a 6-hour incubation period serves as the measure of potential nitrification activity. Because the generation time of ammonia-oxidizing bacteria exceeds 10 hours, the assay measures the activity of the population present at sampling, not population growth.

This method can be used to compare soils (e.g., polluted vs. reference), to screen the effects of management practices or chemical substances on nitrifying communities, and to evaluate the impact of biosolids or waste materials on soil nitrification.

2. Scope

- **Applies to:** Field-moist soils containing a population of nitrifying microorganisms; assessment of nitrification inhibition by chemical substances (except volatiles with Henry's constant $H > 1$), biosolid extracts, and polluted soil mixtures
- **Does not apply to:** Soils with no detectable nitrifying population; measurement of in situ nitrification rates (this is a potential activity assay under optimized conditions); volatile inhibitors
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve experimental design, ensure training compliance
Lab Manager	Prepare reagents, maintain shaking incubator
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Ammonia oxidation:** The first step of nitrification; conversion of NH_4^+ to NO_2^- by ammonia-oxidizing bacteria (AOB) and archaea (AOA); the rate-limiting step of nitrification in most soils.
- **Autotrophic nitrification:** Nitrification carried out by chemolithoautotrophic microorganisms (AOB, AOA, and nitrite oxidizers) that use the energy from ammonia oxidation to fix CO_2; distinguished from heterotrophic nitrification.
- **Chlorate inhibition:** Addition of sodium chlorate (ClO_3^-) to suppress nitrite oxidation, allowing NO_2^- to accumulate and be measured as the product of ammonia oxidation; used in some nitrification potential assays.
- **Nitrite oxidation:** The second step of nitrification; conversion of NO_2^- to NO_3^- by nitrite-oxidizing bacteria (NOB); not rate-limiting under normal conditions.
- **Nitrification potential (NP):** The short-term rate of NO_3^- (or NO_2^- + NO_3^-) production in a soil slurry amended with excess NH_4^+ and incubated aerobically; reflects the size and activity of the extant nitrifier community; expressed as µg NO_3^--N g^{-1} soil h^{-1}.
- **Slurry method:** The standard technique for measuring nitrification potential; soil is suspended in a buffered ammonium sulfate solution at a defined soil-to-solution ratio and shaken aerobically at 25 °C; sequential subsamples are taken and analyzed for NO_2^- + NO_3^-.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Sodium chlorate ($NaClO_3$) is an oxidizer; avoid contact with organic materials and combustible substances. Potassium chloride (4 M KCl) is a mild irritant. Ammonium sulfate is a mild irritant.
- **Physical:** The orbital shaking incubator has moving parts; keep hair and loose clothing clear. Centrifuge operation poses standard mechanical hazards.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For chemical spills, refer to SOP-L003 Hazardous Materials Handling. For sodium chlorate spills, do not use organic absorbents (sawdust, paper towels); use vermiculite or sand.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Orbital shaking incubator	Thermostatically controlled at 25 +/- 2 C; capable of 175 rpm
Erlenmeyer flasks (250 mL)	One per replicate
Centrifuge	Capable of 3,000 x g; see *SOP-L108* or *SOP-L109*
Analytical balance	0.001 g resolution; see SOP-L102
Volumetric flasks (100 mL, 1 L)	Class A
Graduated cylinders	10, 100, 1000 mL
Pipettes	2 mL (for slurry sampling) and assorted
Test tubes with caps	For subsamples
Filter funnels and filter paper	High-filtration-speed paper; chemical purity can be below the highest grade
pH meter	Calibrated per SOP-L300

6.2. Reagents

Reagent	Concentration / Preparation
Distilled or deionized water	From *SOP-L11*
Potassium dihydrogen phosphate (KH_2PO_4)	0.2 mol/L solution
Dipotassium hydrogen phosphate (K_2HPO_4)	0.2 mol/L solution
Sodium chlorate ($NaClO_3$)	0.5 mol/L solution
Ammonium sulfate ($(NH_4)_2SO_4$)	Solid; 0.198 g per liter of test medium
Sodium bicarbonate ($NaHCO_3$)	5 mmol/L solution (optional; see Section 6.2)
Potassium chloride (KCl)	4 mol/L solution (extraction/stop solution)

7. Procedure

7.1. Stock Solution A (Phosphate Buffer)

Combine the following in a volumetric flask and bring to 200 mL with distilled water:

- 28 mL of 0.2 mol/L KH_2PO_4
- 72 mL of 0.2 mol/L K_2HPO_4

This yields a phosphate buffer at approximately pH 7.2.

7.2. Test Medium

Prepare 1 L of test medium as follows:

1. Transfer 10 mL of Stock Solution A to a 1 L volumetric flask
2. Add 10–30 mL of 0.5 mol/L $NaClO_3$ (see note below on concentration selection)
3. Add 0.198 g of $(NH_4)_2SO_4$
4. If a carbon source is needed (e.g., when testing biosolids with high nitrate), add 10 mL of 5 mmol/L $NaHCO_3$
5. Dilute to 1 L with distilled water
6. Verify that the pH is approximately 7.2; adjust if necessary

The final test medium contains approximately 1 mmol/L potassium phosphate buffer, 5–15 mmol/L sodium chlorate, and 1.5 mmol/L ammonium sulfate.

> **Note**
>
> The sodium chlorate concentration should be sufficient to inhibit biological nitrite oxidation without negatively affecting ammonium oxidation. If the appropriate concentration for your soil type is unknown, run a preliminary trial at 5, 10, and 15 mmol/L $NaClO_3$ to identify the optimal level.

7.3. Preparing the 4 M KCl Stop Solution

Dissolve 298.2 g KCl in distilled water and bring to 1 L in a volumetric flask. This solution is used to halt ammonium oxidation at each sampling time.

7.4. Soil Moisture Determination

Determine the gravimetric moisture content of the soil per SOP-L400 (dry at 105 C, 24 h). This value is needed to calculate the volume of water in the initial soil sample and to express results on a dry-mass basis.

7.5. Setting Up the Incubation

1. Weigh approximately 25 g of field-moist soil into each of at least three replicate 250 mL Erlenmeyer flasks
2. Calculate the volume of water already present in the soil:

$$V_{\text{water}} = M_{\text{soil}} \times \theta_g$$

where M_{soil} = mass of moist soil (g) and θ_g = gravimetric moisture content (g water / g dry soil).

3. Calculate the volume of test medium to add:

$$V_{\text{medium}} = V_{\text{total}} - V_{\text{water}}$$

where V_{total} = desired total liquid volume (typically 100 mL).

4. Add the calculated volume of test medium to each flask
5. Place the flasks upright on the orbital shaking incubator set to 25 +/- 2 C and 175 rpm (sufficient to keep solids suspended)

> **!** Important
>
> If testing chemical substances, dissolve them in the phosphate buffer and add to the test medium before diluting to 1 L. For substances with low water solubility, use mechanical dispersion or a low-toxicity vehicle (organic solvent, emulsifier). If a vehicle is used, include an additional control to assess its effect on ammonium oxidation.

7.6. Sampling the Slurry

1. At 2 h and 6 h after the start of incubation, shake each flask well to resuspend solids and withdraw a 2 mL aliquot of the slurry. Ensure the ratio of solution to soil is consistent across all samples.
2. Dispense the 2 mL aliquot into a test tube
3. Immediately add 2 mL of 4 M KCl to stop ammonium oxidation
4. Centrifuge at 3,000 x g for 2 minutes, or filter through high-speed filter paper
5. Transfer the clear supernatant or filtrate to a clean, labeled tube

7.7. Nitrite Analysis

Analyze the extracts for NO_2^--N using a suitable colorimetric method (e.g., ISO 14256-2; diazotization with sulfanilamide and N-(1-naphthyl)ethylenediamine). Extracts may be stored at 4–8 C but must be analyzed within 24 hours.

8. Quality Control and Acceptance Criteria

8.1. Calculations

Potential ammonium oxidation rate:

$$\text{Rate} = \frac{[\text{NO}_2^-\text{-N}]_{6h} - [\text{NO}_2^-\text{-N}]_{2h}}{\Delta t \times M_{\text{dry}}} \times V_{\text{total}}$$

where the NO_2^--N concentrations are in ug/mL (= mg/L), Δt = time interval (4 h), M_{dry} = dry mass of soil (g), and V_{total} = total liquid volume in the flask (mL). Report results as ng NO_2^--N per g dry soil per hour.

Inhibition (%) by a test chemical:

$$\text{Inhibition (\%)} = \frac{R_{\text{control}} - R_{\text{treatment}}}{R_{\text{control}}} \times 100$$

where R = ammonium oxidation rate.

Toxicity assessment for polluted soil mixtures:

When mixing polluted and unpolluted (reference) soils, the polluted soil is considered toxic if:

$$A_m + s_m < 0.9 \times \frac{A_c + A_p}{2}$$

where A_m = mean activity in the mixture, s_m = standard deviation in the mixture, A_c = mean activity in the control soil, and A_p = mean activity in the polluted soil (each tested separately). This threshold applies with a minimum of four replicates.

8.2. QC Requirements

- Use a minimum of three replicates per treatment
- Four replicates are required when applying the toxicity assessment equation above
- Include a negative control (soil with no ammonium addition) and a positive control (reference soil with known nitrification activity) in each batch
- Verify that ammonium oxidation is linear between the 2 h and 6 h sampling points. If non-linearity is suspected, add sampling at 0 h and 4 h to characterize the time course.
- Blank controls (test medium without soil) should show no significant nitrite accumulation
- Report the coefficient of variation among replicates. A CV greater than 15% suggests sampling inconsistency or soil heterogeneity; consider additional replicates.

9. Waste Disposal

Waste Type	Disposal Method
Soil slurries (non-hazardous)	Drain liquid; dispose of soil in standard trash
KCl extracts	Drain with copious water
$NaClO_3$-containing solutions	Collect in oxidizer waste; EH&S pickup. Do not mix with organic waste.
Used filter paper and tubes	Standard trash (if non-hazardous soil)

10. References

International Organization for Standardization. (2012). *Soil quality – determination of potential nitrification and inhibition of nitrification – rapid test by ammonium oxidation* (ISO 15685:2012). International Organization for Standardization. https://www.iso.org/standard/55080.html

11. Training Record

Before performing the nitrification potential assay independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Review the SDS for sodium chlorate
4. Complete SOP-L400 Soil Moisture training
5. Observe a trained operator perform the full assay from slurry setup through nitrite analysis
6. Perform the assay under supervision with acceptable replicate precision (CV less than 15%)
7. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation

```
# Nitrification potential calculation per ISO 15685

# Input data
no2_2h_mg_L <- 0.15       # NO2-N at 2 h (mg/L in the extract)
no2_6h_mg_L <- 0.95       # NO2-N at 6 h (mg/L in the extract)
delta_t_h <- 4             # Time interval (h)
soil_moist_g <- 25.0       # Mass of field-moist soil (g)
theta_g <- 0.22            # Gravimetric moisture (g water / g dry soil)
V_total_mL <- 100          # Total liquid volume in the flask (mL)

# Dry mass of soil
M_dry_g <- soil_moist_g / (1 + theta_g)

# Potential ammonium oxidation rate (ng NO2-N / g dry soil / h)
# Convert mg/L to ug/mL (same units), then multiply by V_total to get
# total ug in the flask, divide by dry mass and time, convert to ng.
rate_ng_per_g_per_h <- ((no2_6h_mg_L - no2_2h_mg_L) *
                        V_total_mL / (delta_t_h * M_dry_g)) * 1000

cat(sprintf("Dry soil mass:            %.2f g\n", M_dry_g))
cat(sprintf("Delta NO2-N:              %.2f mg/L\n",
            no2_6h_mg_L - no2_2h_mg_L))
cat(sprintf("Nitrification potential:  %.1f ng NO2-N / g dry soil / h\n",
            rate_ng_per_g_per_h))
```

13. Appendix B: Reagent Preparation Quick Reference

Reagent	Preparation
0.2 mol/L KH_2PO_4	Dissolve 27.22 g in 1 L distilled water
0.2 mol/L K_2HPO_4	Dissolve 34.84 g in 1 L distilled water
Stock Solution A	28 mL KH_2PO_4 + 72 mL K_2HPO_4 + 100 mL water
0.5 mol/L $NaClO_3$	Dissolve 53.23 g in 1 L distilled water
4 mol/L KCl	Dissolve 298.2 g in 1 L distilled water
5 mmol/L $NaHCO_3$	Dissolve 0.420 g in 1 L distilled water
Test medium (1 L)	10 mL Stock A + 10–30 mL $NaClO_3$ + 0.198 g $(NH_4)_2SO_4$ + water to 1 L

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
No nitrite accumulation	Soil has no active nitrifying population; $NaClO_3$ concentration too high	Test a reference soil in parallel; reduce $NaClO_3$ to 5 mmol/L
Non-linear NO_2^- accumulation	Nitrifier population growing during incubation; substrate depletion	Add intermediate time points (0 h, 4 h); use only the linear portion of the curve
High variability among replicates	Heterogeneous soil; inconsistent slurry sampling	Sieve and mix soil thoroughly; ensure slurry is well-shaken before each aliquot
Nitrite detected in controls (no NH_4^+)	Background nitrification from soil organic N	Note in results; subtract control values from treatment values
$NaClO_3$ appears to inhibit ammonium oxidation	Concentration too high for this soil	Run a $NaClO_3$ dose-response trial (5, 10, 15 mmol/L)

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L413

Compost Maturity – Solvita CO_2/NH_3 Test

Standard Operating Procedure

SOP Number	SOP-L413
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of compost maturity and stability using the Solvita Compost Maturity Test (Woods End Laboratories). The test simultaneously measures carbon dioxide (CO_2) and ammonia (NH_3) emissions from a compost sample during a 4-hour incubation, providing a dual indicator of biological activity and nitrogen stabilization. The intersection of the two readings yields a Compost Maturity Index (CMI) that indicates whether compost is immature, active, curing, or finished (Vargas et al., 2005).

CO_2 evolution reflects ongoing microbial decomposition of organic carbon. NH_3 volatilization indicates incomplete nitrogen stabilization and is associated with odor, phytotoxicity, and high pH. True compost maturity is reached when both gases have declined to low levels. Because the CO_2 and NH_3 cycles do not always track together, the dual-measurement approach provides a more reliable maturity assessment than either gas alone.

This SOP is distinct from SOP-L408 Solvita CO_2 Burst, which measures rewetted *soil* respiration over 24 hours using only a CO_2 paddle.

2. Scope

- **Applies to:** Assessment of maturity and stability of composted organic materials, including green waste, food waste, biosolids compost, manure compost, and vermicompost
- **Does not apply to:** Soil respiration measurements (see SOP-L408); raw feedstock characterization; long-term (7–28 day) laboratory respirometry; finished potting mixes where compost is a minor component
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain Solvita kit inventory, stock gel paddles
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Compost maturity:** A measure of how fully decomposed and stabilized a compost product is; mature compost no longer undergoes rapid decomposition, does not cause phytotoxicity, and can be safely applied to crops or soils.
- **CO_2 index (Solvita):** The Solvita scale value (1–8) corresponding to the CO_2 concentration in the headspace above moist compost; lower values indicate more active decomposition (less mature); higher values indicate stabilization.
- **Maturity index:** A combined score calculated from the CO_2 and NH_3 Solvita indices; the primary output of the Solvita compost maturity test; used by USCC, US Composting Council, and others to certify compost quality.
- **NH_3 index (Solvita):** The Solvita scale value (1–5) corresponding to the NH_3 concentration in the headspace above moist compost; high NH_3 (low index) indicates immature compost with excess ammoniacal nitrogen that can harm plants.
- **Phytotoxicity:** Inhibition of plant germination or growth caused by immature compost; typically results from high NH_3 concentrations, volatile fatty acids, or other metabolites produced during active decomposition.
- **Solvita paddle:** A plastic stick holding two gel-pad sensors (CO_2 and NH_3) inserted into the compost jar headspace during the test incubation; color changes are read with the Solvita DCR reader.

5. Safety and Hazards

⚠ Hazards

- **Biological:** Compost may contain elevated microbial loads, fungal spores, and trace pathogens. Handle as a potential biohazard. Avoid inhaling dust when opening bags or scooping dry compost.
- **Chemical:** NH_3 released from immature compost is an irritant at high concentrations. Work in a well-ventilated area when handling fresh or actively decomposing compost.
- **Physical:** Minor risk of glass breakage from incubation jars.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Lab coat
☐ Dust mask (recommended when handling dry or dusty compost)

5.2. Emergency Procedures

If compost dust is inhaled and respiratory irritation occurs, move to fresh air. For skin contact with compost, wash thoroughly with soap and water. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Solvita Compost Maturity Test kit	Includes CO_2 and NH_3 gel paddles, incubation jars, scoop, and color charts
Solvita Digital Color Reader (DCR)	Recommended for quantitative readings; provides numerical color values
Incubation jars (approx. 475 mL)	Glass or polycarbonate; provided with kit
Timer	4-hour countdown
Thermometer	For recording ambient and sample temperature
Lab notebook or data sheet	For recording results

6.2. Consumables

Item	Notes
Solvita CO_2 gel paddles	One per test; store sealed at room temperature in the dark
Solvita NH_3 gel paddles	One per test; store sealed at room temperature in the dark
Solvita CMI Calculator spreadsheet	Available from Woods End / solvita.com for precise index calculation

7. Procedure

7.1. Sample Collection and Preparation

1. Collect a representative compost sample of at least 500 g from the pile or windrow. Sample from the interior of the pile (at least 30 cm below the surface) to avoid surface drying artifacts.
2. If the compost is very heterogeneous, collect from multiple locations and composite the subsamples
3. The sample should be at "natural" moisture, not artificially wetted or dried. Compost that has been stored at 4 C should be brought to room temperature (20–25 C) before testing. Allow at least 30 minutes.
4. Gently break up large clumps by hand but do not sieve or grind the compost. The test is designed for material in its as-produced condition.

! Important

Do not test compost that is above 40 C (i.e., still in the thermophilic phase). Elevated temperatures accelerate gas production and will give artificially high CO_2 and NH_3 readings

that do not reflect the maturity of the material once cooled.

7.2. Setting Up the Test

1. Fill a clean Solvita incubation jar loosely to the fill line with compost using the supplied scoop. Do not pack or compress the material. The volume should be approximately 150–200 mL.
2. Remove one CO_2 gel paddle and one NH_3 gel paddle from their sealed foil packets
3. Inspect each paddle: the gel should be uniform in color with no cracks, discoloration, or signs of prior exposure. Discard any compromised paddles.
4. Insert both paddles into the jar so that the gel faces are exposed to the headspace above the compost surface. The paddles should stand upright and not be buried in the compost.
5. Seal the jar immediately and securely
6. Record the jar number, sample ID, compost source, ambient temperature, and start time
7. Start the 4-hour timer

7.3. Incubation

1. Place sealed jars at room temperature (20–25 C) in a location shielded from direct sunlight and drafts
2. Do not open, move, or disturb jars during the 4-hour incubation

Note

The standard incubation time is 4 hours. This is shorter than the 24-hour incubation used for the Solvita soil CO_2 burst test (SOP-L408) because compost produces gas at much higher rates than soil.

7.4. Reading Results

1. After exactly 4 hours, open the jar and immediately remove both paddles
2. Read each paddle promptly. Prolonged exposure to ambient air will alter the gel color.

Using the Solvita Color Charts (visual method):

3. Compare the CO_2 paddle to the Solvita CO_2 color chart. Record the color number (1–8 scale, where 1 = highest CO_2 / least stable, 8 = lowest CO_2 / most stable).
4. Compare the NH_3 paddle to the Solvita NH_3 color chart. Record the color number (1–5 scale, where 1 = highest NH_3 / least mature, 5 = lowest NH_3 / most mature).

Using the Solvita Digital Color Reader (DCR, recommended):

3. Insert the CO_2 paddle into the DCR and record the numerical reading
4. Insert the NH_3 paddle into the DCR and record the numerical reading
5. The DCR values can be entered into the Solvita CMI Calculator spreadsheet for a more precise Maturity Index

7.5. Determining the Compost Maturity Index (CMI)

Use the CO_2 and NH_3 color numbers to determine the CMI from the Solvita lookup table (printed in the kit manual) or the CMI Calculator spreadsheet:

CMI	Interpretation	Recommended Action
1	Raw, very immature	Continue active composting
2	Immature, highly active	Continue active composting
3	Immature, active	Continue composting/curing
4	Moderately immature	Begin curing phase
5	Mid-range maturity	Continue curing
6	Moderately mature	Approaching readiness
7	Mature	Ready for most applications
8	Very mature, highly stable	Fully finished compost

Tip

Most regulatory agencies and compost quality programs consider a CMI of 6 or above as "mature" and suitable for unrestricted use. A CMI of 7–8 indicates a fully finished product with minimal phytotoxicity risk. For sensitive applications (seed germination, potting mixes), target CMI 7 or 8.

7.6. Cleanup

1. Dispose of used compost in the designated waste container or return it to the compost pile
2. Wash incubation jars thoroughly with soap and water; rinse with deionized water and allow to air-dry
3. Dispose of used gel paddles in standard trash

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

- Run duplicate tests on at least 10% of samples (two jars from the same homogenized compost). Duplicate CO_2 readings should agree within 1 color number; NH_3 readings within 1 color number.
- Include a blank jar (empty, sealed with both paddles) in each batch to check for ambient CO_2 and NH_3 interference. Both paddles should read at or near their maximum (most stable) values.
- Verify that the compost sample temperature is between 20 and 40 C before testing. Record the temperature.
- Calibrate the DCR per the manufacturer's instructions before each batch if using the digital reader.
- Store gel paddles sealed in their foil packets, at room temperature, away from light. Check expiration dates; do not use expired paddles.

8.2. Complementary Tests

For a comprehensive compost quality assessment, consider pairing the Solvita test with:

Parameter	SOP / Method
pH and electrical conductivity	SOP-L402 (1:5 compost-water extract)
Moisture content	SOP-L400 (105 C, 24 h)
Organic matter (LOI)	SOP-L406
Total C and N (C:N ratio)	SOP-L407 and elemental analysis
Seed germination bioassay	*TBD*

9. Waste Disposal

Waste Type	Disposal Method
Used compost	Return to compost pile or standard trash
Used gel paddles	Standard trash
Jar wash water	Drain

10. References

Vargas, D. C. nas., Monedero, M. S., Urpilainen, S., Kamilaki, A., & Stentiford, E. (2005). Assessing the stability and maturity of compost at large-scale plants. *Ingenier' ia*, *9*(2), 25–30.

11. Training Record

Before performing the Solvita compost maturity test independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the Solvita Compost Maturity Test User's Guide (included in the kit)
4. Observe a trained operator perform the full procedure from sample collection through CMI determination
5. Perform at least one test under supervision with acceptable duplicate precision (within 1 color number)
6. Be signed off by the PI or lab manager

12. Appendix A: Solvita Compost Maturity Index Lookup Table

The following table reproduces the general structure of the Solvita CMI lookup. Consult the kit manual or CMI Calculator spreadsheet for the official version.

	$NH_3 = 1$	$NH_3 = 2$	$NH_3 = 3$	$NH_3 = 4$	$NH_3 = 5$
$CO_2 = 1$	1	1	1	2	2
$CO_2 = 2$	1	2	2	3	3
$CO_2 = 3$	2	2	3	4	4
$CO_2 = 4$	2	3	4	5	5
$CO_2 = 5$	3	4	5	6	6
$CO_2 = 6$	3	4	5	6	7
$CO_2 = 7$	4	5	6	7	7
$CO_2 = 8$	5	5	6	7	8

Note: This table is approximate. Use the official Solvita color charts and CMI Calculator for definitive results.

13. Appendix B: Interpreting Results in Context

13.1. CO_2 Color Number Interpretation

Color No.	CO_2 Level	Stability Interpretation
1–2	Very high	Actively decomposing; unstable
3–4	Moderate to high	Active composting phase
5–6	Low to moderate	Curing; approaching stability
7–8	Very low	Stable; decomposition essentially complete

13.2. NH_3 Color Number Interpretation

Color No.	NH_3 Level	Maturity Interpretation
1	Very high	Strong odor; phytotoxic; immature
2	High	Significant ammonia; not ready
3	Moderate	Some ammonia; continue curing
4	Low	Approaching maturity
5	Very low / none	Nitrogen stabilized; mature

13.3. Common Patterns

CO_2	NH_3	Interpretation
High	High	Actively decomposing with unstable N; classic immature compost
High	Low	Carbon-rich material still decomposing but N is stabilized; may need more time
Low	High	Unusual; possible anaerobic conditions or recent N-rich amendment; investigate

CO_2	NH_3	Interpretation
Low	Low	Both carbon and nitrogen cycles complete; finished compost

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
CO_2 paddle reads 1 (max activity) after 4 h	Compost is very fresh or sample was too warm	Verify sample temperature was below 40 C; confirm composting has progressed past thermophilic phase
NH_3 paddle reads 1 (max ammonia)	High free ammonia; fresh manure or urea input	Continue composting; ensure adequate C:N ratio (target 25:1–30:1)
Both paddles show no color change (max stability)	Compost is fully mature, or paddles expired	Verify paddle expiration date; confirm with a known immature sample
Duplicates disagree by more than 1 color number	Heterogeneous compost; inconsistent fill level	Homogenize sample more thoroughly; fill jars to consistent level
CO_2 high but NH_3 low	High-carbon, low-N material (e.g., wood chip compost)	Normal for C-rich substrates; continue composting until CO_2 declines
CO_2 low but NH_3 high	Possible anaerobic pocket; recent N amendment	Turn the pile to aerate; reduce N inputs; retest in 1–2 weeks

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L414

Soil Carbon and Nitrogen Analysis by Flash EA-IRMS

Standard Operating Procedure

SOP Number	SOP-L414
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the complete workflow for determining $\delta^{13}C$, $\delta^{15}N$, and elemental concentrations (%C, %N) in soil and sediment samples by combustion on the Thermo Scientific Flash IRMS

Elemental Analyzer (EA) coupled to the Delta V IRMS through the ConFlo IV interface (Werner & Brand, 2001). It covers sample preparation, sequence design, the analytical run, and post-analysis data correction including blank correction, linearity correction, scale normalization, and drift correction (Coplen, 2011; Paul et al., 2007).

For general instrument operation, startup/shutdown, gas checks, tuning, and pre-analysis tests, see SOP-L208.

2. Scope

- **Applies to:** Soil, sediment, and plant tissue samples analyzed for δ^{13}C, δ^{15}N, %C, and %N by combustion EA-IRMS in NC (dual reactor) mode.
- **Does not apply to:** Sulfur isotope analysis (NCS mode; requires dedicated reactor and Sulfinert column), GasBench applications (see SOP-L415), TC/EA applications (δ^{2}H, δ^{18}O), or liquid samples.
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Isotope Lab, SGM Rm 135.

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, oversee data quality
Lab Manager	Prepare/verify Isodat methods; maintain reactors
Certified User	All steps from sample prep through data reduction
Student Researcher	May perform sample prep and data reduction under supervision

4. Definitions

- **Blank correction:** Subtraction of the isotopic and elemental contribution of an empty tin capsule from each sample signal; necessary because capsule tin contributes a small amount of C and N (Paul et al., 2007).
- **δ^{13}C:** Stable carbon isotope ratio expressed in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$ where $R = {}^{13}C/{}^{12}C$ (Coplen, 2011).
- **δ^{15}N:** Stable nitrogen isotope ratio expressed in per mil (‰) relative to atmospheric N_2 (AIR): $\delta^{15}N = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$ where $R = {}^{15}N/{}^{14}N$ (Coplen, 2011).
- **Drift correction:** Adjustment for systematic temporal change in measured δ values during a sequence, estimated from bracketing reference materials (Paul et al., 2007).
- **Flash EA:** The Thermo Scientific Flash IRMS Elemental Analyzer peripheral; combusts samples at 1020 °C in an O_2 pulse and separates N_2 and CO_2 chromatographically before delivery to the IRMS via the ConFlo IV.
- **Isodat:** ThermoFisher software used to design analytical sequences, control the instrument, and export raw isotope data.

- **Linearity correction:** Adjustment applied to account for the dependence of measured δ values on peak signal amplitude.
- **Reference material (RM):** A substance with a certified or consensus δ value used to anchor measured values to the VPDB or AIR scale (Werner & Brand, 2001).
- **Scale normalization:** Two-point (or multi-point) correction that maps measured δ values to the international isotope-ratio scale using certified RMs spanning the expected sample range (Paul et al., 2007).
- **Tin capsule:** 5 x 9 mm pressed-tin container used to encapsulate solid samples; tin ignites in the O_2 pulse, raising combustion temperature briefly to ensure complete combustion.

5. Safety and Hazards

See SOP-L208, Section 4 for the full hazard list.

⚠ Application-Specific Hazards

- **Tin capsule handling:** Tweezers must be clean and dry; oils from fingers contaminate capsules and introduce C/N blanks.
- **Compressed gases:** He carrier and O_2 combustion gas; see SOP-L208 for gas handling requirements.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (sample prep and capsule loading)

5.2. Required Training

- SOP-L002 Laboratory Safety
- SOP-L208 IRMS General Instrument Use (must be a certified IRMS User before independent operation)

6. Equipment and Materials

Item	Location	Notes
Tin capsules (5 x 9 mm)	SGM Rm 133	Ultra-clean; handle with tweezers only
Micro-balance (0.001 mg)	SGM Rm 133	Tare and zero before each capsule
Tweezers (stainless)	SGM Rm 133	Clean with EtOH before use
Soil / sediment samples (dried, ground to <150 µm)	SGM Rm 133	See SOP-L116
Flash IRMS EA	SGM Rm 135	NC dual-reactor mode
Tin capsule storage plate	SGM Rm 133	Labeled 96-well plate

Item	Location	Notes
Certified reference materials	SGM Rm 133	e.g., USGS40, USGS41a, IAEA-N1
Lab logbook	SGM Rm 135	

7. Estimated Time

Task	Time
Sample weighing (per capsule)	3–5 min
Sequence setup in Isodat	20–30 min
Analytical run (per sample)	~7 min (N_2 + CO_2 in series)
Data reduction (R script)	30–60 min

8. Procedure

8.1. Sample Preparation

1. Dry samples at 60 °C to constant mass per SOP-L105.
2. Grind and homogenize to <150 µm per SOP-L116.
3. For carbonate removal (inorganic C), acidify with 1 M HCl, re-dry, and record the treatment in the lab notebook before proceeding.
4. Clean the micro-balance pan and zero the balance.
5. Using clean tweezers, place a tin capsule on the balance. Tare.
6. Weigh 10–30 mg of ground sample into the capsule (adjust to keep the N_2 and CO_2 peaks within the instrument's linear range).
7. Fold and crimp the capsule tightly, removing all air.
8. Record the capsule mass in the lab notebook and in the sequence spreadsheet.

8.2. Sequence Design

1. Open Isodat Sequence Editor and load the NC dual-reactor method.
2. Build the sequence in this order:
   - 3–5 conditioning samples (similar matrix to unknowns)
   - 1 blank capsule
   - Bracketing RMs (at least 2 certified materials, e.g., USGS40 + USGS41a for $\delta^{13}C$; IAEA-N1 + IAEA-N2 for $\delta^{15}N$)
   - Unknowns, with 1 RM per 10 samples
   - Check standard at end
   - 1 blank capsule at end
3. Save the sequence file as `YYYYMMDD_Project_SeqID_User.seq`.

8.3. Analytical Run

1. Complete the pre-analysis checklist in SOP-L208.
2. Load the capsule plate into the Flash EA autosampler carousel.
3. Press **Start** in Isodat.
4. Monitor the first 5 injections for expected peak shape and amplitude.

8.4. Post-Analysis Data Correction

Apply corrections in this order using the R script in the project repository:

```
library(tidyverse)

# 1. Import raw Isodat export (.csv)
raw <- read_csv("data/raw/YYYYMMDD_Project_SeqID_User_raw.csv")

# 2. Blank correction
#    Subtract mean blank d13C and blank area from each sample

# 3. Linearity correction
#    Regress RM d-values on peak amplitude; apply slope correction

# 4. Scale normalization (two-point)
#    Use two certified RMs to anchor measured values to VPDB / AIR

# 5. Drift correction
#    Regress RM residuals on run order; apply time-based correction

# 6. QC evaluation
#    Check RM recoveries; flag samples outside ±0.3 permil
```

9. Quality Control and Acceptance Criteria

- Reference material $\delta^{13}C$ and $\delta^{15}N$ must recover within ±0.2‰ of certified values after all corrections.
- Blank capsules must produce peak areas below 0.5 Vs (N_2) and 1 Vs (CO_2).
- Replicate analyses of a check standard (n >= 3) must show SD <= 0.15‰.
- Flag and investigate any sequence where >20% of RMs fall outside acceptance criteria before reporting results.

10. Data Management

1. Export raw data from Isodat immediately after the run as `.csv`.
2. Save under `data/raw/YYYYMMDD_Project_SeqID_User_raw.csv` in the project repository.

3. Commit raw data and the R correction script per SOP-L801.
4. Store all corrected data in `data/processed/`.

11. Waste Disposal

Waste Type	Disposal Method
Used tin capsules	Solid waste (non-hazardous)
Gloves, Kimwipes	Standard trash unless contaminated
Acidified soil residues	Acid waste per SOP-L006

12. References

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, *25*(17), 2538–2560. https://doi.org/10.1002/rcm.5129

Paul, D., Skrzypek, G., & F'orizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry*, *21*(18), 3006–3014. https://doi.org/10.1002/rcm.3185

Werner, R. A., & Brand, W. A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, *15*(7), 501–519. https://doi.org/10.1002/rcm.258

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L208	IRMS General Instrument Use	Instrument-level procedures
L415	Carbonate Isotopes by GasBench-IRMS	Companion application SOP
L802	Quality Control and Quality Assurance	General QC framework

14. Training Record

Before performing soil CN analysis independently, personnel must:

1. Read this SOP and SOP-L208 in full
2. Be a certified IRMS User (SOP-L208, Training Record)
3. Demonstrate capsule preparation under supervision (5 capsules, masses within ±0.05 mg of target)
4. Perform a supervised analytical run with acceptable QC results
5. Complete at least one supervised data reduction session
6. Be signed off by the lab manager or PI



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L415

Carbonate Isotope Analysis by GasBench II-IRMS

Standard Operating Procedure

SOP Number	SOP-L415
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of $\delta^{13}C$ and $\delta^{18}O$ in carbonate minerals and sediments using phosphoric acid digestion and headspace analysis on the GasBench II coupled to the Delta V IRMS (Coplen, 2011; Kim et al., 2007; Swart et al., 1991). It covers sample preparation, vial flushing and acidification, the analytical run, and post-analysis data correction.

For general instrument operation, startup/shutdown, gas checks, tuning, and pre-analysis tests, see SOP-L208.

2. Scope

- **Applies to:** Carbonate-bearing samples (calcite, dolomite, aragonite, shell, foraminifera, soil carbonates, cement, and similar materials) analyzed for $\delta^{13}C$ (VPDB) and $\delta^{18}O$ (VPDB or VSMOW) by phosphoric acid digestion and continuous-flow IRMS.
- **Does not apply to:** DIC in water, breath gas, CO_2 at atmospheric concentrations, or water equilibration methods (these use the GasBench but with different sample preparation and Isodat methods).
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Isotope Lab, SGM Rm 135.

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, oversee data quality
Lab Manager	Prepare/verify Isodat methods; maintain GasBench and PAL
Certified User	All steps from sample prep through data reduction
Student Researcher	May perform sample prep and data reduction under supervision

4. Definitions

- **Acid fractionation factor (α^*):** The equilibrium isotope fractionation between carbonate mineral and CO_2 produced by phosphoric acid digestion; temperature-dependent and mineral-specific (Kim et al., 2007).
- $\delta^{13}C$ (VPDB): Stable carbon isotope ratio in per mil (‰) referenced to Vienna Pee Dee Belemnite (VPDB); the conventional scale for carbonate and organic carbon (Coplen, 2011).
- $\delta^{18}O$ (VPDB or VSMOW): Stable oxygen isotope ratio in per mil (‰); carbonates are commonly reported on VPDB, which relates to VSMOW by $\delta^{18}O_{\text{VPDB}} \approx 0.97001 \times \delta^{18}O_{\text{VSMOW}} - 29.99$ (Coplen, 2011).
- **Exetainer vial:** 12 mL borosilicate glass vial with a septum-capped lid used for sample reaction and headspace analysis.
- **GasBench II:** Continuous-flow peripheral that samples headspace CO_2 from sealed exetainer vials through a flushed needle system and delivers it to the IRMS via the ConFlo IV.

- **PAL autosampler:** CTC Analytics autosampler integrated with the GasBench that positions vials under the injection needle for automated headspace sampling.
- **Phosphoric acid digestion:** Reaction of carbonate with 100% H_3PO_4 at 50 °C to release CO_2 quantitatively from calcite and aragonite (Swart et al., 1991).
- **VPDB (Vienna Pee Dee Belemnite):** The primary international reference scale for $\delta^{13}C$ and $\delta^{18}O$ in carbonates.
- **VSMOW (Vienna Standard Mean Ocean Water):** The primary international reference scale for $\delta^{18}O$ and $\delta^{2}H$ in water and atmospheric gases.

5. Safety and Hazards

See SOP-L208, Section 4 for the full hazard list. Hazards specific to this application:

⚠ Application-Specific Hazards

- **100% phosphoric acid (H_3PO_4):** Corrosive. Handle in the fume hood. Wear nitrile gloves and safety glasses at all times.
- **Pressurized vials:** Flushed vials contain He under slight pressure; use the PAL needle carefully to avoid splashing acid.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (acid handling and vial preparation)

5.2. Required Training

- SOP-L002 Laboratory Safety
- SOP-L003 Hazardous Materials Handling (acid handling)
- SOP-L208 IRMS General Instrument Use (must be a certified IRMS User)

6. Equipment and Materials

Item	Location	Notes
Exetainer vials (12 mL)	SGM Rm 133	Check septum integrity before use
100% phosphoric acid	Fume hood, SGM Rm 133	Corrosive; store in acid cabinet
He gas (ultra-high purity)	SGM Rm 135	For vial flushing
Flushing manifold / needle	SGM Rm 133	10-port manifold preferred
Micro-spatula	SGM Rm 133	For weighing carbonate powder
Micro-balance	SGM Rm 133	Tare before each vial
GasBench II + PAL autosampler	SGM Rm 135	Confirm needle and trap condition

Item	Location	Notes
Certified reference materials	SGM Rm 133	e.g., NBS-18, NBS-19, IAEA-603
Heating block (50 °C)	SGM Rm 133	For overnight reaction

7. Estimated Time

Task	Time
Vial preparation and flushing	1–2 h (for a 40-vial batch)
Acid addition and reaction	1 h (calcite); overnight (dolomite)
Analytical run (per injection)	~8 min x 11 injections/vial
Data reduction (R script)	30–60 min

8. Procedure

8.1. Vial Preparation

1. Label exetainer vials with sample ID and position number.
2. Weigh 0.1–0.5 mg carbonate powder into each vial (adjust for expected calcite content).
3. Cap vials tightly with septum caps.
4. Flush each vial with He (UHP) for 5–8 min using the flushing manifold to remove atmospheric CO_2.

8.2. Acid Addition

1. In the fume hood, add 3–5 drops (~100 µL) of 100% H_3PO_4 to each vial through the septum using a syringe.
2. Incubate at 50 °C for 1 h (calcite and aragonite) or overnight (dolomite) in the heating block.
3. Allow vials to equilibrate to room temperature before loading into the PAL tray.

8.3. Sequence Design

1. Open Isodat Sequence Editor and load the GasBench carbonate method.
2. Build the sequence: 3 blank vials (flushed, no sample), reference materials, unknowns (1 RM per 8 unknowns), check standards.
3. Set the PAL to collect 11 injections per vial; the first 2–3 are discarded in post-processing.
4. Save the sequence file as `YYYYMMDD_Project_SeqID_User.seq`.

8.4. Analytical Run

1. Complete the pre-analysis checklist in SOP-L208.
2. Load the PAL tray with vials in correct order.
3. Press **Start** in Isodat and monitor the first 5 injections.

8.5. Post-Analysis Data Correction

Apply corrections using the R script in the project repository:

```
library(tidyverse)

raw <- read_csv("data/raw/YYYYMMDD_Project_SeqID_User_raw.csv")

# Each vial has 11 injections; discard injections 1-3
clean <- raw |>
  filter(injection >= 4) |>
  group_by(vial_id) |>
  summarise(
    d13C_mean = mean(d13C_raw),
    d18O_mean = mean(d18O_raw),
    d13C_sd   = sd(d13C_raw),
    d18O_sd   = sd(d18O_raw),
    n_inj     = n()
  )

# Scale normalization (two-point) using certified RMs
# Drift correction across run order
# QC: flag vials where within-vial SD > 0.15 permil
```

9. Quality Control and Acceptance Criteria

- Reference materials must recover within ±0.2‰ (δ^{13}C) and ±0.3‰ (δ^{18}O) of certified values after corrections.
- Within-vial SD (injections 4–11) must be <0.15‰ for both isotopes.
- Blank vials must produce no CO_2 peak above noise.

10. Data Management

1. Export raw Isodat data immediately after the run as `.csv`.
2. Save as `data/raw/YYYYMMDD_Project_SeqID_User_raw.csv` in the project repository.
3. Commit raw data and the correction script per SOP-L801.

11. Waste Disposal

Waste Type	Disposal Method
Used exetainer vials and acid	Acid waste carboy per SOP-L006
Gloves, Kimwipes	Standard trash unless acid-contaminated

12. References

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, *25*(17), 2538–2560. https://doi.org/10.1002/rcm.5129

Kim, S.-T., Mucci, A., & Taylor, B. E. (2007). Phosphoric acid fractionation factors for calcite and aragonite between 25 and 75 °C: revisited. *Chemical Geology*, *246*(3–4), 135–146. https://doi.org/10.1016/j.chemgeo.2007.08.005

Swart, P. K., Burns, S. J., & Leder, J. J. (1991). Fractionation of the stable isotopes of oxygen and carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function of temperature and technique. *Chemical Geology: Isotope Geoscience Section*, *86*(2), 89–96. https://doi.org/10.1016/0168-9622(91)90055-2

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials Handling	Acid handling
L208	IRMS General Instrument Use	Instrument-level procedures
L414	Soil CN by Flash EA-IRMS	Companion application SOP
L802	Quality Control and Quality Assurance	General QC framework

14. Training Record

Before performing carbonate isotope analysis independently, personnel must:

1. Read this SOP and SOP-L208 in full
2. Be a certified IRMS User (SOP-L208, Training Record)
3. Complete SOP-L003 Hazardous Materials Handling (acid handling)
4. Observe a GasBench carbonate run from vial preparation through data export
5. Perform a supervised run with acceptable QC results
6. Complete a supervised data reduction session
7. Be signed off by the lab manager or PI



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L416

Cation Exchange Capacity (CEC)

Standard Operating Procedure

SOP Number	SOP-L416
Revision	0.1
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the determination of cation exchange capacity (CEC) and exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in soils using the ammonium acetate (NH_4OAc) saturation and displacement method at pH 7.0 (Chapman, 1965; Sumner & Miller, 1996). CEC quantifies the total capacity of a soil to hold exchangeable cations and is a fundamental indicator of soil fertility, buffering capacity, and nutrient retention (Sparks, 2003).

2. Scope

- **Applies to:** Air-dried, sieved (< 2 mm) mineral soils from all EA Program sites. The method is also applicable to compost and organic amendments, although CEC values for highly organic materials should be interpreted with caution due to pH-dependent charge (Sumner & Miller, 1996).
- **Does not apply to:** Soils dominated by variable-charge minerals (e.g., highly weathered Oxisols, Andisols) where CEC measured at pH 7.0 may overestimate field-effective CEC. For such soils, a compulsive exchange method at field pH may be more appropriate (Gillman & Sumpter, 1979). Does not apply to water samples.
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Prepare extracts, record and report results
New Students	Complete training before independent use

3.1. Required Training

Students using this SOP should be trained on:

- SOP-L002 Laboratory Safety
- SOP-L400 Soil Moisture

4. Definitions

- **Buffered salt solution:** A reagent at a defined pH used to saturate exchange sites with a known index cation (e.g., NH_4^+ at pH 7.0 in the ammonium acetate method); the buffer pH controls which exchange sites are measured.
- **Base saturation:** The percentage of CEC occupied by base cations (Ca^{2+}, Mg^{2+}, K^+, Na^+); calculated as the sum of base cations divided by CEC, expressed as %; important for liming and fertility management.
- **Cation exchange capacity (CEC):** The total capacity of a soil to hold exchangeable cations, expressed in $cmol_c\ kg^{-1}$ (or meq 100 g^{-1}); governed by clay mineralogy and organic matter content; the higher the CEC, the greater the soil's buffering capacity against acidification and nutrient leaching.
- **Effective CEC (ECEC):** CEC measured at the soil's natural pH (using an unbuffered salt such as 1 M KCl or $BaCl_2$); represents the actual exchange capacity at field conditions; lower than pH 7-buffered CEC in acid soils.
- **Exchange sites:** Negatively charged surfaces on clay minerals and organic matter that hold cations electrostatically; released by displacing the sorbed cations with a high-concentration competing ion.
- **Index cation:** The cation used to saturate exchange sites before measurement (e.g., NH_4^+ in the ammonium acetate method, Ba^{2+} in the compulsive exchange method); subsequently displaced and quantified to calculate CEC.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** 1 M ammonium acetate (NH_4OAc) is a mild irritant. Concentrated acetic acid used in reagent preparation is corrosive and produces irritating vapors. Concentrated ammonium hydroxide (NH_4OH) used for pH adjustment is caustic and produces strong vapors. Ethanol (95%) is flammable.
- **Physical:** Reciprocating shaker can pinch fingers if containers are not secured properly.

! Engineering Controls

Prepare 1 M NH_4OAc from concentrated reagents inside a chemical fume hood. Once diluted to working concentration, the solution can be used on the open bench.

5.1. Required PPE

☐ Safety glasses or chemical splash goggles (goggles when preparing reagents)
☐ Laboratory coat
☐ Nitrile gloves
☐ Closed-toed shoes

5.2. Emergency Procedures

For chemical spill or splash, refer to SOP-L002 Laboratory Safety or call campus safety. For acetic acid or ammonium hydroxide contact with eyes, flush immediately with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Reciprocating or end-over-end shaker	For batch extraction
Centrifuge	Capable of 2,000–3,000 x g
Vacuum filtration apparatus	Buchner funnel, flask, and vacuum source
Analytical balance	Readable to 0.01 g
Drying oven	105 °C for moisture subsamples
pH meter	For adjusting NH_4OAc to pH 7.0
Volumetric flasks	1 L and 250 mL

6.2. Reagents and Consumables

Item	Notes
Ammonium acetate (NH_4OAc)	ACS reagent grade; or prepare from glacial acetic acid + NH_4OH
Ethanol (95%)	Reagent grade, to wash excess NH_4^+
Potassium chloride (KCl), 2 M	Or 10% NaCl; for displacing adsorbed NH_4^+
Whatman #42 filter paper	Or equivalent, for filtration
Centrifuge tubes (50 mL)	Polypropylene
Volumetric flasks (250 mL)	For collecting leachates
DI water	

7. Procedure

7.1. Reagent Preparation

7.1.1. M Ammonium Acetate (pH 7.0)

1. In a fume hood, dissolve 77.08 g of ammonium acetate ($NH_4C_2H_3O_2$, MW 77.08) in approximately 800 mL of DI water in a 1 L volumetric flask.
2. Adjust to pH 7.0 using dilute acetic acid (to lower pH) or dilute ammonium hydroxide (to raise pH).
3. Bring to 1 L with DI water and mix thoroughly.

> **Tip**
>
> Prepare this solution the day before extraction. It is stable indefinitely at room temperature in a capped container. Verify pH before each use, as CO_2 absorption can lower the pH over time.

7.1.2. M Potassium Chloride

Prepare per SOP-L403, Section 7.1.

7.2. Moisture Subsample

A companion gravimetric moisture determination is required to express results on an oven-dry soil basis.

1. Set aside approximately 10 g of soil from each sample for moisture determination
2. Dry at 105 °C for 24 hours and record the dry weight
3. Calculate gravimetric moisture content per SOP-L400 Soil Moisture

7.3. Ammonium Acetate Saturation (Exchangeable Cations)

1. Weigh 5.00 g of air-dried, sieved (< 2 mm) soil into a labeled 50 mL centrifuge tube. Record the exact mass to 0.01 g.
2. Add 30 mL of 1 M NH_4OAc (pH 7.0) to the tube. Cap securely.
3. Place on the reciprocating shaker for 30 minutes.
4. Centrifuge at 2,000–3,000 x g for 5 minutes. Decant the clear supernatant into a 250 mL volumetric flask. Retain the soil pellet.
5. Repeat steps 2–4 two additional times (three extractions total), combining all supernatants in the same 250 mL volumetric flask.
6. Bring the combined extract to 250 mL with 1 M NH_4OAc and mix. This extract is used for exchangeable cation analysis (Ca^{2+}, Mg^{2+}, K^+, Na^+) by ICP-OES, AAS, or flame photometry (Helmke & Sparks, 1996).

7.4. Ethanol Wash

The ethanol wash removes excess (non-exchanged) NH_4^+ from the soil without displacing adsorbed NH_4^+ (Sumner & Miller, 1996).

1. Add 30 mL of 95% ethanol to the soil pellet remaining in the centrifuge tube. Cap and shake for 5 minutes.
2. Centrifuge at 2,000–3,000 x g for 5 minutes. Discard the supernatant ethanol wash.

3. Repeat the ethanol wash two additional times (three washes total).

> **!** Critical Step
>
> Incomplete removal of excess NH_4OAc will cause CEC to be overestimated. Ensure three full ethanol washes with clear supernatant before proceeding.

7.5. Displacement of Adsorbed NH_4^+ (CEC Determination)

1. Add 30 mL of 2 M KCl to the washed soil pellet. Cap and shake for 30 minutes.
2. Centrifuge at 2,000–3,000 x g for 5 minutes. Decant the supernatant into a clean 250 mL volumetric flask.
3. Repeat steps 1–2 two additional times (three displacements total), combining all supernatants in the same flask.
4. Bring the combined KCl extract to 250 mL with 2 M KCl and mix.
5. Determine the NH_4^+-N concentration in this extract by colorimetric analysis (indophenol blue method), flow injection, or steam distillation (Mulvaney, 1996). This value represents the CEC.

7.6. Cleanup

1. Discard spent soil in standard solid waste.
2. Rinse all glassware and centrifuge tubes with DI water.
3. Return reagents to labeled storage areas.

8. Calculations

8.1. Cation Exchange Capacity

$$\text{CEC (cmol}_c\text{/kg)} = \frac{C_{NH_4} \times V_{KCl} \times 10^{-3}}{m_{dry} \times 14.01} \times 100$$

where:

- C_{NH_4} = NH_4^+-N concentration in the KCl displacement extract (mg N/L)
- V_{KCl} = total volume of combined KCl extract (mL; typically 250 mL)
- m_{dry} = oven-dry equivalent mass of soil (g), calculated as $m_{air\text{-}dry}/(1 + \theta_g)$
- 14.01 = atomic mass of nitrogen (g/mol)
- The factor of 100 converts from mol_c/g to $cmol_c/kg$

8.2. Exchangeable Cations

Report exchangeable Ca^{2+}, Mg^{2+}, K^+, and Na^+ from the NH_4OAc extract (§6.3) in $cmol_c/kg$:

$$\text{Exchangeable cation (cmol}_c\text{/kg)} = \frac{C_{\text{cation}} \times V_{\text{extract}} \times \text{valence}}{M_{\text{cation}} \times m_{\text{dry}}} \times \frac{1}{10}$$

where C_{cation} is in mg/L, V_{extract} in mL, M_{cation} is the atomic mass (Ca = 40.08, Mg = 24.31, K = 39.10, Na = 22.99), and valence is 2 for Ca and Mg and 1 for K and Na.

8.3. Base Saturation

$$\text{Base Saturation (\%)} = \frac{Ca^{2+} + Mg^{2+} + K^+ + Na^+}{\text{CEC}} \times 100$$

where all values are in $cmol_c/kg$.

8.4. Interpretation

CEC ($cmol_c/kg$)	Classification	Typical Soil Textures
< 5	Very low	Sands, degraded tropicals
5–15	Low	Sandy loams, kaolinitic clays
15–25	Medium	Loams, silt loams
25–40	High	Clay loams, smectitic soils
> 40	Very high	Heavy clays, organic soils

Source: adapted from Sparks (2003) and Brady & Weil (2008).

9. Quality Control and Acceptance Criteria

- Include a reagent blank (NH_4OAc and KCl through the full procedure without soil) in each batch. NH_4^+-N in the blank must be below the method detection limit.
- Run extraction duplicates on at least 10% of samples. Duplicate results should agree within 10% RPD.
- Include a laboratory reference soil with known CEC when available.
- Sum of exchangeable base cations should not exceed CEC. If the sum substantially exceeds CEC, suspect incomplete ethanol washing or analytical error.
- Verify NH_4OAc pH is 7.0 ± 0.05 before each extraction batch.

10. Waste Disposal

Waste Type	Container	Disposal Method
NH_4OAc extracts	Drain with water	Low hazard; drain permitted
Ethanol washes	Flammable waste carboy	EH&S pickup
KCl extracts	Drain with water	Low hazard; drain permitted
Spent soil	Standard solid waste	Trash

11. References

- Sumner & Miller (1996)
- Chapman (1965)
- Sparks (2003)
- Helmke & Sparks (1996)
- Mulvaney (1996)
- Gillman & Sumpter (1979)
- Brady & Weil (2008)
- Ross & Ketterings (1995)
- SOP-L002 Laboratory Safety
- SOP-L400 Soil Moisture
- SOP-L403 Nitrate and Ammonium – KCl Extraction

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Mulvaney, R. L. (1996). Nitrogen: Inorganic forms. In D. L. Sparks, A. L. Page, P. A. Helmke, & R. H. Loeppert (Eds.), *Methods of soil analysis, part 3: Chemical methods* (pp. 1123–1184). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.3.c38

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Sparks, D. L. (2003). *Environmental soil chemistry* (2nd ed.). Academic Press. https://doi.org/10.1016/B978-012656446-4/50001-3

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12. Training Record

Before performing CEC analysis independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the SDS for ammonium acetate, acetic acid, ammonium hydroxide, and ethanol
4. Complete SOP-L400 Soil Moisture training
5. Observe a trained operator perform the full extraction sequence
6. Perform the procedure under supervision with acceptable duplicate precision (RPD $< 10\%$)
7. Be signed off by the PI or lab manager

13. Appendix A: Example Calculation

```
# --- CEC Calculation Example ---

# Input values
nh4_mg_L    <- 42.5    # NH4-N in KCl displacement extract (mg/L)
vol_KCl_mL  <- 250     # Total volume of combined KCl extract (mL)
mass_air_g  <- 5.00    # Air-dried soil mass (g)
moisture    <- 0.032   # Gravimetric moisture content (decimal)

# Oven-dry mass
mass_dry_g <- mass_air_g / (1 + moisture)

# CEC in cmol_c/kg
cec <- (nh4_mg_L * vol_KCl_mL * 1e-3) / (mass_dry_g * 14.01) * 100

cat(sprintf("Oven-dry soil mass: %.3f g\n", mass_dry_g))
cat(sprintf("CEC: %.1f cmol_c/kg\n", cec))

# --- Exchangeable Cation Example (Ca from NH4OAc extract) ---
ca_mg_L      <- 85.2   # Ca concentration in NH4OAc extract (mg/L)
vol_ext_mL   <- 250    # Total volume of NH4OAc extract (mL)
ca_mw        <- 40.08  # Atomic mass of Ca
ca_valence   <- 2

exch_ca <- (ca_mg_L * vol_ext_mL * ca_valence) / (ca_mw * mass_dry_g) / 10

cat(sprintf("Exchangeable Ca: %.1f cmol_c/kg\n", exch_ca))
```

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L417

Soil Aggregate Stability – Wet Sieving Method

Standard Operating Procedure

SOP Number	SOP-L417
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil aggregate stability using the wet sieving method. Aggregate stability quantifies the resistance of soil structural units to disintegration when subjected to disruptive forces such as wetting, raindrop impact, and mechanical disturbance. It is a key

indicator of soil physical quality, erosion susceptibility, and the effects of management practices (tillage, cover cropping, organic amendments) on soil structure (Kemper & Rosenau, 1986; Nimmo & Perkins, 2004).

2. Scope

- **Applies to:** Air-dried mineral soil samples sieved to a defined aggregate size class (typically 1–2 mm or 2–4 mm). Samples must have been collected and processed per SOP-F400 Soil Sampling.
- **Does not apply to:** Sandy soils with negligible aggregation, organic soils (histosols), or aggregate characterization by dry sieving alone
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Lab Manager	Maintain wet sieving apparatus
Trained Personnel	Follow this SOP, report results
New Students	Complete training before independent use

4. Definitions

- **Aggregate:** A cluster of soil particles (sand, silt, clay, organic matter) bound together by organic compounds, fungal hyphae, microbial exudates, and electrostatic forces; the basic structural unit of soil.
- **Aggregate stability (wet-sieve method):** A measure of soil aggregate resistance to disruption by the mechanical action of water immersion and oscillation; expressed as the percentage of aggregates retained on a defined sieve size (e.g., 0.25 mm or 2 mm) after wet sieving.
- **Dispersant (sodium hexametaphosphate):** A chemical added to the control (dispersed) sample to break down all aggregates; used to measure the sand correction in aggregate stability calculations.
- **Mean weight diameter (MWD):** A summary statistic for aggregate size distribution calculated as the weighted mean of aggregate size fractions after wet or dry sieving; higher MWD indicates better structural stability.
- **Slaking:** The rapid collapse of aggregates upon sudden immersion in water, caused by compression of entrapped air; fast wetting promotes slaking and reduces aggregate stability measurements.
- **Water-stable aggregate (WSA):** An aggregate that remains intact after defined wet-sieving; the fraction used to calculate aggregate stability.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Sodium hexametaphosphate (dispersing agent) is a mild irritant. Hydrogen peroxide (if used for organic matter correction) is a strong oxidizer.
- **Physical:** Drying oven operates at 105 C; burn risk from hot containers.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (when handling dispersing solution or H_2O_2)
☐ Heat-resistant gloves (when handling containers from the oven)

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes. Refer to SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Wet sieving apparatus (Yoder-type or Eijkelkamp)	SGM 133	Oscillating sieve holder
Nested sieves (e.g., 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.053 mm)	SGM 133	Stainless steel, 8 cm diameter
Drying oven (105 C)	SGM 133	
Analytical balance (0.001 g)	SGM 133	See SOP-L200
Desiccator with active desiccant	SGM 133	
Pre-weighed aluminum drying tins	SGM 133	

6.2. Reagents and Consumables

Item	Notes
Deionized water	For wetting and sieving
Sodium hexametaphosphate solution (5 g/L)	For dispersing residual aggregates (sand correction)

Item	Notes
Hydrogen peroxide (30%), optional	For removing organic matter if sand correction requires it

7. Procedure

7.1. Sample Preparation

1. Air-dry soil samples at room temperature until friable (typically 48–72 hours)
2. Gently break large clods along natural planes of weakness by hand; do not grind or crush
3. Dry-sieve the sample through a nest of sieves to isolate the desired aggregate size class (e.g., 1–2 mm or 2–4 mm)
4. Discard material outside the target size range for this analysis
5. Weigh out replicate subsamples (typically 4.0 g each, minimum 3 replicates per sample) into labeled containers

7.2. Pre-Wetting (Capillary Wetting)

Rapid wetting causes slaking, which may overestimate instability for some research questions. If slow pre-wetting is desired:

1. Place each subsample on a moistened filter paper in a closed container
2. Allow capillary wetting for 10 minutes (or a duration standardized for the study)
3. Proceed to wet sieving

If the goal is to assess slaking resistance under rapid wetting (the more common approach), omit the pre-wetting step and place air-dried aggregates directly onto the sieve.

7.3. Wet Sieving

1. Place the pre-weighed aggregates on the top sieve of the wet sieving apparatus (e.g., 0.25 mm for a single-sieve method, or the top of a nested stack)
2. Raise the water level in the sieving cans until the sieves are submerged to the appropriate depth (typically 1–2 cm above the sieve mesh)
3. Allow the aggregates to soak for 5 minutes (standardize this time for the study) before starting oscillation
4. Start the wet sieving apparatus. The standard cycle is 3 cm stroke at 34 cycles per minute for 3 minutes (Kemper & Rosenau, 1986). Record the exact settings used.
5. After sieving, carefully raise the sieves from the water

7.4. Collection and Drying

1. Backwash the material retained on each sieve into a pre-weighed aluminum tin using a wash bottle with deionized water

2. Transfer any material that passed through the finest sieve (the material in the sieving can) into a separate tin if size fractionation is desired
3. Dry all tins at 105 C for 24 hours (or until constant mass)
4. Cool in a desiccator and weigh to the nearest 0.001 g

7.5. Sand Correction

The material retained on the sieve after wet sieving includes both water-stable aggregates and primary sand particles that are inherently larger than the sieve openings. To correct for sand:

1. Add 50 mL of 5 g/L sodium hexametaphosphate to the dried residue in each tin
2. Allow to soak for at least 2 hours (or overnight) to fully disperse remaining aggregates
3. Pour the dispersed slurry back through the same sieve used in the wet sieving step
4. Rinse thoroughly with deionized water
5. Backwash the retained sand into a clean tin, dry at 105 C, cool, and weigh

7.6. Calculations

Water-stable aggregates (WSA) as a fraction of the total sample, corrected for sand:

$$\mathrm{WSA}(\%) = \frac{M_{\mathrm{stable}} - M_{\mathrm{sand}}}{M_{\mathrm{total}} - M_{\mathrm{sand}}} \times 100$$

where:

- M_{stable} = oven-dry mass of material retained on the sieve after wet sieving (g)
- M_{sand} = oven-dry mass of sand retained after dispersal (g)
- M_{total} = initial oven-dry equivalent mass of the subsample (g; corrected for moisture using gravimetric moisture from SOP-L400)

If a nested sieve set is used, calculate the mean weight diameter (MWD):

$$\mathrm{MWD} = \sum_{i=1}^{n} \bar{x}_i \, w_i$$

where $\bar{x}_i$ is the mean diameter of the ith size fraction and w_i is the proportion of the total sample weight in that fraction (after sand correction) (Van Bavel, 1950).

8. Quality Control and Acceptance Criteria

- Run a minimum of 3 replicates per sample; report the mean and standard deviation
- The coefficient of variation among replicates should be less than 15%
- Include a laboratory reference soil in each batch to monitor method consistency over time
- Record all apparatus settings (stroke length, frequency, sieving duration) so results are reproducible
- *TBD – identify or prepare a reference soil for long-term QC tracking*

9. Waste Disposal

Waste Type	Disposal Method
Sieved soil and sand	Standard trash or return to sample stock
Sodium hexametaphosphate solution	Drain with copious water (non-hazardous at this concentration)
H_2O_2 waste (if used)	Dilute and drain, or collect for EH&S
Used aluminum tins	Clean and reuse, or recycle

10. References

Kemper, W. D., & Rosenau, R. C. (1986). Aggregate stability and size distribution. In A. Klute (Ed.), *Methods of soil analysis, part 1: Physical and mineralogical methods* (2nd ed., pp. 425–442). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.1.2ed.c17

Nimmo, J. R., & Perkins, K. S. (2004). Aggregate stability and size distribution. In J. H. Dane & G. C. Topp (Eds.), *Methods of soil analysis, part 4: Physical methods* (pp. 317–328). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.4.c14

Van Bavel, C. H. M. (1950). Mean weight-diameter of soil aggregates as a statistical index of aggregation. *Soil Science Society of America Journal*, *14*, 20–23. https://doi.org/10.2136/sssaj1950.036159950014000C0005x

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Complete SOP-L400 Soil Moisture training (required for moisture correction)
4. Observe a trained operator perform the full procedure including sand correction
5. Perform the analysis under supervision with acceptable replicate precision (CV less than 15%)
6. Be signed off by the PI or lab manager



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L418

Water-Extractable Dissolved Organic Carbon (Soil)

Standard Operating Procedure

SOP Number	SOP-L418
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the extraction and quantification of water-extractable dissolved organic carbon (WEOC) from soil. WEOC represents the fraction of soil organic matter that is readily

soluble in water and includes simple sugars, organic acids, amino acids, and other low-molecular-weight compounds. It is a sensitive indicator of labile carbon availability, microbial substrate supply, and changes in soil management or land use (Ghani et al., 2003; Jones et al., 2006).

WEOC is distinct from the K_2SO_4-extractable DOC measured in the chloroform fumigation-extraction method (SOP-L409) and from total soil organic carbon (SOP-L407). The water extraction uses no salt, making it a closer analogue to the dissolved organic carbon that would leach from soil under natural rainfall or irrigation.

2. Scope

- **Applies to:** Mineral and organic soil samples, compost, and organic amendments. Samples should be collected per SOP-F400 Soil Sampling and stored at 4 C (for field-moist analysis) or air-dried.
- **Does not apply to:** Water samples (analyze directly on the TOC-L), K_2SO_4 extracts for microbial biomass carbon (see SOP-L409), or total soil carbon by combustion (see SOP-L407)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Perform extraction and submit samples for TOC analysis
New Students	Complete training before independent use

4. Definitions

- **Dissolved organic carbon (DOC):** Organic carbon that passes through a 0.45 µm filter; in soil extracts, includes low-molecular-weight organic acids, fulvic acids, amino acids, and other water-soluble organic compounds.
- **Hot-water extractable carbon (HWEC):** A more aggressive extraction using hot water (80 °C); releases more labile organic carbon than cold-water extraction; a closer proxy for microbially available carbon and total labile carbon.
- **KHP (potassium hydrogen phthalate):** The primary standard for TOC calibration; a pure, stable organic acid weighed accurately to prepare standards of known carbon concentration.
- **Labile organic carbon:** Readily decomposable organic carbon fractions that turn over on timescales of days to months; includes microbial biomass, water-extractable organic carbon, and dissolved organic carbon; sensitive to management and land use.
- **NPOC (non-purgeable organic carbon):** The organic carbon remaining in a sample after acidification and sparging to remove inorganic carbon; the quantity measured by the Shimadzu TOC-L and the operational definition of DOC.

- **Soil-to-water ratio:** The ratio of soil mass to extraction water volume (typically 1:5 or 1:10 w/v) in the water extraction step; must be reported alongside results and held constant within a study.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Drying oven (105 C) and orbital shaker pose minor physical hazards.
- **Biological:** Soil samples may harbor pathogens. Wash hands after handling soil.
- **Chemical:** Concentrated HCl (if used for acidification before TOC analysis) is corrosive.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For acid splashes, flush with water for at least 15 minutes. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Orbital or end-over-end shaker	SGM 133	
Centrifuge (capable of 4,000 x g)	SGM 133	
Vacuum filtration apparatus or syringe filters	SGM 133	
Shimadzu TOC-L analyzer	SGM 133	See SOP-L204
Analytical balance (0.01 g)	SGM 133	See SOP-L200
Drying oven (105 C)	SGM 133	For gravimetric moisture

6.2. Reagents and Consumables

Item	Notes
Deionized water (Type I or II, 18.2 MOhm)	Carbon-free; verify with TOC blank

Item	Notes
50 mL centrifuge tubes (polypropylene)	Pre-rinsed with DI water
0.45 um membrane or syringe filters	Pre-combusted glass fiber (GF/F) or PVDF; do not use cellulose acetate (leaches DOC)
Pre-combusted 40 mL glass vials with PTFE-lined caps	For TOC-L analysis
2 M HCl	For acidifying extracts if NPOC mode is used

7. Procedure

7.1. Sample Preparation

1. If using field-moist soil, determine gravimetric moisture content on a separate subsample per SOP-L400
2. If using air-dried soil, pass through a 2 mm sieve and determine residual moisture content

7.2. Extraction

1. Weigh 5.0 g (oven-dry equivalent) of soil into a 50 mL centrifuge tube. Record the exact mass.
2. Add 25 mL of deionized water (1:5 soil-to-water ratio) (Ghani et al., 2003; Jones et al., 2006)
3. Cap the tube and shake on an end-over-end or orbital shaker at approximately 150 rpm for 1 hour at room temperature
4. Centrifuge at 4,000 x g for 10 minutes to separate the supernatant

> **Tip**
>
> Some protocols use a hot water extraction (80 C for 16 hours) to capture a larger fraction of labile carbon (Ghani et al., 2003). If hot water extraction is desired, specify the temperature and duration explicitly in the lab notebook and report, as the two methods are not directly comparable.

7.3. Filtration

1. Filter the supernatant through a 0.45 um filter into a pre-combusted glass vial
2. If using syringe filters, discard the first 2–3 mL of filtrate to flush any carbon leached from the filter
3. Collect at least 15 mL of filtrate for TOC analysis (enough for multiple injections plus rinse volume)

7.4. Storage

1. If extracts cannot be analyzed within 24 hours, acidify to pH less than 2 with 2 M HCl (approximately 1 drop per 10 mL) and store at 4 C
2. Analyze within 7 days of extraction; prolonged storage may alter DOC concentrations even when acidified

7.5. DOC Analysis

1. Analyze filtered extracts on the Shimadzu TOC-L in NPOC mode per SOP-L204
2. Prepare a deionized water blank and extraction blanks (DI water processed through the full extraction procedure) alongside samples
3. Run calibration standards (potassium hydrogen phthalate, KHP) as described in SOP-L204

7.6. Calculations

$$\text{WEOC (mg C kg}^{-1}\text{ dry soil)} = \frac{(C_{\text{sample}} - C_{\text{blank}}) \times V_{\text{extract}}}{m_{\text{soil}}}$$

where:

- C_{sample} = DOC concentration in the filtered extract (mg C/L)
- C_{blank} = DOC concentration in the extraction blank (mg C/L)
- V_{extract} = total volume of extractant added (L; e.g., 0.025 L)
- m_{soil} = oven-dry mass of soil extracted (kg)

8. Quality Control and Acceptance Criteria

- Include extraction blanks (DI water only, no soil) in each batch to quantify background carbon from filters, tubes, and water
- Run sample duplicates for at least 10% of samples; CV between duplicates should be less than 10%
- Include a laboratory reference soil in each batch
- Verify that extraction blank DOC is less than 1 mg/L; if higher, troubleshoot water quality and filter blanks
- Instrument QC follows SOP-L204 requirements (calibration verification, instrument blanks, replicate injection CV less than 2%)

9. Waste Disposal

Waste Type	Disposal Method
Soil residue after extraction	Standard trash or return to sample stock
Filtered water extracts	Drain with copious water (non-hazardous)

Waste Type	Disposal Method
Acidified extracts	Neutralize and drain, or collect for EH&S
Used filters	Standard trash

10. References

Ghani, A., Dexter, M., & Perrott, K. W. (2003). Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology and Biochemistry*, *35*(9), 1231–1243. https://doi.org/10.1016/S0038-0717(03)00186-X

Jones, D. L., Willett, V. B., Stockdale, E. A., Sherlock, R., & Murphy, D. V. (2006). Dissolved organic carbon dynamics and fluxes in agricultural soils. *Geoderma*, *135*, 200–211.

11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Complete SOP-L400 Soil Moisture training (required for moisture correction)
4. Be trained on SOP-L204 Shimadzu TOC-L or have extracts analyzed by a trained operator
5. Perform the extraction under supervision with acceptable duplicate precision (CV less than 10%)
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L419

Soil Color – Munsell Lab Confirmation

Standard Operating Procedure

SOP Number	SOP-L419
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil color under controlled laboratory lighting using the Munsell Soil Color Charts. Soil color is a rapid, non-destructive indicator of organic matter content, mineralogy, drainage condition, and degree of weathering. It is a required observation in soil profile descriptions and is used in soil taxonomy (e.g., distinguishing Mollisols by dark surface colors) (Lynn & Pearson, 1993; Soil Survey Division Staff, 2017).

Field color determinations (see SOP-F400) are affected by variable natural lighting, sun angle, and observer fatigue. This laboratory procedure standardizes the observation under a controlled illuminant to improve reproducibility and provide a reference-quality color record.

2. Scope

- **Applies to:** All soil and sediment samples for which a reference-quality Munsell color determination is needed, including both moist and air-dried conditions
- **Does not apply to:** Rock or mineral color determinations using the Munsell Rock Color Chart, instrumental colorimetry or spectrophotometry, or quick field assessments (which remain valuable but are less reproducible)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Perform color determinations, record results
New Students	Complete training before independent use

4. Definitions

- **Chroma:** The colorimetric purity or saturation of a color; the third component of Munsell notation (e.g., the 4 in 10YR 4/4); higher chroma values indicate more saturated, vivid colors; low chroma (less than or equal to 2) in subsurface horizons indicates gleying and periodic saturation.
- **Hue:** The dominant spectral color in Munsell notation (e.g., 10YR, 7.5YR, 2.5Y, Gley 1); corresponds to the wavelength of the dominant reflected light; the first element of a Munsell color designation.
- **Munsell color system:** A standardized color notation developed by A.H. Munsell expressing color as three independent components: Hue, Value, and Chroma; used universally in soil science for objective color description.
- **Redoximorphic features:** Color patterns in soil caused by reduction and oxidation of iron and manganese under fluctuating saturation conditions; include masses (concentrations), depletions (zones of low chroma), and concretions; diagnostic for hydric soils.
- **Standard illuminant (D65):** A standardized daylight spectrum at 6,500 K color temperature; the reference lighting condition for accurate Munsell color matching; overcast north-sky light or a D65 light box approximates this condition.
- **Value:** The lightness or darkness of a color in Munsell notation; ranges from 0 (black) to 10 (white); the second component in Munsell notation (e.g., the 4 in 10YR 4/4).

5. Safety and Hazards

Hazards

- **Biological:** Soil samples may contain pathogens. Wash hands after handling soil.
- **Physical:** No significant hazards. If using a spray bottle to moisten samples, avoid wetting electronic equipment or Munsell charts.

5.1. Required PPE

☐ Nitrile gloves
☐ Lab coat

6. Equipment and Materials

Item	Notes
Munsell Soil Color Charts (current edition)	Replace when pages are faded, stained, or damaged
Daylight-balanced lamp (D65 or equivalent, 5,000–6,500 K)	Position to illuminate the sample and chart evenly without glare
Spray bottle with deionized water	For moistening air-dried samples
White ceramic tile or neutral gray card	For calibrating eye to neutral
Spatula or palette knife	For preparing a smooth sample surface
Lab notebook or standardized data sheet	For recording results

7. Procedure

7.1. Lighting Setup

1. Position the daylight-balanced lamp so it illuminates the sample and the Munsell chart at approximately the same angle and distance
2. Avoid mixing light sources (turn off overhead fluorescent or LED room lights if their color temperature differs significantly from the reference lamp)
3. Allow the lamp to warm up for at least 5 minutes before beginning observations

Note

If a daylight-balanced lamp is not available, work near a north-facing window under overcast sky conditions. Avoid direct sunlight, which changes color temperature with sun angle and clouds.

7.2. Sample Preparation

1. For **moist** color: if the sample is already field-moist, proceed directly. If it has been air-dried, moisten a subsample by adding deionized water drop by drop with a spray bottle or pipette until the sample glistens but no free water pools on the surface (Soil Survey Division Staff, 2017)
2. For **dry** color: use the air-dried sample without adding water
3. Use a spatula to press the soil into a smooth, flat surface (approximately 2–3 cm diameter) on a white or neutral background. A rough surface creates shadows that bias the reading toward darker values.
4. If the sample is heterogeneous (e.g., redoximorphic features), determine the color of the matrix and the color of each distinct mottle or concentration separately

7.3. Color Determination

1. Hold the Munsell chart adjacent to the prepared sample so both are under the same illumination
2. Look through the apertures on the chart page, placing the sample behind each hole, to find the closest match
3. First identify the correct **hue** page (e.g., 10YR, 7.5YR, 2.5Y)
4. Then match the **value** (lightness, vertical axis) and **chroma** (saturation, horizontal axis) within that page
5. Record the Munsell notation in the format: hue value/chroma (e.g., 10YR 4/3)
6. If the sample falls between two chips, record the interpolated notation or the nearest chip with a note (e.g., "10YR 4/3 to 4/4")
7. Repeat the determination for both moist and dry conditions

> **Tip**
>
> Color vision varies between individuals and can shift with fatigue. For critical determinations, have two observers independently determine the color and compare results. If readings differ by more than one chip in any dimension, re-examine jointly.

7.4. Recording Results

Record the following for each sample:

- Sample ID and depth
- Munsell notation (moist)
- Munsell notation (dry)
- Descriptive color name from the chart (e.g., "dark yellowish brown")
- Any redoximorphic features (mottle color, abundance, size, contrast)
- Observer name(s)
- Date and lighting conditions

7.5. Special Cases

Redoximorphic features: When mottles or concentrations are present, record the matrix color and the color, abundance (few, common, many), size (fine, medium, coarse), and contrast (faint, distinct, prominent) of each type of feature per the standards in the Soil Survey Manual (Soil Survey Division Staff, 2017).

Very dark samples (value 2 or less): These may be difficult to distinguish on the chart. Prepare a thinner smear and confirm under slightly brighter illumination.

Effervescent or calcareous samples: Note the presence of carbonates (tested with dilute HCl per SOP-L402), as carbonate coatings affect apparent color.

8. Quality Control and Acceptance Criteria

- Perform duplicate readings (by the same or a second observer) on at least 10% of samples
- Acceptable agreement: readings should match within one chip in value and one chip in chroma on the same hue page
- Inspect the Munsell chart for fading or staining at the start of each session; replace if the neutral chip (N/) no longer appears true gray
- Record the lamp model and approximate color temperature in the lab notebook

9. Waste Disposal

Waste Type	Disposal Method
Used soil subsamples	Return to sample container or standard trash
No chemical waste generated	–

10. References

Lynn, W., & Pearson, M. J. (1993). Soil color, mineralogy, and classification. In J. M. Bigham & E. J. Ciolkosz (Eds.), *Soil color* (pp. 71–91). Soil Science Society of America. https://doi.org/10.2136/sssaspecpub31.c5

Soil Survey Division Staff. (2017). *Soil survey manual*. Government Printing Office. https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-survey-manual

11. Training Record

Before performing Munsell color determinations independently, personnel must:

1. Read this SOP in full
2. Review the introductory pages of the Munsell Soil Color Charts

3. Practice matching at least 10 samples under supervision and compare readings with a trained observer
4. Demonstrate agreement within one chip in value and chroma on duplicate determinations
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L420

Soil Labile Amino-Nitrogen – Solvita SLAN Test

Standard Operating Procedure

SOP Number	SOP-L420
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the determination of soil labile amino-nitrogen (amino-N) using the Solvita SLAN test (Woods End Laboratories) (Brinton, 2016b). The SLAN test measures a chemically labile fraction of organic nitrogen present in soil humus as amino-sugars and related amino compounds. These forms represent a "potentially available" nitrogen pool that lies between the immediately available mineral nitrogen (NH_4^+ and NO_3^-) measured by conventional extraction and the total nitrogen pool measured by combustion or Kjeldahl digestion.

The test uses alkali hydrolysis with 2 mol L^{-1} NaOH to release NH_3 from amino-N compounds in soil (Mulvaney & Khan, 2001). The liberated NH_3 diffuses into a Solvita gel probe during a 24-hour room-temperature incubation. The color change of the gel is proportional to the quantity of labile amino-N and is quantified using the Solvita Digital Color Reader (DCR) in NH_3 mode, yielding results in mg kg^{-1} amino-N.

The SLAN procedure is chemically analogous to the Illinois Soil Nitrogen Test (ISNT), which uses the same 2 mol L^{-1} NaOH reagent but requires heating. The SLAN test offers two practical advantages over the ISNT: heating is not needed, and fewer reagents are required (Moore et al., 2019a). In combination with the Solvita CO_2 Burst test (SOP-L408), SLAN provides a complementary assessment of biological soil fertility by pairing a measure of microbial activity (CO_2 respiration) with a measure of organic nitrogen reserves (Brinton, 2016a).

This SOP is distinct from SOP-L408, which measures microbial CO_2 respiration from rewetted soil, and from SOP-L403, which measures immediately available mineral nitrogen by KCl extraction.

2. Scope

- **Applies to:** Air-dried, sieved mineral soils from all EA Program sites; assessment of organic nitrogen reserves and humus quality; screening for management effects on labile nitrogen pools
- **Does not apply to:** Measurement of immediately available mineral nitrogen (NH_4^+ or NO_3^-; see SOP-L403 or SOP-L404); total soil nitrogen determination (see SOP-L407 and elemental analysis); compost maturity testing (see SOP-L413); field-moist soils (samples must be air-dried before testing)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance

Role	Responsibility
Lab Manager	Maintain SLAN kit inventory, prepare and store NaOH solution, verify probe expiration dates
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Alkali-hydrolyzable nitrogen (AHN):** The fraction of organic nitrogen released by hydrolysis in sodium hydroxide solution; operationally defined by the extraction conditions; highly correlated with amino acid nitrogen and microbially available N (Mulvaney & Khan, 2001).
- **Amino acid nitrogen:** Organic nitrogen in the form of free amino acids and simple peptides; the most labile fraction of soil organic nitrogen; the primary substrate measured by the Solvita SLAN test.
- **Diffusion cell:** The sealed incubation jar used in the Solvita SLAN test in which NaOH releases NH_3 from hydrolyzed soil nitrogen into the headspace, where it is captured and measured by a gel probe.
- **Digital Color Reader (DCR):** The Solvita handheld device that reads gel probe color and converts it to a numerical amino-N concentration (mg kg^{-1}); set to NH_3 mode for SLAN measurements.
- **Gel probe (SLAN):** The colorimetric sensor in the Solvita SLAN test that changes color in proportion to accumulated NH_3; read with the DCR after exactly 24 hours of incubation.
- **Labile amino nitrogen (LAN):** The nitrogen fraction estimated by the Solvita SLAN test; a proxy for readily mineralizable organic nitrogen; expressed as mg N kg^{-1} dry soil (Moore et al., 2019b).
- **Nitrogen mineralization:** The biological conversion of organic nitrogen to inorganic forms (NH_4^+ and eventually NO_3^-) by soil microorganisms; the primary process by which plant-available N is replenished from organic matter; positively correlated with LAN (Moore et al., 2019a).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Sodium hydroxide (NaOH) at 2 mol L^{-1} is strongly corrosive and can cause severe burns to skin, eyes, and mucous membranes. Handle with care; refer to the NaOH Safety Data Sheet (SDS) posted in the lab. The SLAN test requires use of a strong reagent and is not intended for casual or unsupervised use.
- **Biological:** Soil samples may contain soil-borne pathogens. Wash hands thoroughly after handling soil.
- **Physical:** Minor risk of glass breakage from incubation jars. Handle jars carefully.

5.1. Required PPE

☐ Nitrile gloves (chemical-resistant)
☐ Safety glasses or splash goggles
☐ Lab coat

5.2. Emergency Procedures

- For NaOH contact with skin, immediately flush the affected area with copious water for at least 15 minutes. Remove contaminated clothing.
- For NaOH contact with eyes, flush with water at the eyewash station for at least 15 minutes and seek medical attention.
- For spills, neutralize with dilute acid (e.g., 0.1 M HCl or vinegar) or contain with absorbent material, then clean up and dispose per institutional guidelines.
- Refer to SOP-L002 Laboratory Safety for general emergency procedures.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Solvita SLAN test kit	Includes NH_3 gel probes, incubation jars with silicone-gasketed lids, 50 mL plastic beakers, color chart, and manual
Solvita Digital Color Reader (DCR)	Set to NH_3 mode; calibrate per manufacturer instructions
Incubation jars (~250 mL)	Glass with silicone-gasketed screw-top lids; provided with kit
Analytical balance (0.01 g)	See SOP-L102
Graduated cylinder (25 or 50 mL)	For dispensing NaOH solution
Timer	24-hour countdown
Lab notebook or data sheet	For recording results

6.2. Consumables and Reagents

Item	Notes
Solvita NH_3 gel probes	One per test; store sealed at room temperature in the dark; check expiration dates
50 mL plastic beakers (cups)	Provided with kit; one per test
Sodium hydroxide (NaOH), 2 mol L^{-1}	Prepare from ACS-grade NaOH pellets; see Section 5.1

6.3. Reagent Preparation

2 mol L^{-1} NaOH solution:

1. Weigh 80.0 g of ACS-grade NaOH pellets.
2. Dissolve in approximately 800 mL of deionized water in a 1 L volumetric flask or heat-resistant beaker. The dissolution is exothermic; add pellets slowly and stir.

> **Warning**
>
> Always add NaOH to water, never water to NaOH. Use a heat-resistant container and allow the solution to cool to room temperature before bringing to final volume.

3. Bring to a final volume of 1000 mL with deionized water.
4. Transfer to a labeled, tightly sealed polyethylene bottle. NaOH solutions absorb CO_2 from the atmosphere; keep sealed when not in use.
5. Label with: reagent name, concentration, date prepared, preparer's initials, and expiration date (6 months from preparation).

7. Procedure

7.1. Sample Preparation

1. Use soil that has been air-dried and sieved through a 2 mm sieve per SOP-F400. Do not grind the soil.
2. If samples were stored at 4 °C after sieving, allow them to equilibrate to room temperature (20–25 °C) for at least 30 minutes before beginning the assay.
3. Gently mix each sample to ensure homogeneity before weighing.

> **Note**
>
> The SLAN test is designed for air-dried soil. Results from field-moist soil are not comparable to published SLAN norms or the manufacturer's interpretation guidelines (Brinton, 2016b).

7.2. Incubation Setup

1. Weigh 4.0 g of air-dried, sieved soil into a clean 50 mL plastic beaker. Record the exact mass to 0.01 g.
2. Place the beaker containing soil inside a clean, dry 250 mL incubation jar.
3. Using the graduated cylinder, add 20 mL of 2 mol L^{-1} NaOH directly to the soil in the beaker.
4. Remove a fresh NH_3-sensitive gel probe from its sealed foil packet. Inspect the gel: it should be uniform in color with no cracks, discoloration, or signs of prior exposure. Discard any compromised probes.

5. Place the gel probe in the air space surrounding the plastic beaker. The gel face must be exposed to the headspace and must not contact the NaOH solution or the soil.
6. Seal the jar immediately and securely with the silicone-gasketed lid.
7. Record the jar number, sample ID, soil mass, and start time.

> **Important**
>
> Work efficiently once the NaOH is added. NH_3 begins volatilizing immediately upon contact with the soil; seal the jar as quickly as possible to capture the full emission.

7.3. Incubation

1. Place sealed jars at room temperature (20–25 °C), shielded from direct sunlight and drafts.
2. Incubate undisturbed for 24 hours.
3. Do not open, move, or disturb jars during incubation.

7.4. Reading Results

1. After exactly 24 hours (±30 minutes), open the jar and immediately remove the gel probe.
2. Read the probe promptly; prolonged exposure to ambient air will alter the gel color.

Using the Solvita Digital Color Reader (DCR – recommended):

3. Set the DCR to NH_3 mode.
4. Insert the gel probe into the DCR and record the numerical reading.
5. The DCR reading corresponds to amino-N concentration in mg kg^{-1} per the manufacturer's calibration (Brinton, 2016b).

Using the Solvita Color Chart (visual – semi-quantitative only):

3. Compare the gel probe to the Solvita NH_3 color chart.
4. Record the color number.

> **Tip**
>
> Read probes within 2 minutes of opening the jar. The DCR is strongly recommended for all quantitative work; the color chart provides semi-quantitative screening only.

7.5. Recording Results

Record the following for each test:

- Date and time (start and end of incubation)

- Sample ID, site, and depth
- Soil mass (g)
- NaOH volume added (mL)
- DCR reading (NH_3 mode) or color chart number
- Amino-N concentration (mg kg^{-1})
- Any anomalies (unusual soil color or odor, probe defects)

7.6. Cleanup

1. Carefully pour the NaOH-soil slurry into the designated waste container. Do not pour corrosive solutions down the drain without neutralization (see Section 8).
2. Rinse plastic beakers and incubation jars with deionized water. Dry completely before reuse.
3. Dispose of used gel probes in standard trash.

8. Quality Control and Acceptance Criteria

8.1. QC Requirements

QC Element	Requirement
Duplicates	At least 1 per 10 samples; RPD within 15%
Blank	One jar with 20 mL NaOH, no soil, and a gel probe per batch; probe should show no or minimal color change
DCR calibration	Calibrate per manufacturer instructions before each batch; set to NH_3 mode
Gel probe storage	Store sealed in foil at room temperature, away from light; check expiration dates; never use expired probes
NaOH solution	Verify 2 mol L^{-1}; check expiration; keep tightly sealed

8.2. Corrective Actions

- If the blank reading exceeds baseline by more than one color increment, check for NH_3 contamination sources (nearby ammonium reagents, compost, inadequate ventilation) and repeat the batch.
- If duplicate RPD exceeds 15%, re-run the sample pair. Persistent poor precision may indicate sample heterogeneity; increase homogenization effort before weighing.
- If NaOH solution appears cloudy or shows precipitate, prepare a fresh solution.

8.3. Limitations

The SLAN test does not detect nitrate or urea, and normal ammonium fertilizer rates do not interfere with the measurement. Known limitations include:

- The test may not be sensitive in very low-organic-matter soils (e.g., sandy soils with less than 1% SOM), where results may fall below the detection limit.

- Color matching by visual chart is subjective; the DCR is required for quantitative reporting.
- SLAN measures a chemically defined labile N pool, not the biologically available fraction directly; interpret alongside other soil health indicators.

8.4. Interpretation Guide

SLAN Amino-N (mg kg^{-1})	Interpretation	Implication
Less than 30	Very low	Severely depleted organic N reserves; management intervention warranted
30–60	Low	Limited labile N pool; consider cover crops or organic amendments
60–120	Moderate	Adequate organic N reserves for many soils
120–200	High	Good labile N pool; indicative of healthy humus
Greater than 200	Very high	Abundant organic N reserves; common in high-SOM, no-till, or prairie soils

Thresholds are approximate guidelines adapted from Woods End Laboratories and published field studies (Moore et al., 2019c, 2019b). Interpret SLAN values in the context of soil organic matter content, soil texture, and management history.

8.5. Complementary Tests

Parameter	SOP / Method
Microbial respiration (CO_2 Burst)	SOP-L408
Mineral nitrogen (NO_3^-, NH_4^+)	SOP-L403
Nitrate quick test	SOP-L404
Soil organic matter (LOI)	SOP-L406
Total C and N	SOP-L407 and elemental analysis
pH and electrical conductivity	SOP-L402

9. Waste Disposal

Waste Type	Disposal Method
NaOH-soil slurry	Neutralize with dilute HCl to ~pH 7 before draining, or collect in labeled corrosive waste container per SOP-L006
Used gel probes	Standard trash
Deionized water rinses	Drain
Incubation jars and beakers	Wash, rinse with DI water, and reuse
Unused NaOH solution (expired)	Neutralize before disposal per SOP-L006

10. References

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11. Related SOPs

SOP	Title	Relationship
F400	Soil Sampling and Processing	Sample collection and preparation
L002	Laboratory Safety	Required safety training
L400	Soil Moisture (Gravimetric)	Moisture correction for dry-mass basis
L403	Nitrate and Ammonium (KCl)	Complementary mineral N test
L404	Nitrate Quick Test	Complementary field N screening
L406	Loss on Ignition (SOM)	Complementary organic matter index
L407	Total Carbon	Complementary total C and N
L408	Solvita CO_2 Burst	Paired biological activity test

12. Training Record

Before performing the Solvita SLAN test independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the Solvita SLAN Official Instructions manual (included in the kit or available from solvita.com)
4. Demonstrate safe handling and preparation of 2 mol L^{-1} NaOH solution
5. Observe a trained operator perform the full procedure from sample weighing through DCR reading

6. Perform at least one test under supervision with acceptable QC results (duplicate RPD within 15%; blank within acceptable range)
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L475

Flash EA Soil Combustion for IRMS Analysis

Standard Operating Procedure

SOP Number	SOP-L475
Revision	1.1
Author(s)	Marc Los Huertos, Kyle McCarty
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the complete workflow for analyzing soil and sediment samples for δ^{15}N, δ^{13}C, and elemental C and N content using the Thermo Scientific Flash IRMS Elemental Analyzer (EA) coupled to the Delta V Isotope Ratio Mass Spectrometer (IRMS) through the ConFlo IV continuous-flow interface (Thermo Fisher Scientific, 2008b, 2010a). It covers sample preparation, instrument warm-up, sequence creation, running the analysis, shutdown, and data reduction.

For general instrument operation, gas checks, and pre-analysis tests, see SOP-L208. For the full data correction workflow (blank, linearity, scale normalization, and drift correction), see SOP-L414.

2. Scope

- **Applies to:** Soil, sediment, and plant tissue samples analyzed for δ^{13}C, δ^{15}N, and elemental concentrations (%C, %N) by combustion EA-IRMS in NC (dual reactor) mode
- **Does not apply to:** Sulfur isotope analysis (NCS mode), GasBench carbonate analysis (see SOP-L415), TC/EA hydrogen and oxygen isotope analysis, or liquid samples
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Environmental Isotope Lab, SGM Rm 135

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, ensure training compliance
Lab Manager	Prepare and verify instrument methods; maintain reactors
Super-User	Supervise trainees; perform minor maintenance
Certified User	Prepare samples, build sequences, run analyses
Student Researcher	Sample weighing and data reduction (no unsupervised runs)

4. Definitions

- **Blank correction:** Subtraction of the isotopic and elemental contribution of an empty tin capsule from each sample signal; necessary because capsule tin contributes a small amount of C and N (Paul et al., 2007; Thermo Fisher Scientific, 2010a).
- **Certified user:** A student, staff, or faculty member qualified to prepare samples and run the IRMS without supervision, as designated by the lab PI.
- **ConFlo IV:** Interface hub connecting the Flash EA and other peripherals to the Delta V IRMS; handles reference-gas delivery and peak switching (Thermo Fisher Scientific, 2008a).
- δ^{13}**C:** Stable carbon isotope ratio expressed in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ where $R = {}^{13}C/{}^{12}C$ (Coplen, 2011).
- δ^{15}**N:** Stable nitrogen isotope ratio expressed in per mil (‰) relative to atmospheric N_2 (AIR): $\delta^{15}N = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ where $R = {}^{15}N/{}^{14}N$ (Coplen, 2011).

- **Drift correction:** Adjustment for systematic temporal change in measured δ values during a sequence, estimated from bracketing reference materials (Paul et al., 2007).
- **Flash EA:** The Thermo Scientific Flash IRMS Elemental Analyzer peripheral; combusts samples at 1020 °C in an O_2 pulse and separates N_2 and CO_2 chromatographically before delivery to the IRMS via the ConFlo IV (Thermo Fisher Scientific, 2010a).
- **Isodat:** Thermo Fisher software for analytical sequence design, instrument control, and raw isotope data export (Thermo Fisher Scientific, 2010b).
- **Linearity correction:** Adjustment applied to account for the dependence of measured δ values on peak amplitude at the detector (Paul et al., 2007).
- **Reference material (RM):** A substance with certified or consensus δ values used to anchor measured values to the VPDB or AIR scale; at least two RMs spanning the expected sample range are required for two-point scale normalization (Coplen, 2011; Werner & Brand, 2001).
- **Scale normalization:** Two-point (or multi-point) correction that maps measured δ values to the international isotope-ratio scale using certified reference materials (Coplen, 2011; Paul et al., 2007).
- **Super-user:** A staff or faculty member qualified to run the IRMS and perform minor maintenance (gas replacement, reactor exchange, etc.).
- **Tin capsule:** 5 × 9 mm pressed-tin container used to encapsulate solid samples; tin ignites in the O_2 pulse, raising combustion temperature briefly to ensure complete combustion.
- **Zero-enrichment test:** Reference gas on/off pulses used to verify that the IRMS measures consistent δ values before analysis; performed during instrument warm-up.

5. Safety and Hazards

⚠ Hazards

- **Pressurized gases:** He, O_2, N_2, CO_2. Verify cylinder pressures before each session. He tank pressure must be above 500 psi, regulated to 50 psi.
- **Hot surfaces:** The Flash EA combustion reactor operates above 1000 °C. Use caution when handling ash fingers or reactor tubes.
- **Sharps:** Syringes, tweezers, and capsule-crimping tools can cause puncture or cut injuries.
- **Chemical:** Chromium oxide, silvered cobaltous/cobaltic oxide, and reduced copper used in the reactor packing are irritants; follow standard laboratory handling precautions.

❗ User Responsibilities

The lab manager verifies that methods are working properly, but **Certified Users** are responsible for preparing their own samples, creating run sequences, overseeing runs, and conducting data reduction.

5.1. Required PPE

☐ Safety glasses

☐ Laboratory coat
☐ Nitrile gloves (especially when handling reactor packing materials)

5.2. Emergency Procedures

Refer to SOP-L208 for instrument-specific emergency procedures and SOP-L002 for general lab safety.

6. Required Materials

6.1. Consumables and Reagents (NC Dual Reactor)

Item	ThermoFisher P/N	EA Consumables P/N
Quartz reactor (set of 2)	468 20070	C1069
Ash finger (quartz insert)	N/A	C1098
Silvered cobaltous/cobaltic oxide	338 24500	B1120
Chromium oxide	338 22900	B1099
Reduced copper wires	338 35300	B1016
Quartz wool	338 22200	—
Magnesium perchlorate (Anhydrone)	281 13102	—

6.2. Capsules and Weighing Supplies

- Tin capsules (appropriate size for your sample mass)
- Tungsten oxide (WO_3) – approximately 2 parts per sample for combustion aid (if required by method)
- Aluminum crucibles (for oven-drying soils)
- Weighing paper, cell wells, tweezers, spatulas
- Kimwipes, gloves

6.3. Standard Reference Materials

Prepare calibrated internal standards for every batch. Confirm which standards you need **before** approaching the lab. Standards on hand (certified values):

Reference Standard	δ^{13}C (VPDB)	δ^{15}N (AIR)
LOCSS	–26.66	7.30
HOCSS	–26.27	4.42
Urea	–43.26	–0.56
USGS24	–16.05	—
USGS40	–26.39	–4.52
USGS41a	36.55	47.6

(Coplen, 2011; Werner & Brand, 2001)

> **!** Important
>
> Lab standards are shared resources. Do not use them without approval from a super-user, the lab technician, or the PI. You are expected to replace what you use.

7. Sample Preparation

7.1. Drying and Grinding

1. Weigh soil or sediment samples into aluminum crucibles.
2. Oven-dry at 105 °C until constant mass.
3. Grind dried samples to a fine, homogeneous powder.

7.2. Elemental Pre-screening

Submit a subset of samples to elemental analysis (EA) to determine %C and %N before committing the full batch. This determines the correct sample mass to target the optimal signal range.

7.3. Sample Weighing

The target sample mass depends on %C and %N concentrations (Thermo Fisher Scientific, 2010a):

Typical %C	Target Sample Mass	Notes
$>$ 5% C	3–5 mg	Dilute if signal is too high
1–5% C	7–15 mg	Standard range
$<$ 1% C	15–25 mg	May require longer run times

1. Fold a tin capsule using tweezers (clean hands; no skin oils).
2. Weigh the target sample mass into the capsule.
3. Add WO_3 if required for combustion aids.
4. Crimp the capsule tightly so no sample can escape during handling.
5. Place crimped capsules in sequence order in a cell-well plate.
6. Record the exact mass of each sample in the data reduction spreadsheet.

> Tip: Clean Tweezers and Capsules
>
> Oils and residue from fingers contaminate tin capsules, introducing C and N blanks. Handle capsules only with clean, dry tweezers (Thermo Fisher Scientific, 2010a).

8. Instrument Warm-Up and Pre-Analysis Checks

See SOP-L208 for the complete warm-up procedure, gas pressure verification, zero-enrichment test, and linearity check. The Flash EA should be at operating temperature and passing all pre-analysis tests before loading samples.

9. Sequence Design

1. Open Isodat on the acquisition computer (Thermo Fisher Scientific, 2010b).
2. Create a new sequence with the following elements in order:
    - **Empty capsule blanks** (4–6): establish blank baseline
    - **Reference materials** (2–3 each of two or more certified RMs): for scale normalization (Coplen, 2011)
    - **Conditioning samples** (2–3): first samples in the run; typically sacrificial or low-priority
    - **Unknown samples** (bracketed by RMs every 10–12 unknowns)
    - **Closing reference materials** (2–3 of each RM): for drift correction (Paul et al., 2007)
3. Enter sample names, masses, and expected %C/%N for each row.
4. Assign the correct Isodat method to the sequence.
5. Save the sequence file before starting.

10. Running the Analysis

1. Verify carrier gas pressure (He $>$ 500 psi, regulated to 50 psi).
2. Load capsules into the autosampler carousel in sequence order.
3. Press **Start Sequence** in Isodat.
4. Monitor the first 3–4 samples to confirm:
    - N_2 and CO_2 peaks are within the expected mass range
    - Peak shapes are symmetric and well-separated
    - Reference material δ values are within $\pm 0.3‰$ of expected values (Werner & Brand, 2001)
5. If values are drifting, stop the run, identify the cause, and restart after correction.

11. Shutdown

1. After the final sample, allow the sequence to complete fully.
2. Follow the shutdown procedure in SOP-L208.
3. Export the raw data file from Isodat and save to the project directory.

12. Data Reduction

Data reduction follows the protocol in SOP-L414, which covers:

1. Blank correction (empty tin capsule contributions) (Paul et al., 2007)
2. Linearity correction (δ vs. peak area regression)
3. Two-point scale normalization to the VPDB / AIR scale using certified RMs (Coplen, 2011)
4. Drift correction (linear regression through bracketing RMs)
5. Uncertainty estimation and quality flags

13. Definitions of Certification Levels

Level	Capabilities
Lab PI	Unrestricted access; certify super-users
Super-User	Independent operation; certify certified users; replace He, O_2 cylinders; change reactors
Certified User	All steps from sample prep through data reduction; no unsupervised maintenance
Student Researcher	Sample prep and data reduction only, under supervision

14. References

- Thermo Fisher Scientific (2010a)
- Thermo Fisher Scientific (2008b)
- Thermo Fisher Scientific (2008a)
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15. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L208	Delta V IRMS General Instrument	Instrument startup and QC
L414	Soil CN by Flash EA-IRMS	Full data correction workflow
L415	Carbonate GasBench IRMS	Complementary IRMS application

16. Training Record

Before running analyses independently, personnel must:

1. Read this SOP and SOP-L208 in full
2. Be assigned a certification level by the Lab PI
3. Observe a Super-User or Certified User run a complete sequence from sample loading through data reduction
4. Run a supervised batch of at least 10 unknown samples with RM checks, achieving results within ±0.3‰ of expected values for both C and N
5. Be signed off by the Lab PI or designated Super-User

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L601

Preparing a Wet Mount for Microscopy

Standard Operating Procedure

SOP Number	SOP-L601
Revision	1.1
Author(s)	Dmaia Curry, Aparna Chintapalli
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes how to prepare a wet mount slide for light microscopy. A wet mount suspends a specimen in a thin layer of water (or other mounting medium) beneath a cover slip, preserving the natural shape and motility of living cells and small organisms. It is the standard preparation method for examining fresh biological samples under bright field or phase contrast illumination (Murphy & Davidson, 2012).

2. Scope

- **Applies to:** Preparation of temporary wet mount slides for examination of algae, protozoa, bacteria, fungi, plant cells, soil microorganisms, and other small specimens in the EA Program laboratory
- **Does not apply to:** Permanent slide preparation (fixed, stained, and sealed mounts); dry mounts; hanging-drop preparations for motility studies; fluorescence specimen preparation (see SOP-L211)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Prepare slides following this procedure
New Students	Complete training before independent use

4. Definitions

- **Cover slip (coverslip):** A thin (0.13–0.17 mm) square or circular piece of optical glass placed over the specimen on a microscope slide; standardizes the optical path length and protects the objective from liquid samples; the EA Program standard is No. 1.5 (0.17 mm), matched to the objectives on the Echo Revolve microscope (Murphy & Davidson, 2012).
- **Immersion medium:** Liquid placed between the specimen and the coverslip to improve optical contact, reduce refraction artifacts, and support live organisms during observation; options include water, glycerol, or immersion oil depending on the application.
- **Live mount:** A wet mount prepared without fixation or staining; allows observation of living organisms and motile behavior; must be examined quickly before evaporation or oxygen depletion affects the specimen.
- **Mounting medium:** The liquid in which the specimen is suspended; distilled or deionized water is standard; saline, glycerol, or specific staining solutions may be used depending on the application (Murphy & Davidson, 2012).
- **Refraction artifact:** Optical distortion caused by differences in refractive index between the sample, mounting medium, coverslip, and objective; minimized by matching refractive indices and maintaining thin, uniform wet mounts.

- **Wet mount:** A temporary microscopy preparation method in which a specimen is suspended in a drop of liquid (usually water) on a glass slide and covered with a coverslip; the standard preparation for aquatic microorganisms, algae, macroinvertebrates, and other specimens examined under transmitted light (Murphy & Davidson, 2012).

5. Safety and Hazards

⚠ Hazards

- **Physical:** Microscope slides and cover slips are fragile glass. Broken glass can cause cuts. Handle by the edges and dispose of chipped or cracked glass in the broken-glass box.
- **Biological:** Environmental samples (water, soil, compost) may contain unknown microorganisms. Treat all samples as potentially infectious and wash hands after handling.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves

5.2. Emergency Procedures

For cuts from broken glass, apply direct pressure and rinse with water. Seek first aid if bleeding is significant. Refer to SOP-L002 Laboratory Safety.

6. Required Materials

Item	Catalog No.	Notes
Microscope slides (75 × 25 mm)	TBD	Standard glass, pre-cleaned
Cover slips (No. 1.5, 22 × 22 mm)	TBD	0.17 mm thickness; matched to objectives on the Revolve microscope (Murphy & Davidson, 2012)
Squeeze bottle with DI water		Or distilled water
Disposable transfer pipettes or eyedroppers		For placing water drops
Forceps (fine-tipped)		For handling specimens and cover slips
Toothpicks		For lowering cover slips
Lint-free wipes (Kimwipes)		For blotting excess water

Catalog Numbers Needed

The original SOP flagged that catalog numbers for slides and cover slips should be added. Update this table when part numbers are confirmed with the lab manager.

7. Estimated Time

This procedure requires approximately 5 minutes per slide.

8. Procedure

8.1. Placing the Specimen

1. Clean a microscope slide with a lint-free wipe to remove dust and fingerprints.
2. Using forceps, place the specimen in the center of the slide. For liquid samples (pond water, culture broth), skip to Step 3 and pipette the sample directly.

8.2. Adding the Mounting Liquid

3. Using a transfer pipette or eyedropper, place one to two drops of water onto the specimen. Adjust the volume based on specimen size: larger or thicker specimens may need slightly more water, while very small specimens need less.

8.3. Applying the Cover Slip

4. Pick up a cover slip by its edges with forceps or clean, dry fingers. Never touch the flat surfaces.
5. Place one edge of the cover slip on the slide adjacent to the specimen, holding it at approximately a 45-degree angle.
6. Slowly lower the cover slip using the tip of a toothpick or forceps, allowing the water to spread gradually beneath the glass. Lowering at an angle lets air escape and minimizes bubble formation (Murphy & Davidson, 2012).

Avoiding Air Bubbles

Air bubbles distort the image and can be mistaken for cellular structures. If bubbles are present after placing the cover slip, gently tap the cover slip with the eraser end of a pencil or press lightly with a toothpick to work bubbles toward the edge. If large bubbles persist, remove the cover slip, add a fresh drop of water, and try again.

8.4. Adjusting Water Volume

7. **Too much water:** If the cover slip floats or slides freely, the water layer is too thick. Hold the edge of a lint-free wipe against the edge of the cover slip and let capillary action draw off excess water until the cover slip sits flat.
8. **Too little water:** If dry areas are visible beneath the cover slip, place a small drop of water at the edge and let capillary action draw it underneath. Do not lift the cover slip.

8.5. Examining the Slide

9. Place the prepared slide on the microscope stage and examine following SOP-L211 Echo Labs Revolve Microscope. Start at low magnification (4× or 10×) and increase as needed.
10. For unstained, transparent specimens, phase contrast mode typically provides better visibility than bright field (see SOP-L211, Section 6.5).

9. Quality Control

- Verify that the cover slip lies flat and is not tilted by air bubbles or excess sample material.
- Confirm that the water layer is thin enough for the specimen to remain in a single focal plane at high magnification.
- If examining motile organisms, note that excessive pressure from the cover slip can restrict movement; use slightly more water to provide adequate space.

10. Waste Disposal

Waste Type	Disposal Method
Used glass slides and cover slips	Broken-glass box
Used toothpicks and wipes	Standard trash
Biological specimen waste	Biohazard bag if potentially infectious; otherwise standard trash

11. Troubleshooting

Problem	Probable Cause	Corrective Action
Air bubbles under cover slip	Cover slip lowered too quickly	Remove cover slip, re-wet, lower at 45° slowly
Cover slip slides around	Too much water	Blot excess with lint-free wipe
Dry patches under cover slip	Too little water	Add a drop at the edge; capillary action fills the gap
Specimen drifts out of view	Water current under cover slip	Wait for flow to stop; blot if excess water is present

Problem	Probable Cause	Corrective Action
Image blurry at high magnification	Thick water layer or dirty slide	Blot excess water; clean slide and re-prepare

12. References

- Murphy & Davidson (2012)

Murphy, D. B., & Davidson, M. W. (2012). *Fundamentals of light microscopy and electronic imaging* (2nd ed.). Wiley. https://doi.org/10.1002/9781118382905

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L211	Revolve Microscope	Instrument used to examine slides
L602	Macroinvertebrate Sorting	Uses wet mounts for ID
L604	Algae Identification	Uses wet mounts for ID

14. Training Record

Before preparing wet mount slides independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Complete SOP-L211 Revolve Microscope training
4. Prepare at least two wet mount slides under supervision and demonstrate proper cover slip technique (no air bubbles, correct water volume)
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L602

Macroinvertebrate Sorting, Subsampling, and Identification

Standard Operating Procedure

SOP Number	SOP-L602
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the laboratory procedures for sorting, subsampling, and identifying benthic macroinvertebrates (BMI) collected from wadeable streams per SOP-F610. The procedure follows

the California Surface Water Ambient Monitoring Program (SWAMP) laboratory processing protocols to ensure compatibility with statewide bioassessment datasets (Barbour et al., 1999; Ode et al., 2016). Taxonomic identifications are used to calculate biological condition metrics, including the California Stream Condition Index (CSCI) (Mazor et al., 2016).

2. Scope

- **Applies to:** Laboratory processing of ethanol-preserved BMI samples from wadeable streams, including subsampling, sorting, identification to the lowest practical taxonomic level, and enumeration
- **Does not apply to:** Field collection of BMI (see SOP-F610); marine or estuarine macroinvertebrates; meiofauna (organisms passing through a 500 μm sieve); molecular identification (eDNA metabarcoding)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Approve taxonomic level targets; arrange external QC
Trained Personnel	Sort, identify, and enumerate organisms per this SOP
New Students	Complete training before independent sorting

4. Definitions

- **Benthic macroinvertebrate (BMI):** An invertebrate organism inhabiting the bottom substrate of streams, lakes, or wetlands that is retained on a 500 µm mesh sieve; includes aquatic insects, crustaceans, worms, mollusks, and other taxa.
- **Fixed count subsample:** A randomly selected subset of a BMI sample from which a fixed number of organisms (e.g., 500) is picked and identified; reduces processing time for large samples while maintaining statistical reliability.
- **Functional feeding group (FFG):** A classification of macroinvertebrates by feeding mode (shredders, collectors-gatherers, collectors-filterers, scrapers/grazers, predators); used to assess stream ecosystem function from invertebrate assemblage data.
- **Qualitative identification:** Determination of taxa present in a sample without quantifying abundances; used for rapid bioassessment and presence/absence monitoring.
- **Quantitative sample:** A sample in which all organisms are counted and identified to calculate density (organisms per unit area or volume); requires accurate recording of sampled area and volume.
- **Taxonomic resolution:** The level of taxonomic identification applied to BMI specimens; options range from phylum/class to order, family, genus, or species; standard SWAMP protocol requires family-level identification for most groups.

- **Voucher specimen:** A representative specimen of a taxon retained in preservative as a reference for future verification; essential for quality assurance and resolving taxonomic uncertainties.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Preserved samples contain approximately 70% ethanol, which is flammable and an inhalation irritant. Work in a well-ventilated area away from ignition sources.
- **Physical:** Forceps and dissecting needles are sharp. Use caution when handling small organisms.
- **Biological:** Stream organisms may harbor parasites or pathogens. Wear gloves when handling preserved samples.

5.1. Required PPE

☐ Nitrile gloves
☐ Safety glasses
☐ Lab coat

5.2. Emergency Procedures

For ethanol spills, ventilate the area, absorb with paper towels, and dispose in flammable waste. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Dissecting microscope (stereo)	7–45x magnification; illuminated base
Compound microscope	For slide-mounted specimens requiring higher magnification
Gridded Caton sorting tray	White, subdivided into numbered cells
Fine-tipped forceps (2 pairs)	Dumont #5 or equivalent
Dissecting needles	For separating tangled organisms
Petri dishes	For isolating specimens during ID
Wash bottles (ethanol and DI water)	

6.2. Consumables

Item	Notes
70% ethanol	For topping off vials
Scintillation vials (20 mL)	For archiving sorted taxa
Labels (pencil on waterproof paper)	Internal and external labels
Depression slides	For compound microscope work

7. Procedure

7.1. Sample Receiving and Logging

1. Verify that the sample jar label matches the chain-of-custody or field data sheet: site ID, date, collector, preservative.
2. Log the sample into the laboratory tracking spreadsheet.
3. Inspect the sample for adequate preservative (organisms should not appear soft or discolored). If the ethanol is diluted below approximately 50%, decant carefully and replace with fresh 70% ethanol before proceeding.

7.2. Subsampling

The SWAMP standard requires identification of a minimum of 500 organisms per sample. A fixed-count random subsampling procedure is used (Caton, 1991):

1. Pour the entire sample through a 500 μm sieve. Rinse the jar into the sieve to recover all material.
2. Transfer the sieved material to the gridded Caton sorting tray. Distribute the material as evenly as possible across all grid cells.
3. Use a random number generator or random number table to select grid cells for sorting.
4. Under the dissecting microscope, remove all organisms from the selected cell using forceps, transferring them to a labeled petri dish or vial. Count each organism as it is picked.
5. Continue selecting and sorting random cells until the cumulative organism count reaches or exceeds 500. Complete the cell in progress even if the count exceeds 500 mid-cell.
6. Record the number of cells sorted, the total cells in the tray, and the total organism count.

> **Tip**
>
> Keep the residue (unsorted cells) in a labeled jar with 70% ethanol. The residue is needed for the re-sort QC check and may be required if additional identifications are needed later.

7.3. Identification

1. Separate sorted organisms into major taxonomic groups (e.g., Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, non-insect taxa) under the dissecting microscope.

2. Identify each organism to the lowest practical taxonomic level using standard keys (Merritt et al., 2019; Thorp & Rogers, 2015):
    - Insects: genus (family for early instars or damaged specimens)
    - Non-insect invertebrates: family or order (depending on group and specimen condition)
3. For specimens requiring higher magnification (e.g., chironomid head capsules, oligochaete chaetae), prepare slide mounts and examine under the compound microscope.
4. Record counts by taxon on the laboratory data sheet or directly into the data entry spreadsheet.

7.4. Voucher Specimens

1. Retain at least one representative specimen of each taxon as a voucher in a labeled scintillation vial with 70% ethanol.
2. Label each vial with: site ID, date, taxon name, identifier initials, identification date.
3. Store voucher vials in the BMI reference collection.

7.5. Data Entry and Metric Calculation

1. Enter identification data into the standardized spreadsheet or database.
2. Calculate summary metrics:
    - Total taxa richness
    - EPT (Ephemeroptera, Plecoptera, Trichoptera) richness
    - Percent tolerant taxa
    - Percent dominant taxon
    - Shannon diversity index (H')
3. If sufficient data are available, calculate the CSCI score using the CSCI R package or the online CSCI calculator (Mazor et al., 2016).

```
# Example: Shannon diversity from BMI count data
library(vegan)

# counts is a named vector of organism counts by taxon
counts <- c(Baetis = 120, Hydropsyche = 85, Chironomidae = 210,
            Simulium = 45, Optioservus = 30, Physa = 10)

H_prime <- diversity(counts, index = "shannon")
cat(sprintf("Shannon H': %.3f\n", H_prime))
cat(sprintf("Total taxa: %d\n", length(counts)))
cat(sprintf("Total organisms: %d\n", sum(counts)))
cat(sprintf("EPT richness: count EPT genera from full dataset\n"))
```

8. Quality Control

- **Sorting efficiency:** Re-sort 10% of processed samples (randomly selected). The re-sort technician examines all residue material from the original subsampling. Sorting efficiency must be greater than or equal to 90% (i.e., fewer than 10% of total organisms were missed).
- **Taxonomic verification:** Have a second identifier verify identifications for 10% of specimens. Agreement should be greater than or equal to 90% at the operational taxonomic level.
- **External QC:** For data submitted to SWAMP or CEDEN, submit 10% of samples to a SWAMP-certified taxonomic laboratory for independent re-identification.
- Record all QC results in the QC log.

9. Waste Disposal

Waste Type	Disposal Method
Ethanol from samples	Evaporate in fume hood or collect in flammable waste
Sorted residue (organic material)	Drain ethanol; dispose in standard trash
Used gloves and paper towels	Standard trash

10. References

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish* (2nd ed.). U.S. Environmental Protection Agency, Office of Water. https://www.epa.gov/cwa-methods/rapid-bioassessment

Caton, L. W. (1991). Improved subsampling methods for the EPA "rapid bioassessment" benthic protocols. *Bulletin of the North American Benthological Society*, *8*(3), 317–319.

Mazor, R. D., Rehn, A. C., Ode, P. R., Engeln, M., Schiff, K. C., Stein, E. D., Gillett, D. J., Herbst, D. B., & Hawkins, C. P. (2016). A revised framework for modeling and implementing bioassessment in California: The California Stream Condition Index (CSCI). *Ecological Indicators*, *69*, 653–669. https://doi.org/10.1016/j.ecolind.2016.04.029

Merritt, R. W., Cummins, K. W., & Berg, M. B. (2019). *An introduction to the aquatic insects of North America* (5th ed.). Kendall Hunt Publishing.

Ode, P. R., Fetscher, A. E., & Busse, L. B. (2016). *Standard operating procedures for the collection of field data for bioassessments of california wadeable streams: Benthic macroinvertebrates, algae, and physical habitat.* California State Water Resources Control Board, Surface Water Ambient Monitoring Program. https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.html

Thorp, J. H., & Rogers, D. C. (2015). *Ecology and general biology* (4th ed., Vol. 1). Academic Press. https://doi.org/10.1016/C2010-0-65590-8

11. Related SOPs

SOP	Title	Relationship
F610	Benthic Macroinvertebrate Sampling	Upstream: field collection
L002	Laboratory Safety	Required safety training
L601	Wet Mount for Microscopy	Slide preparation for ID

12. Training Record

Before sorting and identifying BMI independently, personnel must:

1. Read this SOP and SOP-F610 in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the major taxonomic keys (Merritt et al., 2019; Thorp & Rogers, 2015)
4. Complete a supervised sorting exercise achieving greater than 90% sorting efficiency
5. Correctly identify a training set of at least 30 taxa under supervision (greater than 85% agreement with reference identifications)
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L603

Plant Tissue Drying, Grinding, and Preparation for Analysis

Standard Operating Procedure

SOP Number	SOP-L603
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the laboratory procedures for drying, grinding, and preparing plant tissue samples for subsequent elemental, isotopic, or nutrient analysis. Proper sample preparation is essential for obtaining representative, reproducible analytical results. The procedures follow standard methods for plant analysis (Jones, 2001; Kalra, 1998; Mills & Jones, 2004).

2. Scope

- **Applies to:** Leaf, stem, root, litter, and other plant tissue samples collected from EA Program field sites, prepared for total carbon and nitrogen (EA-IRMS), total phosphorus, metal analysis (ICP-MS after digestion), or other elemental determinations
- **Does not apply to:** Fresh tissue assays that require unfixed material (e.g., chlorophyll extraction from fresh leaves, enzyme activity assays); seed or fruit tissue with high oil content (requires freeze-drying); soil organic matter (see SOP-L406)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve drying protocol and grinding method
Trained Personnel	Process samples per this SOP
New Students	Complete training before independent use

4. Definitions

- **Acid washing (plant tissue):** Cleaning tissue with dilute HCl to remove surface contamination (dust, soil particles, fertilizer residues) before analysis for trace metals or nutrients; may be inappropriate for samples requiring analysis of acid-labile compounds.
- **Air drying:** Drying plant tissue at ambient temperature (typically 20–40 °C) to a stable moisture content; used as a preliminary step before oven drying; preserves enzyme activity and RNA better than direct oven drying.
- **Ball milling:** Size reduction by grinding samples between hardened steel or ceramic balls inside a rotating vessel; the preferred method for producing a homogeneous powder from fibrous plant tissue for EA-IRMS and elemental analysis.
- **Critical tissue concentration:** The minimum concentration of a nutrient required for maximum growth; below this level the plant is deficient; above it, growth is normal or the nutrient may be toxic at very high levels.
- **Lyophilization (freeze drying):** Removal of water from tissue under vacuum after rapid freezing; preserves cell structure, volatile compounds, and biological activity better than oven drying; required for enzyme, RNA, and some metabolite analyses.
- **Subsampling:** Drawing a representative small portion from a bulk dried, ground sample for analysis; requires thorough mixing and homogenization of the bulk powder before subsampling to ensure the subsample is representative.

5. Safety and Hazards

⚠ Hazards

- **Physical:** Drying ovens operate at 60–105 °C; burn risk from hot trays and glassware. The Wiley mill and ball mill contain rapidly moving parts; keep hands, hair, and loose clothing away from the grinding chamber.
- **Noise:** The Wiley mill generates noise above 85 dBA. Wear hearing protection during operation.
- **Dust:** Grinding produces fine particulate matter. Work in a well-ventilated area or use a dust mask, especially when processing large batches.
- **Biological:** Field-collected plant tissue may harbor ticks, spiders, or allergenic compounds (e.g., poison oak resin). Inspect samples before handling; wear gloves.

5.1. Required PPE

- ☐ Safety glasses or goggles
- ☐ Lab coat
- ☐ Nitrile gloves
- ☐ Hearing protection (Wiley mill operation)
- ☐ Dust mask or N95 respirator (grinding large batches)

5.2. Emergency Procedures

For burns from the oven, cool affected area under running water. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Drying oven	Forced-air convection; set to 60 °C (standard) or 105 °C (rapid dry)
Wiley mill (Thomas Scientific)	With 1 mm (20-mesh) screen
Ball mill or mortar and pestle	For fine grinding (< 250 μm) for IRMS
Analytical balance	0.0001 g readability (for weighing into tin capsules)
Desiccator with desiccant	For cooling dried samples

6.2. Consumables

Item	Notes
Paper bags or aluminum foil trays	For oven drying; labeled with pencil
Labeled sample bags (polyethylene or paper)	For storing ground tissue
Tin capsules (5 x 9 mm)	For EA-IRMS analysis
Kimwipes	For cleaning mill between samples
70% ethanol (spray bottle)	For decontaminating equipment

7. Procedure

7.1. Sample Receiving and Cleaning

1. Log incoming samples in the laboratory tracking spreadsheet. Verify site ID, date, species or tissue type, and collector.
2. If tissue was not rinsed in the field, briefly rinse leaf or stem material with deionized water to remove surface soil, dust, and epiphytes. Do not soak, as leaching of soluble compounds will occur (Kalra, 1998).
3. Blot excess water with paper towels.
4. For root samples, remove adhering soil by gentle agitation in DI water; multiple rinses may be required.

7.2. Drying

1. Place cleaned tissue in labeled paper bags or foil trays. Do not overload bags; tissue should be loosely arranged to allow air circulation.
2. Dry at 60 °C in the forced-air oven for a minimum of 48 hours, or until constant mass is achieved (two consecutive weighings 4 hours apart differ by less than 0.1% of sample mass) (Jones, 2001).
3. For samples intended only for total C and N by combustion (not volatile compound analysis), drying at 70 °C is acceptable and reduces drying time.

> **!** Important
>
> Do not exceed 70 °C for samples intended for nitrogen analysis, as volatile nitrogen compounds may be lost at higher temperatures. Do not exceed 60 °C for samples intended for analysis of volatile organic compounds, essential oils, or lipids (Kalra, 1998).

4. Remove dried samples from the oven and cool in a desiccator for at least 30 minutes before weighing or grinding.
5. Record the dry mass if gravimetric moisture content is needed.

7.3. Coarse Grinding (Wiley Mill)

1. Inspect the mill: confirm the 1 mm (20-mesh) screen is installed and the cutting blades are sharp and properly seated.

2. Turn on the mill and allow it to reach full speed before feeding material.
3. Feed dried tissue into the hopper in small amounts, pushing material gently with the wooden plunger. Do not force tissue into the blades.
4. Collect the ground material passing through the screen in a clean, labeled container or bag.
5. Between samples, disassemble the grinding chamber and clean thoroughly:
    - Brush all surfaces with a stiff brush to remove residual material
    - Wipe with 70% ethanol
    - Run a small amount of acid-washed quartz sand through the mill as a blank grind if cross-contamination of trace metals is a concern
    - Allow to dry before the next sample

7.4. Fine Grinding (for IRMS or Homogeneity-Critical Analyses)

For EA-IRMS analysis, the Wiley-milled material (less than 1 mm) must be further ground to a fine, homogeneous powder (less than 250 μm):

1. Transfer approximately 1–2 g of coarsely ground tissue to a clean ball mill vial with grinding balls.
2. Grind for 2–3 minutes until the material is a uniform fine powder.
3. Alternatively, use an agate mortar and pestle, grinding until no gritty texture remains.
4. Transfer the powder to a labeled vial or sample bag.
5. Clean the ball mill vial and balls between samples with 70% ethanol and Kimwipes.

7.5. Weighing into Tin Capsules (for EA-IRMS)

1. Using the analytical balance, tare a tin capsule on the balance pan.
2. Weigh 3–5 mg of finely ground tissue into the capsule (Brodie et al., 2011). Record the mass to 0.001 mg.
3. Fold the capsule into a tight pellet using forceps, ensuring no material escapes.
4. Place the pellet in a labeled well of a microplate or sample tray.
5. Include blanks (empty tin capsules) and reference materials (e.g., acetanilide, USGS-40) in the run sequence per SOP-L414.

8. Quality Control

- Grind and analyze duplicate subsamples at a rate of at least 1 per 10 samples to assess preparation variability. Analytical duplicates should agree within 5% RPD for C and N concentrations.
- Include a laboratory reference material (e.g., NIST SRM 1515 Apple Leaves or NIST SRM 1547 Peach Leaves) in each analytical batch to verify accuracy (Kalra, 1998).
- Document all cleaning steps in the laboratory notebook, especially the blank grind between samples when trace metal analysis is planned.
- Confirm drying completeness by re-weighing a subset of samples after an additional 4 hours of drying.

9. Waste Disposal

Waste Type	Disposal Method
Plant tissue waste	Standard trash (non-hazardous)
Ethanol cleaning rinses	Evaporate in fume hood or flammable waste
Used tin capsules (unfired)	Standard trash
Quartz sand (blank grind)	Standard trash

10. References

Brodie, C. R., Casford, J. S. L., Lloyd, J. M., Sherlock, S. C., Sherlock, S. C., Sherlock, S. C., & Sherlock, S. C. (2011). A comparison of GasBench II and elemental-analyser-based methodologies for measuring the stable carbon isotope composition of soil organic carbon. *Rapid Communications in Mass Spectrometry*, *25*(16), 2413–2421. https://doi.org/10.1002/rcm.5149

Jones, J. B. (2001). *Laboratory guide for conducting soil tests and plant analysis*. CRC Press. https://doi.org/10.1201/9781420025293

Kalra, Y. P. (1998). *Handbook of reference methods for plant analysis*. CRC Press. https://doi.org/10.1201/9781420049398

Mills, H. A., & Jones, J. B. (2004). *Plant analysis handbook II: A practical sampling, preparation, analysis, and interpretation guide*. Micro-Macro Publishing.

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L102	Using Balances	Balance operation
L406	Loss on Ignition	Related preparation for SOM
L414	Flash EA Combustion (IRMS)	Downstream analytical method
L410	Acid Digestion	Downstream digestion for metals

12. Training Record

Before processing plant tissue independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Demonstrate safe Wiley mill operation (startup, feeding, cleaning, shutdown) under supervision
4. Process at least five samples under supervision, including decontamination steps between samples

5. If preparing samples for IRMS, demonstrate fine grinding and accurate weighing into tin capsules (target mass 3–5 mg, recorded to 0.001 mg)
6. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L604

Algae / Periphyton Identification and Enumeration

Standard Operating Procedure

SOP Number	SOP-L604
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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12 Training Record **7**

1. Purpose

This SOP describes the laboratory procedures for identifying and enumerating benthic algae (periphyton) from stream bioassessment samples. Periphyton assemblages are sensitive indicators of water quality, nutrient enrichment, and physical habitat condition, and are a required component of the California SWAMP bioassessment protocol (Barbour et al., 1999; Ode et al., 2016). This SOP covers soft-bodied algae (green algae, cyanobacteria, and other non-diatom taxa) and diatoms, which are processed separately due to different preparation requirements (Charles et al., 2002; Stevenson & Bahls, 1996).

2. Scope

- **Applies to:** Identification and enumeration of benthic periphyton from composite epilithic, epiphytic, or epidendric samples collected per SWAMP protocols at EA Program stream sites
- **Does not apply to:** Phytoplankton (water column) identification; chlorophyll *a* extraction (see SOP-L310); algal culture or toxin analysis; molecular identification (eDNA metabarcoding)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Approve taxonomic protocols; arrange external QC
Trained Personnel	Process samples, identify and enumerate algae
New Students	Complete training before independent identification

4. Definitions

- **Acid washing (plant tissue):** Cleaning tissue with dilute HCl to remove surface contamination (dust, soil particles, fertilizer residues) before analysis for trace metals or nutrients; may be inappropriate for samples requiring analysis of acid-labile compounds.
- **Air drying:** Drying plant tissue at ambient temperature (typically 20–40 °C) to a stable moisture content; used as a preliminary step before oven drying; preserves enzyme activity and RNA better than direct oven drying.
- **Ball milling:** Size reduction by grinding samples between hardened steel or ceramic balls inside a rotating vessel; the preferred method for producing a homogeneous powder from fibrous plant tissue for EA-IRMS and elemental analysis.
- **Critical tissue concentration:** The minimum concentration of a nutrient required for maximum growth; below this level the plant is deficient; above it, growth is normal or the nutrient may be toxic at very high levels.
- **Lyophilization (freeze drying):** Removal of water from tissue under vacuum after rapid freezing; preserves cell structure, volatile compounds, and biological activity better than oven drying; required for enzyme, RNA, and some metabolite analyses.

- **Subsampling:** Drawing a representative small portion from a bulk dried, ground sample for analysis; requires thorough mixing and homogenization of the bulk powder before subsampling to ensure the subsample is representative.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Lugol's iodine solution (preservative) contains potassium iodide and iodine; it is a mild irritant and stains skin and clothing. Nitric acid (used in diatom cleaning) is corrosive; handle in the fume hood. The mounting medium Naphrax contains toluene, which is flammable and toxic by inhalation.
- **Physical:** Hot plates used for diatom cleaning and slide preparation pose burn risk. Coverslips and slides are fragile.
- **Biological:** Stream samples may contain pathogens. Wear gloves.

5.1. Required PPE

☐ Safety glasses or goggles
☐ Lab coat
☐ Nitrile gloves
☐ Fume hood (required for nitric acid cleaning and Naphrax mounting)

5.2. Emergency Procedures

For acid splashes, flush immediately with copious water for at least 15 minutes. For Naphrax/toluene spills, ventilate and absorb. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Compound microscope (brightfield)	400x and 1000x (oil immersion) for diatoms
Inverted microscope (optional)	For soft-bodied algae in settling chambers
Palmer-Maloney counting cell	For soft-bodied algae enumeration
Hot plate	For diatom acid cleaning
Centrifuge	For concentrating cleaned diatom material
Slide warmer	For drying diatom mounts

6.2. Consumables

Item	Notes
Lugol's iodine solution	Preservative for soft-bodied algae
Concentrated HNO_3 (trace metal grade)	For diatom frustule cleaning
30% H_2O_2	Alternative oxidizer for cleaning
Naphrax mounting medium	High refractive index (n = 1.73); for permanent diatom slides
Coverslips (22 x 22 mm, No. 1)	
Glass microscope slides	
Immersion oil	

7. Procedure

7.1. Sample Processing Overview

Periphyton samples are processed in two parallel workflows:

1. **Soft-bodied algae:** Examined alive or preserved in Lugol's solution using a Palmer-Maloney cell or settling chamber at 400x magnification.
2. **Diatoms:** Cleaned with acid or peroxide to remove organic matter, mounted in Naphrax on permanent slides, and identified at 1000x (oil immersion).

7.2. Soft-Bodied Algae Identification

1. Homogenize the preserved sample by gently inverting the jar 20 times.
2. Pipette a known volume (typically 0.1 mL) into a Palmer-Maloney counting cell.
3. Allow cells to settle for 5 minutes.
4. Scan the entire cell at 400x magnification.
5. Identify and count a minimum of 300 natural units (cells, colonies, or filament lengths of 10 μm). A natural unit is the smallest entity that can be identified: a single cell for unicellular taxa, a colony for colonial taxa, or a 10 μm filament segment for filamentous taxa (Stevenson & Bahls, 1996).
6. Record counts by taxon. Identify to genus where possible; use higher-level groupings (family or order) for taxa that cannot be resolved without specialized preparation.
7. Calculate cell density:

$$\text{Cells/cm}^2 = \frac{N \times V_{\text{sample}}}{V_{\text{counted}} \times A_{\text{substrate}}}$$

where N = number of cells counted, V_{sample} = total sample volume (mL), V_{counted} = volume examined in the counting cell (mL), and $A_{\text{substrate}}$ = total area of substrate scraped in the field (cm^2).

7.3. Diatom Cleaning

1. Transfer approximately 5 mL of well-mixed sample to a labeled glass beaker or test tube.
2. In the fume hood, add an equal volume of concentrated HNO_3 (or 30% H_2O_2 as a less hazardous alternative).
3. Heat on the hot plate at low-to-medium heat (approximately 80 °C) until the sample clears and all organic material is digested (30–60 minutes). Do not boil.
4. Allow to cool. Centrifuge at 1500 x g for 5 minutes and decant the supernatant.
5. Wash the pellet with DI water, centrifuge, and decant. Repeat the wash 3–5 times until the pH of the supernatant is near neutral.
6. Resuspend the cleaned frustules in a small volume of DI water (approximately 1–2 mL).

7.4. Diatom Slide Preparation

1. Pipette a drop of cleaned diatom suspension onto a clean coverslip. Allow to air-dry completely on the slide warmer.
2. Place a drop of Naphrax on a labeled glass slide (work in the fume hood; Naphrax contains toluene).
3. Invert the coverslip (diatom side down) onto the Naphrax drop.
4. Heat gently on the hot plate or slide warmer to drive off the toluene until no more bubbles appear.
5. Allow to cool. The slide is now a permanent mount.

7.5. Diatom Identification and Enumeration

1. Examine the slide at 1000x under oil immersion.
2. Count a minimum of 600 diatom valves, traversing random transects across the coverslip (Charles et al., 2002).
3. Identify each valve to the lowest practical taxonomic level (species where possible) using regional diatom floras and SWAMP reference keys (Krammer, 2000; Spaulding et al., 2021).
4. Record counts by taxon.

7.6. Data Entry and Metric Calculation

1. Enter soft-bodied and diatom counts into the standardized data entry spreadsheet.
2. Calculate summary metrics: total taxa richness, relative abundance of indicator taxa (e.g., pollution-tolerant vs. sensitive diatoms), and community-level indices.
3. If submitting to SWAMP/CEDEN, format data per SWAMP requirements.

8. Quality Control

- **Soft-bodied algae:** Re-count 10% of samples. Counts should agree within 15% RPD for dominant taxa.
- **Diatoms:** Have a second identifier verify 10% of slides. Taxonomic agreement should be greater than or equal to 85% at the species level.

- For SWAMP submissions, submit 10% of diatom slides to a SWAMP-certified taxonomist for external QC.
- Include a reference slide set in the laboratory for training and calibration.

9. Waste Disposal

Waste Type	Disposal Method
Nitric acid waste	Neutralize and collect in acid waste; EH&S pickup
H_2O_2 waste	Dilute and drain with copious water
Naphrax / toluene waste	Collect in organic waste; EH&S pickup
Lugol's iodine waste	Collect in designated container; EH&S pickup
Used slides and coverslips	Standard sharps/glass waste

10. References

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish* (2nd ed.). U.S. Environmental Protection Agency, Office of Water. https://www.epa.gov/cwa-methods/rapid-bioassessment

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11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials Handling	Acid and solvent safety
L310	Chlorophyll Extraction	Complementary biomass metric
L601	Wet Mount for Microscopy	General microscopy technique
F610	BMI Sampling	Co-collected bioassessment data

12. Training Record

Before identifying and enumerating algae independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety and SOP-L003 Hazardous Materials Handling training
3. Review the SDS for nitric acid and Naphrax
4. Study the diatom and soft-bodied algae reference collections and identification keys
5. Complete a supervised identification exercise (minimum 100 diatom valves and 50 soft-bodied algae natural units) with greater than 80% agreement with reference identifications
6. Demonstrate safe acid cleaning and Naphrax mounting under supervision
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L605

Soil Enzyme Assays (Fluorometric Microplate Method)

Standard Operating Procedure

SOP Number	SOP-L605
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the fluorometric microplate method for measuring the potential activity of extracellular enzymes in soils. Extracellular enzymes mediate the decomposition of organic matter and the cycling of carbon, nitrogen, phosphorus, and sulfur in soils. Assays use fluorogenic substrates based on 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC), which release a fluorescent product upon enzymatic hydrolysis (Bell et al., 2013; German et al., 2011; Sinsabaugh & Moorhead, 1994). The method is high-throughput (96-well microplate format), sensitive, and widely used in soil biogeochemistry and ecosystem ecology.

2. Scope

- **Applies to:** Measurement of potential hydrolytic enzyme activities in field-moist, sieved (less than 2 mm) mineral and organic soils from EA Program sites, using MUB- and AMC-linked fluorogenic substrates in a 96-well microplate format
- **Does not apply to:** Oxidative enzyme assays (e.g., phenol oxidase, peroxidase), which use colorimetric substrates (L-DOPA); enzyme assays in aquatic samples (requires modified dilution); commercially available enzyme activity kits with different substrates
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Select target enzymes; approve substrate concentrations
Trained Personnel	Prepare reagents, run assays, analyze data
New Students	Complete training before independent use

4. Definitions

- **Fluorometric enzyme assay:** An enzyme activity assay in which substrate hydrolysis releases a fluorescent product (typically methylumbelliferone, MUB, or methylcoumarin, MCA) measurable at low concentrations by a fluorometer; more sensitive than colorimetric assays.
- **Maximum velocity (V_{max}):** The maximum rate of an enzyme-catalyzed reaction when substrate is saturating; expressed in units of nmol product g^{-1} dry soil h^{-1}; reflects the potential activity of the enzyme in soil.
- **Methylumbelliferone (MUB):** A fluorescent product released by many hydrolytic enzymes when they cleave a methylumbelliferyl-linked substrate; detected at excitation ~365 nm and emission ~450 nm; the basis of most fluorometric soil enzyme assays.
- **Potential enzyme activity:** The enzyme activity measured under optimized laboratory conditions (excess substrate, controlled pH, temperature 25 °C); an index of the extant enzyme pool rather than actual in situ activity.

- **Quench correction:** A correction applied to fluorometric assays to account for reduction in fluorescence signal caused by soil colloids and dissolved organic matter; calculated from samples spiked with a known amount of MUB standard.
- **Substrate:** The molecule acted upon by an enzyme; in fluorometric assays, a synthetic substrate linked to MUB or MCA is cleaved by the target enzyme to release the fluorescent product.
- **Turnover rate:** The number of substrate molecules converted per enzyme molecule per unit time; an intrinsic property of the enzyme that depends on temperature, pH, and substrate concentration.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** MUB and AMC substrates are dissolved in dimethyl sulfoxide (DMSO), which is readily absorbed through the skin and can carry dissolved solutes into the body. Sodium acetate buffer and sodium hydroxide (for pH adjustment) are mild to moderate irritants. Concentrated NaOH is corrosive.
- **Biological:** Soil samples may contain pathogens. Handle with gloves.
- **Physical:** Microplate reader UV lamp may cause eye strain with prolonged direct viewing.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (double glove when handling DMSO solutions)

5.2. Emergency Procedures

For DMSO contact with skin, wash immediately with soap and water (do not use ethanol, which enhances absorption). For NaOH contact, flush with water for 15 minutes. Refer to SOP-L002 Laboratory Safety.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Fluorescence microplate reader	Excitation 365 nm, emission 450 nm
Multichannel micropipette (8-channel)	50–300 μL range
Single-channel micropipettes	10–100 μL and 100–1000 μL

Item	Notes
96-well black flat-bottom microplates	Non-treated; opaque walls to prevent bleedthrough
Orbital shaker or plate shaker	
Analytical balance	For weighing soil
pH meter	For buffer preparation
Centrifuge (optional)	For clarifying soil slurries

6.2. Consumables

Item	Notes
4-Methylumbelliferyl substrates	See enzyme table (Section 6)
7-Amino-4-methylcoumarin substrates	For leucine aminopeptidase
4-Methylumbelliferone (MUB) standard	For calibration curves
7-Amino-4-methylcoumarin (AMC) standard	For calibration curves
DMSO (ACS grade)	Solvent for substrate stocks
Sodium acetate buffer (50 mM, pH 5.0)	Or Tris buffer pH 7.8 for alkaline soils
NaOH (1 N)	To stop reactions and enhance fluorescence
DI water (Type II)	
Reagent reservoirs	For multichannel pipetting
Adhesive plate seals	For incubation

7. Target Enzymes and Substrates

The following enzymes are routinely assayed in the EA Program. Other MUB- or AMC-linked substrates can be added using the same general protocol.

Table 1: Target enzymes, substrates, and associated nutrient cycles.

Enzyme	Abbreviation	Substrate	Nutrient Cycle	Substrate Conc.
β-1,4-glucosidase	BG	4-MUB-β-D-glucopyranoside	Carbon	200 μM
Cellobiohydrolase	CBH	4-MUB-β-D-cellobioside	Carbon	200 μM
β-1,4-N-acetylglucosaminidase	NAG	4-MUB-N-acetyl-β-D-glucosaminide	Carbon / Nitrogen	200 μM
Acid (alkaline) phosphatase	AP	4-MUB-phosphate	Phosphorus	200 μM
β-1,4-xylosidase	BX	4-MUB-β-D-xylopyranoside	Carbon	200 μM
Leucine aminopeptidase	LAP	L-Leucine-7-AMC	Nitrogen	200 μM
Sulfatase	SUL	4-MUB-sulfate	Sulfur	200 μM

8. Procedure

8.1. Soil Slurry Preparation

1. Weigh 1.0 g of field-moist, sieved (less than 2 mm) soil into a clean container.
2. Add 125 mL of 50 mM sodium acetate buffer (pH adjusted to match the soil pH, typically 5.0 for acidic soils or 7.8 for alkaline soils) (German et al., 2011).
3. Homogenize with a magnetic stir bar or by vortexing for 1 minute.
4. Keep the slurry stirring continuously during pipetting to maintain a uniform suspension.

8.2. Substrate Stock Preparation

1. Dissolve each MUB or AMC substrate in DMSO to prepare a 10 mM stock solution. Store at –20 °C in amber vials; stable for at least 6 months.
2. Prepare working solutions by diluting the DMSO stock in buffer to the target concentration (typically 200 μM final concentration in the well).
3. Prepare MUB and AMC calibration standards in buffer at concentrations of 0, 0.5, 1, 2.5, 5, 10, and 25 μM.

8.3. Microplate Layout

For each soil sample, set up the following wells (8 analytical replicates per enzyme) (Bell et al., 2013):

Well Type	Contents	Purpose
Sample assay (n = 8)	200 μL slurry + 50 μL substrate	Enzyme activity
Sample control (n = 8)	200 μL slurry + 50 μL buffer	Soil background fluorescence
Substrate control (n = 8)	200 μL buffer + 50 μL substrate	Substrate autofluorescence
MUB/AMC standard curve	200 μL buffer + 50 μL standard	Calibration
Blank	200 μL buffer + 50 μL buffer	Instrument blank

8.4. Incubation

1. Seal the plate with an adhesive seal.
2. Incubate at 20 °C (or field-relevant temperature) in the dark on a plate shaker at low speed for 2–4 hours. The incubation time depends on expected activity; 3 hours is a standard starting point (Sinsabaugh & Moorhead, 1994).

> **Tip**
>
> For soils with unknown activity, run a pilot plate at 1, 2, and 4 hours to identify the linear range. Activity calculations assume linearity over the incubation period; if the reaction plateaus, shorten the incubation.

8.5. Stopping and Reading

1. After incubation, add 10 μL of 1 N NaOH to each well to stop the reaction and maximize fluorescence of the MUB/AMC product (the fluorophore is brightest above pH 10).
2. Read fluorescence on the microplate reader at excitation 365 nm / emission 450 nm within 5 minutes of adding NaOH.
3. Export the raw fluorescence data for analysis.

9. Data Analysis and Calculations

9.1. Activity Calculation

For each enzyme, calculate the net fluorescence:

$$F_{\text{net}} = F_{\text{sample}} - F_{\text{soil control}} - F_{\text{substrate control}} + F_{\text{blank}}$$

Convert net fluorescence to nmol of product released using the MUB or AMC standard curve (linear regression of fluorescence vs. concentration).

Calculate enzyme activity:

$$\text{Activity (nmol g}^{-1}\text{h}^{-1}) = \frac{F_{\text{net}}/m \times V_{\text{well}}}{M_{\text{soil}} \times t}$$

where m = slope of the standard curve (fluorescence per nmol), V_{well} = total well volume (L), M_{soil} = dry mass equivalent of soil in the well (g, corrected for moisture content per SOP-L400), and t = incubation time (h).

```
# Example enzyme activity calculation
library(tidyverse)

# Standard curve data
standards <- tibble(
  conc_uM = c(0, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0),
  fluorescence = c(120, 850, 1580, 3750, 7200, 14100, 34500)
)

# Fit linear model
cal_model <- lm(fluorescence ~ conc_uM, data = standards)
```

```
slope <- coef(cal_model)[2]  # fluorescence units per uM

# Sample data (means of 8 replicates)
F_sample <- 5200
F_soil_ctrl <- 350
F_sub_ctrl <- 180
F_blank <- 125

F_net <- F_sample - F_soil_ctrl - F_sub_ctrl + F_blank

# Convert to concentration (uM in well)
conc_well <- F_net / slope

# Calculate activity
V_well_L <- 250e-6          # 250 uL total well volume in liters
soil_fresh_g <- 1.0         # g fresh soil in 125 mL slurry
frac_in_well <- 200 / (125 * 1000)  # fraction of slurry in well
soil_in_well_g <- soil_fresh_g * frac_in_well
moisture <- 0.25            # gravimetric moisture content
soil_dry_g <- soil_in_well_g * (1 - moisture)
incubation_h <- 3

activity <- (conc_well * V_well_L * 1e6) / (soil_dry_g * incubation_h)
# Result in nmol / g dry soil / h

cat(sprintf("Net fluorescence: %.0f\n", F_net))
cat(sprintf("Activity: %.1f nmol g-1 h-1\n", activity))
```

10. Quality Control

- Run 8 analytical replicates per enzyme per sample. Report the mean and coefficient of variation; CV should be less than 20%.
- Include substrate and soil quench controls on every plate.
- Run the MUB/AMC standard curve on each plate (do not carry over calibrations between plates, as reader sensitivity may drift).
- Include a laboratory reference soil in each batch to track inter-batch variability.
- Verify that activity is linear over the incubation period by running a time-course pilot for new soil types.

11. Waste Disposal

Waste Type	Disposal Method
Plate contents (soil slurry + substrates)	Drain with copious water (dilute, non-hazardous at assay concentrations)
DMSO substrate stock waste	Collect in organic waste; EH&S pickup
NaOH waste	Neutralize and drain
Used microplates	Standard trash
Used pipette tips	Standard trash

12. References

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments*, (81), e50961. https://doi.org/10.3791/50961

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Sinsabaugh, R. L., & Moorhead, D. L. (1994). Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. *Soil Biology and Biochemistry*, *26*(10), 1305–1311. https://doi.org/10.1016/0038-0717(94)90211-9

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L400	Soil Moisture (Gravimetric)	Moisture correction for dry-mass calculations
L409	Microbial Biomass Carbon	Complementary microbial activity measure
L408	Solvita CO_2 Burst	Related microbial activity metric
F400	Soil Sampling	Upstream: sample collection

14. Training Record

Before performing soil enzyme assays independently, personnel must:

1. Read this SOP in full
2. Complete SOP-L002 Laboratory Safety training
3. Review the SDS for DMSO, NaOH, and the fluorogenic substrates
4. Observe a trained operator perform a complete assay (slurry preparation through plate reading and data export)
5. Perform the assay under supervision with acceptable QC results (replicate CV less than 20%; standard curve $r^2 > 0.99$)

6. Demonstrate correct use of the multichannel pipette and microplate reader
7. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L700

DNA Extraction, Quantification, and Purity Assessment

Standard Operating Procedure

SOP Number	SOP-L700
Revision	1.0
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the extraction of genomic DNA from environmental samples (soil, sediment, water filters, and plant tissue), followed by quantification of DNA concentration and assessment of DNA purity using spectrophotometric and fluorometric methods. The protocol is based on commercial bead-beating extraction kits and is suitable for downstream applications including PCR amplification, qPCR, and amplicon sequencing (Qiagen, 2024a, 2024b).

2. Scope

- **Applies to:** Genomic DNA extraction from soil, sediment, water filter membranes, and plant tissue using the DNeasy PowerSoil Pro Kit (Qiagen) or equivalent bead-beating kit; DNA quantification using a NanoDrop spectrophotometer and Qubit fluorometer
- **Does not apply to:** RNA extraction, plasmid isolation, ancient DNA, or clinical/diagnostic samples
- **Location:** Molecular biology bench, Pomona College Biogeochemistry Lab

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **A_{260}/A_{280} ratio:** The ratio of absorbance at 260 nm to 280 nm; pure dsDNA yields ~1.8; values below 1.7 indicate protein or phenol contamination that may inhibit PCR amplification.
- **A_{260}/A_{230} ratio:** The ratio of absorbance at 260 nm to 230 nm; values of 2.0–2.2 indicate pure nucleic acids; low values indicate contamination with chaotropic salts, humic acids, or carbohydrates.
- **Bead beating:** Mechanical cell lysis in which sample is agitated at high speed with ceramic or glass beads in a tube; disrupts cell walls and membranes including the tough walls of fungal spores and gram-positive bacteria.
- **Chaotropic salt:** A salt (e.g., guanidinium isothiocyanate, guanidinium hydrochloride) that disrupts hydrogen bonds and hydrophobic interactions; used in DNA extraction to denature proteins and facilitate binding of DNA to silica membranes.
- **Environmental DNA (eDNA):** Genetic material shed into the environment by organisms through mucus, feces, skin cells, and gametes; collected from soil, water, or air without organism capture.
- **Inhibitor:** A substance co-extracted with DNA that reduces PCR amplification efficiency; common inhibitors in soil and water eDNA include humic acids, tannins, heavy metals, and residual ethanol.

- **Silica column (spin column):** A miniaturized chromatography column containing a silica membrane that selectively binds DNA in the presence of chaotropic salts; DNA is washed and then eluted with low-salt buffer or water.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Lysis buffer contains guanidine salts, which are harmful if swallowed or inhaled and can cause skin and eye irritation. Solution C4 contains ethanol. Review the Safety Data Sheets (SDS) for all kit reagents before use.
- **Physical:** Bead-beating generates aerosols; keep tubes sealed during vortexing. Centrifuge rotor imbalance can cause equipment damage.
- **Biological:** Environmental samples may contain unknown pathogens. Treat all samples as potentially infectious (Centers for Disease Control and Prevention & National Institutes of Health, 2020).

5.1. Required PPE

☐ Safety glasses or goggles
☐ Lab coat
☐ Nitrile gloves (change frequently to avoid cross-contamination)
☐ Face shield (if working with large volumes of lysis buffer)

5.2. Emergency Procedures

In case of chemical splash or skin/eye contact, flush affected area with water for at least 15 minutes and consult the SDS. For spills of lysis buffer, absorb with paper towels, clean with water, and dispose as chemical waste. Refer to SOP-L003 Hazardous Materials Handling for general chemical spill response.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Vortex adapter for bead tubes	Mol bio bench	Holds 24 tubes horizontally
Vortex-Genie 2	Mol bio bench	Set to maximum speed
Microcentrifuge	Mol bio bench	Must reach 10,000 x g minimum
NanoDrop One spectrophotometer	Instrument bench	UV-Vis micro-volume
Qubit 4 fluorometer	Instrument bench	For dsDNA quantification
Micropipettes (10, 100, 1000 uL)	Mol bio bench	Calibrated annually

Item	Location	Notes
–80 C freezer	Cold room	For long-term DNA storage
–20 C freezer	Lab	For short-term DNA storage

6.2. Reagents and Consumables

Item	Catalog No.	Storage
DNeasy PowerSoil Pro Kit (Qiagen)	47014 (50 prep)	Room temperature
Qubit dsDNA BR Assay Kit	Q32850	Room temperature
Qubit dsDNA HS Assay Kit	Q32851	Room temperature
Qubit assay tubes (0.5 mL)	Q32856	Room temperature
Nuclease-free water	Various	Room temperature
Molecular-grade ethanol (100%)	Various	Flammables cabinet
1.5 mL microcentrifuge tubes (DNase-free)	Various	Room temperature
Filter pipette tips (10, 100, 1000 uL)	Various	Room temperature

7. Procedure

7.1. Sample Preparation

1. Record sample identity, collection date, and mass/volume in the lab notebook and in the Freezer Inventory Google Sheet
2. For soil/sediment: weigh 0.20–0.25 g of sample into the PowerBead Pro tube. Record exact mass.
3. For water filters: use sterile forceps to transfer the membrane filter into the PowerBead Pro tube. Cut the filter into strips if needed to fit inside the tube.
4. For plant tissue: weigh 0.02–0.10 g of tissue (fresh or flash-frozen) into the PowerBead Pro tube. Use less tissue for highly mucilaginous samples (Doyle & Doyle, 1987).

> **!** Important
>
> Keep samples on ice during preparation to minimize DNA degradation by endogenous nucleases. Process all samples within a single batch if possible to reduce extraction variability (Frosteg aard et al., 1999).

7.2. Cell Lysis and DNA Extraction

Follow the DNeasy PowerSoil Pro Kit protocol (Qiagen, 2024b):

1. Add 800 uL of Solution CD1 to the PowerBead Pro tube
2. Secure tubes horizontally on the vortex adapter and vortex at maximum speed for 10 minutes
3. Centrifuge at 15,000 x g for 1 minute

4. Transfer the supernatant to a clean 2 mL collection tube
5. Add 200 uL of Solution CD2, vortex briefly (5 seconds), and centrifuge at 15,000 x g for 1 minute
6. Avoiding the pellet, transfer the supernatant to a clean 2 mL collection tube
7. Add 600 uL of Solution CD3, vortex briefly (5 seconds)
8. Load 650 uL onto an MB Spin Column seated in a 2 mL collection tube. Centrifuge at 15,000 x g for 1 minute. Discard flow-through. Repeat to load remaining lysate.
9. Add 500 uL of Solution EA. Centrifuge at 15,000 x g for 1 minute. Discard flow-through.
10. Add 500 uL of Solution C5 (wash buffer). Centrifuge at 15,000 x g for 1 minute. Discard flow-through.
11. Centrifuge again at 16,000 x g for 2 minutes to remove residual wash buffer
12. Transfer the MB Spin Column to a clean 1.5 mL microcentrifuge tube
13. Add 50–100 uL of Solution C6 (elution buffer) or nuclease-free water to the center of the membrane. Allow to stand for 1 minute at room temperature, then centrifuge at 15,000 x g for 1 minute.

> **Tip**
>
> Use a smaller elution volume (50 uL) when higher DNA concentration is needed for downstream applications. A second elution with an additional 50 uL can recover 10–20% more DNA but at lower concentration (Qiagen, 2024b).

7.3. DNA Quantification – NanoDrop Spectrophotometry

The NanoDrop measures DNA concentration and provides absorbance ratios used to assess purity (Desjardins & Bhatt, 2010):

1. Clean the pedestal with 2 uL of nuclease-free water; wipe with a lint-free tissue
2. Blank the instrument with 2 uL of the elution buffer (Solution C6 or nuclease-free water, matching your elution)
3. Load 2 uL of DNA extract onto the pedestal
4. Record the concentration (ng/uL), A_{260}/A_{280} ratio, and A_{260}/A_{230} ratio for each sample

7.4. DNA Quantification – Qubit Fluorometry

The Qubit fluorometer uses fluorescent dyes that bind selectively to dsDNA, providing more accurate quantification than spectrophotometry, especially at low concentrations or in the presence of contaminants (Thermo Fisher Scientific, 2024):

1. Prepare the Qubit working solution: dilute the Qubit dsDNA BR (or HS) reagent 1:200 in Qubit buffer. Prepare enough for all samples plus two standards (e.g., 200 uL per assay tube).
2. Prepare standards: add 190 uL of working solution to each of two Qubit assay tubes. Add 10 uL of Standard 1 to one tube and 10 uL of Standard 2 to the other. Vortex briefly.
3. Prepare samples: add 198 uL of working solution to a Qubit assay tube. Add 2 uL of DNA extract. Vortex briefly.

4. Incubate all tubes at room temperature for 2 minutes (but not longer than 30 minutes)
5. Calibrate the Qubit with the two standards, then read each sample
6. Record the concentration (ng/uL, corrected for dilution by the instrument)

7.5. DNA Purity Assessment

Evaluate DNA purity using the NanoDrop absorbance ratios (Desjardins & Bhatt, 2010; Wilfinger et al., 1997):

Ratio	Ideal Range	Interpretation
A_{260}/A_{280}	1.8–2.0	Values below 1.8 suggest protein or phenol contamination
A_{260}/A_{230}	2.0–2.2	Values below 2.0 suggest contamination with humic acids, carbohydrates, salts, or residual guanidine from extraction buffers

Note

For environmental DNA extracts, especially from humic-rich soils, A_{260}/A_{230} ratios are frequently below 2.0. If downstream applications (e.g., PCR) fail or show inhibition, consider additional cleanup using a OneStep PCR Inhibitor Removal Kit or gel purification (Young et al., 2014).

7.6. Storage and Freezer Inventory

1. Label each tube with: sample ID, extraction date, elution volume, and concentration (in ng/uL)
2. For short-term storage (less than 1 month): store DNA extracts at –20 C
3. For long-term storage: store DNA extracts at –80 C in the designated molecular biology section of the freezer
4. Record the storage location (freezer, rack, box, and position) in the Freezer Inventory Google Sheet
5. Update the Google Sheet immediately upon placing samples in the freezer or removing them

–80 C Freezer Protocol

- Minimize the time the freezer door is open. Retrieve the correct box before opening the freezer.
- Place DNA extracts in designated cryoboxes labeled “DNA Extracts” with the project name and date range.
- Do not store DNA extracts in the same box as tissue samples or reagents.
- Avoid repeated freeze-thaw cycles. If multiple downstream analyses are planned, prepare aliquots at the time of extraction (Shao et al., 2012).

8. Quality Control and Acceptance Criteria

- Include one extraction blank (empty PowerBead Pro tube processed through the full protocol) per extraction batch to detect contamination (Salter et al., 2014)
- Run Qubit standards with each new batch of working solution
- Acceptable DNA yield from 0.25 g of soil: typically 1–20 ng/uL in a 100 uL elution, depending on soil type (Dineen et al., 2010)
- Acceptable A_{260}/A_{280}: 1.7–2.0. Samples outside this range should be noted and may require re-extraction.
- Acceptable A_{260}/A_{230}: greater than 1.5 for most downstream applications. Values below 1.0 indicate significant contamination and the extract should be purified or re-extracted.
- If NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; use the Qubit value for planning downstream reactions (Nakayama et al., 2016).

9. Waste Disposal

Waste Type	Container	Disposal Method
Used PowerBead Pro tubes	Biohazard bag	Autoclave, then standard trash
Flow-through from spin columns	Chemical waste carboy	EH&S hazardous waste pickup (contains guanidine salts)
Used spin columns	Chemical waste bag	EH&S hazardous waste pickup
Pipette tips and tubes	Biohazard sharps/trash	Standard biohazard disposal
Empty reagent bottles	Rinse and recycle	Check SDS for specific instructions

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11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP and SOP-L003 Hazardous Materials Handling in full
2. Review the SDS for all kit components (especially Solutions CD1, CD2, and CD3)
3. Complete the Qiagen DNeasy PowerSoil Pro Kit quick-start guide
4. Demonstrate competence with micropipetting, the NanoDrop, and the Qubit fluorometer
5. Perform a full extraction under supervision, including quantification and freezer logging
6. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Very low DNA yield	Insufficient lysis; too little starting material	Increase vortexing time to 15 min; verify sample mass
Low A_{260}/A_{280} (below 1.6)	Protein carryover	Re-extract with fresh kit reagents; ensure full removal of CD2 pellet

Symptom	Probable Cause	Corrective Action
Low A_{260}/A_{230} (below 1.0)	Humic acid or guanidine carryover	Add a second C5 wash step; use inhibitor removal kit
NanoDrop reads much higher than Qubit	Co-extracted contaminants inflating UV absorbance	Trust Qubit value; consider cleanup before downstream use
PCR inhibition despite good yield	Co-extracted humic acids or polysaccharides	Dilute template 1:10; use inhibitor-tolerant polymerase; clean up with OneStep kit (Young et al., 2014)
Extraction blank shows amplification	Reagent or cross-contamination	Discard batch; use fresh reagents; clean workspace with 10% bleach then ethanol

13. Appendix B: DNA Concentration Calculation Worksheet

```
# Calculate total DNA yield from extraction
elution_vol_uL <- 100        # Volume of elution buffer used (uL)
qubit_conc_ng_uL <- 8.5      # Qubit reading (ng/uL)
sample_mass_g <- 0.25         # Starting sample mass (g)

total_dna_ng <- qubit_conc_ng_uL * elution_vol_uL
dna_per_g <- total_dna_ng / sample_mass_g

cat(sprintf("Total DNA yield: %.1f ng\n", total_dna_ng))
```

```
Total DNA yield: 850.0 ng
```

```
cat(sprintf("DNA yield per gram of sample: %.1f ng/g\n", dna_per_g))
```

```
DNA yield per gram of sample: 3400.0 ng/g
```

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L701

Primer Selection and Design for Nitrogen Cycle Genes – ddPCR

Standard Operating Procedure

SOP Number	SOP-L701
Revision	0.2
Author(s)	Marc Los Huertos
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1. Purpose

This procedure describes the selection, design, and validation of primers and probes targeting functional genes of the nitrogen cycle for use with droplet digital PCR (ddPCR). The nitrogen cycle genes covered include those encoding nitrification (*amoA* for both ammonia-oxidizing bacteria and archaea), denitrification (*nirK*, *nirS*, *nosZ*), nitrogen fixation (*nifH*), and dissimilatory nitrate reduction to ammonium (*nrfA*). The SOP covers literature-based primer selection, in silico evaluation, and bench validation on the Bio-Rad QX200 ddPCR system (Bio-Rad Laboratories, 2024; Hindson et al., 2011).

2. Scope

- **Applies to:** Design and selection of primers and hydrolysis probes (TaqMan) or EvaGreen-based assays for quantifying nitrogen cycle functional genes from environmental DNA extracts (soil, sediment, water)
- **Does not apply to:** Amplicon sequencing library preparation, metagenomics, or RNA-based transcript quantification (RT-ddPCR)
- **Location:** Molecular biology bench, Pomona College Biogeochemistry Lab

3. Responsibilities

Role	Responsibility
Lab PI	Approve primer selections and assay designs
Trained Personnel	Conduct in silico analysis, order primers, perform validation runs
New Students	Complete training before independent use

4. Definitions

- **Amplicon length:** The size in base pairs (bp) of the PCR product generated by a primer pair; for ddPCR, optimal amplicon length is 60–200 bp; shorter amplicons partition more efficiently into droplets and produce cleaner positive/negative separation (Bio-Rad Laboratories, 2024; Hindson et al., 2011).
- **amoA (ammonia monooxygenase alpha subunit):** The gene encoding the alpha subunit of ammonia monooxygenase; used as a functional marker for ammonia-oxidizing bacteria (AOB; *Nitrosomonas*-like) and ammonia-oxidizing archaea (AOA; Thaumarchaeota); quantified separately because AOB and AOA primers differ (Francis et al., 2005; Rotthauwe et al., 1997).
- **Annealing temperature (T_a):** The temperature at which primers bind to their complementary template sequences during PCR cycling; determined by gradient PCR optimization; typically 55–65 °C for nitrogen cycle gene assays; primer pairs should be within 2 °C of each other (Bio-Rad Laboratories, 2024).
- **ddPCR (droplet digital PCR):** A third-generation PCR technology that partitions a reaction into approximately 20,000 nanoliter droplets and amplifies target DNA to endpoint within each droplet; the fraction of positive droplets is fit to a Poisson distribution to calculate absolute target concentration without a standard curve (Hindson et al., 2011).
- **DNRA (dissimilatory nitrate reduction to ammonium):** A microbial process that reduces NO_3^- to NH_4^+, retaining nitrogen in the ecosystem rather than losing it as gas; marker gene: *nrfA* (Levy-Booth et al., 2014).
- **EvaGreen:** A fluorescent intercalating dye used as a non-specific reporter in ddPCR and qPCR; binds double-stranded DNA; less specific than hydrolysis probes but useful for assay development and for targets without validated probe sequences (Bio-Rad Laboratories, 2024).
- **FunGene:** A curated functional gene database (http://fungene.cme.msu.edu/) that provides reference sequences, primer coverage analysis, and taxonomy for nitrogen cycle genes including *amoA*, *nirK*, *nirS*, *nosZ*, and *nifH* (Fish et al., 2013).

- **gBlock (synthetic DNA fragment):** A double-stranded DNA fragment (IDT) with a sequence matching the target amplicon; used as a positive control for ddPCR assay validation and as a template for limit-of-detection determination (Hindson et al., 2011).
- **Hydrolysis probe (TaqMan probe):** A short oligonucleotide labelled with a 5' fluorophore (e.g., FAM) and a 3' quencher (e.g., Iowa Black FQ); cleaved by Taq polymerase during extension, releasing the fluorophore and generating a signal; provides higher specificity than intercalating dyes and is required for multiplexed ddPCR (Bio-Rad Laboratories, 2024).
- **In silico primer evaluation:** Computational assessment of primer specificity, coverage, and secondary structure using tools such as NCBI Primer-BLAST, IDT OligoAnalyzer, and FunGene; a required step before ordering primers for bench validation (Fish et al., 2013; Ye et al., 2012).
- **nifH (nitrogenase reductase):** The gene encoding nitrogenase iron protein; the universal marker for biological nitrogen fixation (diazotrophy); highly conserved across diverse diazotrophic bacteria and archaea (Levy-Booth et al., 2014; Poly et al., 2001).
- **nirK / nirS:** Genes encoding the two structurally distinct nitrite reductases in denitrifiers: *nirK* encodes a copper-containing (Cu) enzyme; *nirS* encodes a cytochrome cd_1 enzyme; the two forms serve the same function and are often both targeted to capture the full denitrifying community (Braker et al., 2000; Henry et al., 2006).
- **nosZ:** The gene encoding nitrous oxide reductase, which reduces N_2O to N_2 in the final step of denitrification; divided into Clade I (classical denitrifiers; Henry et al. (2006)) and Clade II (diverse non-denitrifiers; Jones et al. (2013)); the sum of both clades estimates the community capacity to consume the greenhouse gas N_2O.
- **nrfA:** The gene encoding cytochrome c nitrite reductase, the enzyme catalyzing DNRA; a functional marker for organisms that retain nitrogen in the ecosystem as NH_4^+ rather than releasing it as N_2O or N_2 (Welsh et al., 2014).
- **Rain (ddPCR):** Droplets with intermediate fluorescence amplitude that fall between the positive and negative clusters in a ddPCR amplitude plot; indicates suboptimal primer binding, non-specific amplification, or partial inhibition; reduced by increasing annealing temperature or decreasing primer concentration (Lievens et al., 2016).

5. Safety and Hazards

⚠ Hazards

- **Chemical:** ddPCR droplet generation oil contains proprietary surfactants; avoid skin and eye contact. EvaGreen and other intercalating dyes are potential mutagens at high concentrations; handle with gloves.
- **Physical:** The QX200 Droplet Generator uses pressurized cartridges. Follow manufacturer instructions for cartridge handling.
- **Biological:** Environmental DNA extracts may originate from samples containing pathogens. Handle with standard precautions.

5.1. Required PPE

☐ Safety glasses

☐ Lab coat
☐ Nitrile gloves (changed frequently)

5.2. Emergency Procedures

For chemical splash, flush with water for 15 minutes. Refer to SOP-L003 Hazardous Materials Handling.

6. Target Genes Overview

The following table summarizes the nitrogen cycle gene targets, the enzymatic steps they represent, and key references for established primer sets (Braker et al., 2000; Henry et al., 2006; Poly et al., 2001; R"osch et al., 2002; Rotthauwe et al., 1997; Welsh et al., 2014):

Gene	Enzyme	Process	Organism Group
amoA	Ammonia monooxygenase	Ammonia oxidation (nitrification)	AOB, AOA
nirK	Copper-containing nitrite reductase	Denitrification (NO_2^- to NO)	Denitrifiers
nirS	Cytochrome cd_1 nitrite reductase	Denitrification (NO_2^- to NO)	Denitrifiers
nosZ	Nitrous oxide reductase	Denitrification (N_2O to N_2)	Denitrifiers (Clade I and II)
nifH	Nitrogenase reductase	Nitrogen fixation (N_2 to NH_3)	Diazotrophs
nrfA	Cytochrome c nitrite reductase	DNRA (NO_2^- to NH_4^+)	DNRA organisms

7. Procedure

7.1. Literature-Based Primer Selection

1. Search the published literature for validated primer sets targeting each gene of interest. Prioritized references include Rotthauwe et al. (1997), Francis et al. (2005), Braker et al. (2000), Henry et al. (2006), Poly et al. (2001), Jones et al. (2013), and Welsh et al. (2014).
2. For each candidate primer pair, record: primer names, sequences (5' to 3'), amplicon length, annealing temperature, and the original publication.
3. Evaluate published primer sets against the following criteria:
    - Amplicon length between 60 and 200 bp (Bio-Rad Laboratories, 2024; Hindson et al., 2011)
    - Demonstrated use in environmental samples similar to your matrix
    - Broad coverage of the target gene clade verified by in silico analysis (see §6.3)
    - Minimal off-target amplification in non-target organisms

> **Note**
>
> Many classic nitrogen cycle primers (e.g., *nosZ* primers from Henry et al. (2006)) were designed for conventional qPCR with amplicons of 250–400 bp. These may need to be redesigned or replaced with shorter alternatives optimized for ddPCR (Bio-Rad Laboratories, 2024).

7.2. Recommended Primer Sets

The following table summarizes commonly used primer sets for nitrogen cycle genes. Confirm suitability for your specific sample type and ddPCR platform before use.

> **Important**
>
> This table provides a starting point. Primer sequences, annealing temperatures, and amplicon sizes should be verified against current literature and databases before ordering. Some primer sets listed below produce amplicons longer than ideal for ddPCR and may require redesign.

Gene	Primer Name	Sequence (5' to 3')	Amplicon (bp)	Reference	ddPCR Suitability
amoA (AOB)	amoA-1F / amoA-2R	GGGGTTTCTACTGGTGGT / CCCCTCKGSAAAGCCTTCTTC	491	Rotthauwe et al. (1997)	Redesign needed (too long)
amoA (AOA)	Arch-amoAF / Arch-amoAR	STAATGGTCTGGCTTAGACG / GCGGCCATCCATCTGTATGT	635	Francis et al. (2005)	Redesign needed (too long)
nirK	nirK876 / nirK1040	ATYGGCGGVCAYGGCGA / GCCTCGATCAGRTTRTGGTT	165	Henry et al. (2006)	Suitable
nirS	cd3aF / R3cd	GTSAACGTSAAGGARACSGG / GASTTCGGRTGSGTCTTGA	410	Braker et al. (2000)	Redesign needed
nosZ (Clade I)	nosZ2F / nosZ2R	CGCRACGGCAASAAGGTSMSSGT / CAKRTGCAKSGCRTGGCAGAA	267	Henry et al. (2006)	Marginal; consider shorter alternative
nosZ (Clade II)	nosZ-II-F / nosZ-II-R	CTIGGICCIYTKCAYAC / GCIGARCARAAITCBGTRC	698	Jones et al. (2013)	Redesign needed

Gene	Primer Name	Sequence (5' to 3')	Amplicon (bp)	Reference	ddPCR Suitability
nifH	PolF / PolR	TGCGAYCC-SAARGCBGACTC / ATSGCCAT-CATYTCRCCGGA	360	Poly et al. (2001)	Redesign needed
nrfA	nrfAF2aw / nrfAR1	CARTGYCAYGTB-GARTA / TWNGGCATRTGR-CARTC	251	Welsh et al. (2014)	Marginal

7.3. In Silico Primer Evaluation

1. Check primer specificity using NCBI Primer-BLAST against the nr/nt database, restricting the search to bacteria and/or archaea as appropriate (Ye et al., 2012).
2. Verify primer coverage against curated functional gene databases:
   - FunGene (http://fungene.cme.msu.edu/) for *amoA*, *nirK*, *nirS*, *nosZ*, *nifH* (Fish et al., 2013)
   - *TBD – identify additional databases for nrfA coverage analysis*
3. Check for primer dimers and secondary structures using IDT OligoAnalyzer (https://www.idtdna.com/calc/analyzer) or Primer3 (https://primer3.ut.ee/).
4. Confirm that the melting temperature (T_m) of each primer falls between 55 and 65 °C, with no more than 2 °C difference between forward and reverse primers.
5. For hydrolysis probe (TaqMan) assays, design the probe to have a T_m 5–10 °C higher than the primers, with no G at the 5' end (quenches FAM fluorescence), and a length of 15–30 bases (Bio-Rad Laboratories, 2024).

7.4. Primer and Probe Ordering

1. Order desalted primers at 25 nmol scale from IDT (Integrated DNA Technologies) or equivalent supplier.
2. Order hydrolysis probes HPLC-purified with 5'-FAM (or HEX for multiplexing) and 3'-Iowa Black FQ quencher.
3. For positive controls, order synthetic double-stranded DNA fragments (gBlocks) matching the target amplicon sequence. Include at least 50 bp of flanking sequence on each side of the primer binding sites.
4. Resuspend lyophilized primers in nuclease-free water to a stock concentration of 100 µM. Store at –20 °C.
5. Prepare 10 µM working stocks by diluting 1:10 in nuclease-free water. Store at –20 °C.

7.5. ddPCR Thermal Cycling Optimization

1. Set up a temperature gradient experiment on the Bio-Rad C1000 Touch to determine the optimal annealing temperature for each primer set.

2. Use a thermal gradient spanning $T_m \pm 5$ °C (typically 55–65 °C).
3. Use positive control gBlock DNA diluted to approximately 1,000 copies per µL as template.
4. Recommended cycling protocol (Bio-Rad Laboratories, 2024):
    - Enzyme activation: 95 °C for 10 min
    - 40 cycles: 94 °C for 30 s, then annealing/extension at gradient temperature for 60 s
    - Signal stabilization: 98 °C for 10 min
    - Hold at 4 °C (indefinite)
    - Ramp rate: 2 °C/s for all steps
5. Read droplets on the QX200 Droplet Reader.
6. Select the annealing temperature that produces the clearest separation between positive and negative droplet clusters in the QuantaSoft amplitude plot.

> **Tip**
>
> If positive and negative droplet populations overlap (rain), try increasing the annealing temperature by 1–2 °C or reducing the primer concentration from 900 nM to 450 nM (Lievens et al., 2016).

7.6. Assay Validation

1. **Specificity:** Run no-template controls (NTC) and extraction blanks from SOP-L700 to confirm the absence of false positives.
2. **Dynamic range:** Prepare a serial dilution of gBlock positive control (10 to 100,000 copies per reaction) and verify linearity of the ddPCR response across the range (Huggett et al., 2013).
3. **Limit of detection (LOD):** Determine the lowest copy number that produces a statistically significant positive call above NTC background; typically 1–10 copies per 20 µL reaction (Hindson et al., 2011).
4. **Matrix effects:** Run environmental DNA extracts at multiple dilutions (undiluted, 1:5, 1:10) to check for PCR inhibition. If the 1:10 dilution value (back-calculated) disagrees by more than 20% from the 1:5 value, inhibition is likely present (Ra vcki et al., 2014).
5. Document all validation results in the primer/probe registry (Appendix A) before approving the assay for routine use.

8. Primer and Probe Registry

Maintain a lab registry of all validated and candidate assays. The registry must include at minimum: gene target, primer names and sequences, probe sequence (if applicable), amplicon length, validated annealing temperature, LOD, matrix tested, validation date, and analyst initials. Store the registry as a version-controlled file in the lab GitHub repository.

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10. Related SOPs

SOP	Title	Relationship
L700	DNA Extraction	Upstream: DNA template source
L702	ddPCR Applications Guide	Downstream: data analysis
L703	PCR and Gel Electrophoresis	Gel verification of primer products
L802	Quality Control and QA	General QA/QC framework

11. Training Record

Before designing or ordering primers independently, personnel must:

1. Read this SOP in full
2. Complete the Bio-Rad ddPCR Foundations eLearning module
3. Demonstrate competent use of Primer-BLAST and IDT OligoAnalyzer
4. Perform a supervised gradient optimization experiment and select the annealing temperature with guidance from the PI
5. Be signed off by the PI

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L702

ddPCR Applications Guide – Absolute Quantification of Soil Nitrogen-Cycle Functional Genes

Standard Operating Procedure

SOP Number	SOP-L702
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the workflow for exporting raw data from the Bio-Rad QX200 ddPCR system, performing quality control checks, calculating gene copy numbers normalized to soil mass or DNA concentration, and conducting statistical analyses of nitrogen cycle gene abundance data. The SOP links the instrument output from SOP-L701 to reproducible analysis scripts written in R (R Core Team, 2024).

2. Scope

- **Applies to:** Interpretation and analysis of QX200 ddPCR data (QuantaSoft or QuantaSoft Analysis Pro output) for nitrogen-cycle functional genes (*amoA*, *nirK*, *nirS*, *nosZ*, *nifH*, *nrfA*) from environmental soil samples, quantified in absolute units (copies per gram dry soil or copies per nanogram DNA)
- **Does not apply to:** Operating the instrument (see SOP-L214); assay or primer design and validation (see SOP-L701); amplicon sequencing, metagenomics, or qPCR data from other platforms
- **Location:** Instrument computer (QuantaSoft) for export and threshold review; RStudio Server for analysis (see SOP-L801 RStudio and GitHub)

3. Responsibilities

Role	Responsibility
Lab PI	Approve analysis pipeline and interpretation
Trained Personnel	Export data, run QC, perform analysis
New Students	Complete training before independent analysis

4. Definitions

- **Amplitude threshold:** The fluorescence intensity boundary set in QuantaSoft (or QuantaSoft Analysis Pro) that separates positive droplets (containing amplified target) from negative droplets; correct threshold placement is the single most critical step in ddPCR data analysis and must be reviewed manually for each plate (Bio-Rad Laboratories, 2024).
- **Copies per microliter (copies/µL):** The absolute concentration of target DNA in the final ddPCR reaction volume as calculated by Poisson statistics from the fraction of positive droplets; must be back-calculated through all dilution steps to report concentration in the original sample (Hindson et al., 2011).
- **Droplet count filter:** A minimum threshold of accepted droplets per well (10,000 recommended) below which the Poisson estimate is unreliable; wells below this threshold must be excluded from analysis (Bio-Rad Laboratories, 2024).
- **DMINQ (Digital Minimum Information for Publication of Quantitative Digital PCR Experiments):** The community standard for reporting ddPCR data (The dMIQE Group & Huggett, 2020); specifies required metadata including droplet count, threshold method, NTC results, and normalization approach.
- **NTC (no-template control):** A reaction well containing all reagents except DNA template; run on every plate to detect reagent or consumable contamination; must show 0 or fewer than 3 positive droplets per well (Bio-Rad Laboratories, 2018).
- **Normalization:** The process of expressing gene copy numbers relative to a common denominator (total DNA concentration in ng/µL, or dry soil mass in g) to allow comparison across samples and studies; both normalizations should be reported when possible.

- **Poisson correction:** The statistical calculation applied by QuantaSoft to convert the fraction of positive droplets to an absolute copy number per microliter, correcting for the probability that a single droplet may contain more than one target molecule (Hindson et al., 2011).
- **QLp file:** The proprietary binary data file format produced by the Bio-Rad QX200 system; contains raw droplet amplitude data, QuantaSoft threshold settings, and computed results; must be archived unmodified before any downstream analysis.
- **Rain:** Droplets with intermediate fluorescence amplitude falling between the positive and negative clusters in the amplitude plot; indicates suboptimal amplification, partial inhibition, or primer issues; quantified as the percentage of events in the intermediate cluster and should be documented (Bio-Rad Laboratories, 2018; Lievens et al., 2016).

5. Why ddPCR for This Application

Quantitative PCR (qPCR) infers target abundance from amplification kinetics against a standard curve, so its accuracy depends on amplification efficiency and on curve quality. ddPCR instead partitions each reaction into roughly 20,000 nanoliter droplets, amplifies to endpoint within each droplet, and counts droplets as positive or negative. The fraction of positive droplets is fit to a Poisson distribution to recover the starting concentration in copies per microliter, with no standard curve required (Bio-Rad Laboratories, 2018; Hindson et al., 2011).

For soil nitrogen-cycle work, this matters for several reasons:

- **Absolute, comparable counts.** Copy numbers are returned directly in copies per microliter, which can be normalized to soil mass or DNA and compared across plates, runs, and studies without a shared standard curve.
- **Tolerance of inhibitors.** Soil DNA extracts carry humic acids and other PCR inhibitors. Endpoint partitioning is less sensitive to partial inhibition than the kinetic measurement that qPCR depends on, although inhibition should still be checked (§6) (Hindson et al., 2011).
- **Precision for small differences.** The large number of partitions lets ddPCR resolve modest fold-changes in gene abundance between treatments (Hindson et al., 2011).
- **Low-abundance targets.** Genes such as *nosZ* or *nifH* are often present at low copy numbers relative to bulk DNA; partitioning improves the signal-to-noise ratio for rare targets.

These properties are the reason functional-gene abundance is increasingly reported in absolute units when relating microbial communities to nitrogen cycling processes (Levy-Booth et al., 2014).

6. The Soil Nitrogen Cycle and Functional Gene Markers

Soil nitrogen transformations are carried out by distinct microbial guilds, each defined by the enzymes (and therefore the genes) it carries. Quantifying these marker genes provides a direct, culture-independent estimate of the genetic potential for each process (Kuypers et al., 2018; Levy-Booth et al., 2014).

Gene	Process / guild	What the abundance indicates	Primary reference
amoA (AOB)	Ammonia oxidation, bacterial	Bacterial ammonia-oxidizer potential; first, rate-limiting step of nitrification	Rotthauwe et al. (1997)
amoA (AOA)	Ammonia oxidation, archaeal	Archaeal ammonia-oxidizer potential; often dominant in low-N, acidic soils	Francis et al. (2005)
nirK	Nitrite reduction (denitrification)	Cu-type nitrite-reductase potential	Henry et al. (2006)
nirS	Nitrite reduction (denitrification)	cd_1-type nitrite-reductase potential	Braker et al. (2000)
nosZ	Nitrous oxide reduction	N_2O-reductase potential; capacity to reduce N_2O to N_2	Henry et al. (2006)
nifH	Nitrogen fixation	Diazotroph potential; biological N_2 fixation	Levy-Booth et al. (2014)
nrfA	DNRA	Retains N in system as NH_4^+	Levy-Booth et al. (2014)

7. From Instrument Output to Quality-Controlled Data

7.1. Reviewing and Exporting Data

Instrument operation and the QuantaSoft interface are covered in SOP-L214. For analysis, the entry point is the exported well-level data.

1. Open the completed experiment file (`.qlp`) in QuantaSoft and confirm the positive/negative threshold for each well. Re-apply thresholds manually in QuantaSoft Analysis Pro if needed, and record any change in the lab notebook.
2. Export with **Options > Export CSV** to produce a well-level results file. Key columns include `Well`, `Sample`, `Target`, `Concentration` (Poisson-corrected copies/µL (Hindson et al., 2011)), `Positives`, `Negatives`, `AcceptedDroplets`, `MeanAmplitudeOfPositives`, and `MeanAmplitudeOfNegatives`.
3. Name the file `YYYY-MM-DD_gene_project_plate.csv`.
4. Archive both the `.qlp` and the exported `.csv` to the lab shared drive under `ddPCR_raw_data/` before any analysis begins.

> **Data Integrity**
>
> - Never overwrite raw exported data files. Work only with copies.
> - Back up raw `.qlp` files immediately after each run.
> - All analysis scripts must be version-controlled in the lab GitHub repository.

7.2. Quality Control and Acceptance Criteria

Apply these checks before any analysis, following the data-quality expectations set out for digital PCR reporting (Bio-Rad Laboratories, 2018; The dMIQE Group & Huggett, 2020).

1. **Droplet count filter.** Exclude wells with fewer than 10,000 accepted droplets; below this, the Poisson estimate is unreliable (Bio-Rad Laboratories, 2024).
2. **No-template controls (NTC).** NTC wells must show 0 or fewer than 3 positive droplets. More than 3 positives indicates contamination; rerun the affected target or plate.
3. **Extraction blanks.** Blanks carried from DNA extraction (SOP-L700) must fall below the NTC threshold. Elevated counts point to contamination in the extraction workflow. Note that direct soil extraction is itself a known source of quantitative bias (Frosteg aard et al., 1999), so blanks and recovery checks matter for absolute numbers.
4. **Positive controls.** gBlock serial dilution positive controls must return concentrations within 20% of expected values across the linear range (Huggett et al., 2013).
5. **Rain.** Document the percentage of rain droplets per well. Assays with persistent high rain (> 10–15% of events) should be flagged and the primer or cycling conditions reviewed (Lievens et al., 2016).

8. Calculating Gene Copy Numbers

8.1. From Reaction to Sample

The concentration reported by QuantaSoft is in copies per microliter of the final ddPCR reaction. To convert to copies per gram dry soil:

$$N_{\text{soil}} = C_{\text{rxn}} \times V_{\text{rxn}} \times D \times \frac{V_{\text{elution}}}{V_{\text{rxn input}}} \times \frac{1}{m_{\text{dry soil}}}$$

where:

- C_{rxn} = QuantaSoft-reported concentration (copies/µL)
- V_{rxn} = total ddPCR reaction volume (typically 20 µL)
- D = dilution factor applied to the extract before the reaction
- V_{elution} = total DNA extract volume (µL)
- $V_{\text{rxn input}}$ = volume of extract added to the reaction (µL)
- $m_{\text{dry soil}}$ = mass of dry soil extracted (g)

8.2. R Implementation

```r
# ============================================================================
# ddPCR copy number normalization (SOP-L702)
# ============================================================================

library(tidyverse)

# Import well-level QuantaSoft export
raw <- read_csv("2025-05-14_amoA_wetland_plate1.csv")

# Parameters (adjust per run)
rxn_vol       <- 20     # µL total ddPCR reaction volume
input_vol     <- 5      # µL extract added to reaction
elution_vol   <- 100    # µL total DNA extract
dilution      <- 10     # dilution factor applied to extract before reaction

# Dry soil mass (g) per sample -- join from sample metadata
metadata <- read_csv("sample_metadata.csv")  # cols: Sample, dry_soil_g

results <- raw |>
  filter(AcceptedDroplets >= 10000) |>      # droplet count filter
  left_join(metadata, by = "Sample") |>
  mutate(
    copies_per_g = Concentration *           # copies/µL in reaction
      rxn_vol *                              # total reaction volume
      dilution *                             # dilution correction
      (elution_vol / input_vol) *            # extraction recovery
      (1 / dry_soil_g)                       # normalize to dry soil
  )

# Flag QC issues
results <- results |>
  mutate(
    qc_flag = case_when(
      AcceptedDroplets < 10000              ~ "low_droplets",
      Concentration == 0 & !is.na(copies_per_g) ~ "no_signal",
      TRUE                                  ~ "OK"
    )
  )

write_csv(results, "2025-05-14_amoA_wetland_plate1_processed.csv")
```

9. Interpreting Relative Gene Abundances

Individual gene copy numbers are most informative when examined in relation to each other and to the soil's total microbial biomass (often estimated as 16S rRNA gene copies) (Kuypers et al., 2018; Levy-Booth et al., 2014).

Suggested derived metrics:

Metric	Formula	Ecological interpretation
AOB : AOA ratio	*amoA*(AOB) / *amoA*(AOA)	Relative dominance of bacterial vs. archaeal nitrifiers
Denitrification potential	*nirK* + *nirS* + *nosZ*	Combined capacity for N loss as gas
N_2O consumer : producer	*nosZ* / (*nirK* + *nirS*)	Capacity to complete denitrification; low values predict N_2O accumulation
Diazotroph index	*nifH* / 16S	Fraction of community with N fixation capacity
DNRA : denitrification	*nrfA* / (*nirK* + *nirS*)	N retention vs. N loss balance

10. References

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11. Related SOPs

SOP	Title	Relationship
L700	DNA Extraction	Upstream: DNA template source
L701	ddPCR Primer Design	Primer validation and assay registry
L801	RStudio and GitHub	Version-controlled analysis
L802	Quality Control and QA	General QA/QC framework

12. Training Record

Before performing ddPCR data analysis independently, personnel must:

1. Read this SOP and SOP-L701 in full
2. Review the DMINQ reporting standard (The dMIQE Group & Huggett, 2020)

3. Demonstrate correct QuantaSoft threshold setting on at least two plates under supervision
4. Run the copy number normalization R script on a test dataset and reproduce the trainer's results
5. Be signed off by the PI or lab manager

13. Appendix A: DMINQ Checklist

When reporting ddPCR data from this workflow, include the following metadata per The dMIQE Group & Huggett (2020):

Item	Required by DMINQ	Notes
Instrument model and serial	Yes	Bio-Rad QX200
Software version	Yes	QuantaSoft version
Droplet count per well	Yes	Must be 10,000
Threshold setting method	Yes	Manual or automated; document both
NTC result	Yes	Copies/µL; must be 0 or < 3 positive droplets
Positive control	Yes	gBlock; expected and measured concentration
Rain percentage	Yes	Document even if low
Dilution factor	Yes	
Normalization denominator	Yes	Dry soil mass (g) and/or DNA (ng)
Inhibition check result	Yes	1:5 vs. 1:10 dilution agreement
Primer/probe sequences	Yes	Or cite SOP-L701 registry

Item	Required by DMINQ	Notes
Cycling conditions	Yes	Annealing temperature, cycles
Reference for target gene	Yes	Cite from SOP-L701 registry

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L702

ddPCR Data Export and Analysis – Nitrogen Cycle Gene Abundance

Standard Operating Procedure

SOP Number	SOP-L702
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the workflow for exporting raw data from the Bio-Rad QX200 ddPCR system, performing quality control checks, calculating gene copy numbers normalized to soil mass or DNA concentration, and conducting statistical analyses of nitrogen cycle gene abundance data. The SOP links the instrument output from SOP-L701 to reproducible analysis scripts written in R (R Core Team, 2024).

2. Scope

- **Applies to:** Processing and analysis of ddPCR data (QuantaSoft or QuantaSoft Analysis Pro output) for nitrogen cycle functional genes (*amoA*, *nirK*, *nirS*, *nosZ*, *nifH*, *nrfA*) from environmental samples
- **Does not apply to:** Amplicon sequencing data, metagenomics, or qPCR data processed with different software
- **Location:** Instrument computer (QuantaSoft) and RStudio Server (see SOP-L801 RStudio and GitHub)

3. Responsibilities

Role	Responsibility
Lab PI	Approve analysis pipeline and interpretation
Trained Personnel	Export data, run QC, perform analysis
New Students	Complete training before independent analysis

4. Definitions

- **Amplitude threshold:** The fluorescence intensity boundary set in DropletQuant (or equivalent software) that separates positive droplets (containing amplified target) from negative droplets; correct threshold placement is the single most critical step in ddPCR data analysis.
- **Copies per microliter (copies/µL):** The absolute concentration of target DNA in the final ddPCR reaction volume as calculated by Poisson statistics from the fraction of positive droplets; must be back-calculated through all dilution steps to report concentration in the original sample.
- **DropletQuant / QuantaSoft:** Bio-Rad software used to read the QX200 droplet reader output, set amplitude thresholds, and calculate target concentrations; raw .qlp files must be archived unmodified.
- **Duplex assay:** A ddPCR assay in which two targets (e.g., a gene of interest and a reference gene) are quantified simultaneously using two spectrally distinct fluorescent probes (FAM and HEX/VIC) in the same reaction; requires careful threshold setting for each channel.

- **Fractional abundance:** The proportion of positive droplets relative to total accepted droplets: $\lambda = -\ln(1-p)$ where p is the positive fraction; used by Poisson correction to calculate copies/µL.
- **No-template control (NTC):** A reaction containing all ddPCR components except sample DNA; must yield zero or near-zero positive droplets; persistent positive droplets in NTCs indicate reagent contamination.
- **Poisson correction:** The statistical adjustment applied by DropletQuant to account for the probability that some droplets contain more than one target molecule; assumes random partitioning and is essential for accurate absolute quantification.
- **Rain:** Droplets with intermediate fluorescence amplitude between the positive and negative clusters; caused by partial amplification, degraded DNA, or probe competition; must be assigned to positive or negative by threshold adjustment and noted in data records.
- **Rejected droplets:** Droplets excluded from analysis by the instrument because they fall outside acceptable size or fluorescence quality criteria; runs with fewer than 10,000 accepted droplets per well should be flagged and potentially repeated.

5. Safety and Hazards

No physical or chemical hazards are associated with this procedure. Data integrity and reproducibility are the primary concerns.

> **Data Integrity**
>
> - Never overwrite raw exported data files. Work only with copies.
> - All analysis scripts must be version-controlled in the lab GitHub repository.
> - Back up raw `.qlp` files from QuantaSoft to the lab shared drive immediately after each run.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
QX200 instrument computer	Instrument room	Runs QuantaSoft or QuantaSoft Analysis Pro
RStudio Server	Remote (see SOP-L801)	For R-based analysis
USB drive or network share	Lab	For transferring `.qlp` and `.csv` files

6.2. Software

Software	Version	Purpose
QuantaSoft or QuantaSoft Analysis Pro	1.7+ or 1.0+	Threshold setting, data export
R	4.3+	Statistical analysis
RStudio	Current	IDE for R scripts
`tidyverse` R package	Current	Data wrangling and visualization
`ggplot2` R package	Current	Plotting
TBD – identify additional R packages for ddPCR analysis (e.g., `ddpcr`, `twoddpcr`)		

7. Procedure

7.1. Data Export from QuantaSoft

1. Open the completed experiment file (`.qlp`) in QuantaSoft
2. Review the droplet plots for each well. Confirm that the positive/negative threshold was set appropriately during the run (see SOP-L701, Section 6.6)
3. If threshold adjustment is needed, use QuantaSoft Analysis Pro to re-apply thresholds manually. Document any threshold changes in the lab notebook.
4. Export data using **Options > Export CSV** to generate a comma-separated file containing well-level results
5. The exported CSV includes the following key columns:
    - `Well`: plate position (e.g., A01)
    - `Sample`: sample name as entered during plate setup
    - `Target`: gene target name
    - `Concentration`: copies per uL of the final ddPCR reaction (Poisson-corrected) (Hindson et al., 2011)
    - `Positives`: number of positive droplets
    - `Negatives`: number of negative droplets
    - `AcceptedDroplets`: total accepted droplets
    - `MeanAmplitudeOfPositives`: mean fluorescence of positive cluster
    - `MeanAmplitudeOfNegatives`: mean fluorescence of negative cluster
6. Save the CSV with the naming convention: `YYYY-MM-DD_gene_project_plate.csv` (e.g., `2025-05-14_amoA_wetland_plate1.csv`)
7. Copy the `.qlp` file and the exported `.csv` to the lab shared drive under `ddPCR_raw_data/`

7.2. Quality Control Checks

Perform the following checks on the exported data before proceeding to analysis. These can be scripted in R for reproducibility.

1. **Droplet count filter:** Exclude any well with fewer than 10,000 accepted droplets. Wells below this threshold have unreliable Poisson statistics (Bio-Rad Laboratories, 2024).
2. **No-template controls (NTC):** NTC wells must show 0 or fewer than 3 positive droplets. If NTC wells show more than 3 positives, the entire plate or the affected target should be considered contaminated and rerun.
3. **Extraction blanks:** Extraction blanks (from SOP-L80) must show fewer positive droplets than the NTC acceptance threshold. Elevated counts in extraction blanks indicate contamination in the DNA extraction workflow.
4. **Positive controls:** Positive control gBlock wells should fall within 20% of the expected concentration based on the dilution prepared.
5. **Technical replicate agreement:** If technical replicates were run, calculate the coefficient of variation (CV) across replicates. A CV greater than 25% suggests pipetting error or sample heterogeneity.

> **Tip**
>
> Create a QC report template in R Markdown or Quarto that automatically flags wells failing any of the criteria above. This makes batch QC efficient and ensures nothing is missed.

7.3. Calculating Gene Copy Numbers

The QuantaSoft `Concentration` output is in copies per uL of the ddPCR reaction mixture. To express results per gram of dry soil (or per unit of extracted DNA), apply the following conversions:

$$\text{Copies per g dry soil} = \frac{C_{\text{ddPCR}} \times V_{\text{reaction}} \times DF \times V_{\text{elution}}}{V_{\text{template}} \times m_{\text{soil}} \times (1 - \theta_g)}$$

where:

- C_{ddPCR} = concentration from QuantaSoft (copies/uL)
- V_{reaction} = total ddPCR reaction volume (typically 20 uL)
- DF = dilution factor of the DNA template (e.g., 10 if diluted 1:10)
- V_{elution} = total DNA elution volume from extraction (uL; see SOP-L80)
- V_{template} = volume of DNA template added to the ddPCR reaction (uL)
- m_{soil} = mass of soil extracted (g)
- θ_g = gravimetric soil moisture (as a decimal; see SOP-L400)

To express results per ng of extracted DNA instead:

$$\text{Copies per ng DNA} = \frac{C_{\text{ddPCR}} \times V_{\text{reaction}}}{V_{\text{template}} \times C_{\text{DNA}}}$$

where C_{DNA} is the Qubit DNA concentration (ng/uL) from SOP-L80.

```r
# Example calculation: copies per g dry soil
c_ddpcr <- 52.3         # copies/uL (from QuantaSoft)
v_reaction <- 20         # uL
dilution_factor <- 10    # 1:10 dilution of template
v_elution <- 100         # uL (from SOP-L80)
v_template <- 2          # uL added to ddPCR reaction
soil_mass_g <- 0.25      # g soil extracted
moisture_decimal <- 0.35 # gravimetric moisture as decimal

copies_per_g <- (c_ddpcr * v_reaction * dilution_factor * v_elution) /
                (v_template * soil_mass_g * (1 - moisture_decimal))

cat(sprintf("Gene copies per g dry soil: %.0f\n", copies_per_g))
```

```
Gene copies per g dry soil: 3218462
```

```r
cat(sprintf("Gene copies per g dry soil (log10): %.2f\n", log10(copies_per_g)))
```

```
Gene copies per g dry soil (log10): 6.51
```

7.4. Data Organization and Merging

1. Merge the QC-filtered ddPCR results with the sample metadata (site, treatment, depth, date, soil moisture, DNA concentration) using a shared sample ID column
2. Organize the merged data in a tidy (long) format with one row per sample-by-gene combination (Wickham, 2014)
3. Save the merged analysis-ready dataset as a `.csv` in the project GitHub repository under `data/processed/`
4. *TBD – define the standard column naming convention for the lab's ddPCR datasets (e.g., `sample_id`, `gene`, `copies_per_g_dry`, `copies_per_ng_dna`, `site`, `treatment`, `depth_cm`, `date`)*

7.5. Statistical Analysis

> **Note**
>
> The choice of statistical approach depends on the experimental design (e.g., paired vs. independent sites, factorial treatments, time series). The guidance below covers common analyses for nitrogen cycle gene abundance. Consult the PI before selecting a final analytical approach.

7.5.1. Data Exploration and Visualization

1. Plot gene copy numbers ($\log_{10}$-transformed) by treatment, site, or depth using boxplots or dot plots

2. Assess normality of log-transformed data using Shapiro-Wilk tests and Q-Q plots
3. Check for outliers and investigate whether they represent true biological variation or analytical artifacts

7.5.2. Comparing Gene Abundance Across Treatments

- For two-group comparisons (e.g., amended vs. control): use Welch's t-test on $\log_{10}$-transformed copies per g dry soil, or the non-parametric Wilcoxon rank-sum test if normality assumptions are not met (Frosteg aard et al., 1999)
- For multi-group comparisons: use one-way ANOVA on $\log_{10}$-transformed data with Tukey HSD post hoc tests, or Kruskal-Wallis with Dunn's test for non-parametric data
- For factorial designs (e.g., treatment x depth): use two-way ANOVA or linear mixed-effects models with site as a random effect (Pinheiro & Bates, 2000)

7.5.3. Gene Ratios

Ratios of functional gene abundances can provide insight into the balance of nitrogen cycling processes (Hallin et al., 2009):

- *nirK*/*nirS*: relative contribution of the two nitrite reductase pathways to denitrification
- *nosZ*/(*nirK* + *nirS*): completeness of denitrification (higher values suggest greater N_2O reduction capacity)
- *amoA* AOA/*amoA* AOB: relative dominance of archaeal vs. bacterial ammonia oxidation
- *nrfA*/(*nirK* + *nirS*): relative importance of DNRA vs. denitrification

TBD – determine which gene ratios are most relevant for the lab's current research questions and establish standard visualization formats.

7.5.4. Correlations with Environmental Variables

- Correlate gene abundances with soil physicochemical data (pH, moisture, total C, total N, nitrate, ammonium) using Pearson or Spearman correlations
- *TBD – evaluate whether multivariate approaches (e.g., PCA, redundancy analysis) are appropriate given the number of samples and variables*
- *TBD – determine whether gene abundance data should be related to process rate measurements (e.g., N_2O flux, nitrification potential) and define the analytical framework for doing so*

7.5.5. Reporting

- Report gene abundance as copies per g dry soil (mean +/- standard error) and/or copies per ng DNA
- Present data on a $\log_{10}$ scale in figures; report back-transformed means and confidence intervals in tables where appropriate (Bolker et al., 2009)
- Include all QC metrics (droplet counts, NTC results, positive control recovery) as supplementary tables
- *TBD – create a standardized R Markdown/Quarto reporting template for ddPCR results that the lab can reuse across projects*

8. Quality Control and Acceptance Criteria

- Raw `.qlp` files must be archived on the lab shared drive before any analysis proceeds
- All R scripts must be committed to the lab GitHub repository with meaningful commit messages
- QC filtering must be applied before any statistical analysis; unfiltered data should never be reported
- Positive control recovery within 20% of expected value
- *TBD – define minimum sample sizes required for each statistical test based on power analysis*
- *TBD – establish whether and how to handle values below the limit of detection (e.g., substitution with LOD/2, censored regression, or Tobit models) (Helsel, 2011)*

9. Waste Disposal

No physical waste is generated by this procedure. Data management practices are described in Section 6.4.

10. References

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11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP, SOP-L701 Primer Design, and SOP-L700 DNA Extraction
2. Complete SOP-L801 RStudio and GitHub training
3. Demonstrate competence exporting data from QuantaSoft
4. Successfully run the QC and analysis pipeline on a practice dataset under supervision
5. Be signed off by the PI or lab manager

12. Appendix A: Example QC Filtering Script

```
library(tidyverse)

# Read exported QuantaSoft CSV
raw <- read_csv("data/raw/2025-05-14_amoA_wetland_plate1.csv")

# QC filtering
qc_filtered <- raw %>%
  filter(
    AcceptedDroplets >= 10000        # Minimum droplet threshold
  ) %>%
  mutate(
    qc_flag = case_when(
      Sample == "NTC" & Positives > 3 ~ "NTC_contamination",
      Sample == "Extraction_Blank" & Positives > 3 ~ "Extraction_contamination",
      TRUE ~ "Pass"
    )
  )

# Flag and report QC failures
qc_failures <- qc_filtered %>%
  filter(qc_flag != "Pass")

if (nrow(qc_failures) > 0) {
  warning("QC failures detected:")
  print(qc_failures %>% select(Well, Sample, Target, Positives, qc_flag))
}

# Retain only passing sample wells
analysis_ready <- qc_filtered %>%
```

```
  filter(qc_flag == "Pass",
         !Sample %in% c("NTC", "Extraction_Blank", "Positive_Control"))
```

13. Appendix B: Visualization Template

```
library(tidyverse)

# Assumes analysis_ready has columns: sample_id, gene, copies_per_g_dry, treatment
analysis_ready %>%
  ggplot(aes(x = treatment, y = log10(copies_per_g_dry), fill = treatment)) +
  geom_boxplot(alpha = 0.7, outlier.shape = NA) +
  geom_jitter(width = 0.2, alpha = 0.5, size = 1.5) +
  facet_wrap(~ gene, scales = "free_y") +
  labs(
    x = "Treatment",
    y = expression(log[10]~"(gene copies g"^{-1}~"dry soil)"),
    title = "Nitrogen Cycle Gene Abundance by Treatment"
  ) +
  theme_minimal() +
  theme(legend.position = "none")
```

14. Appendix C: Sending Samples to External Laboratories

TBD – if ddPCR analysis is outsourced to an external service provider (e.g., university core facility, commercial lab), document the following:

- *Preferred service provider(s) and contact information*
- *Sample submission requirements (volume, concentration, format)*
- *Data format and file types returned by the provider*
- *Turnaround time expectations*
- *Cost per sample and funding source/account number*
- *How to reconcile externally generated data with in-house QC standards*

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L703

PCR Amplification and Gel Electrophoresis

Standard Operating Procedure

SOP Number	SOP-L703
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standard polymerase chain reaction (PCR) amplification of target DNA fragments and visualization of PCR products by agarose gel electrophoresis. PCR is used in the EA Program for confirming DNA extraction success, screening for target genes before ddPCR or sequencing, and verifying amplicon size and specificity prior to library preparation (Lorenz, 2012; Mullis, 1990). Gel electrophoresis separates DNA fragments by size, providing a visual confirmation that amplification produced the expected product.

2. Scope

- **Applies to:** Standard endpoint PCR amplification of environmental DNA extracts (soil, water, tissue) and visualization of products on agarose gels; applicable to gene-specific screening, amplicon verification, and colony PCR
- **Does not apply to:** Quantitative PCR (qPCR) or digital PCR (ddPCR; see SOP-L701 and SOP-L702); RT-PCR for RNA targets; polyacrylamide gel electrophoresis (PAGE)
- **Location:** Molecular biology bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve primer selections and cycling protocols
Trained Personnel	Set up PCR reactions and run gels per this SOP
New Students	Complete training before independent use

4. Definitions

- **Agarose gel:** A porous polymer matrix cast from dissolved agarose in TAE or TBE buffer; DNA fragments migrate through the gel under an electric field and are separated by size; typical concentrations are 1–2% (w/v).
- **Amplicon:** The specific DNA fragment produced by PCR between a defined pair of primers; its length (in base pairs) is determined by the primer binding positions and is used to verify PCR specificity on a gel.
- **Annealing temperature (T_a):** The temperature during the PCR extension cycle at which primers bind to the template; typically 3–5 °C below the calculated primer melting temperature (T_m); incorrect T_a causes non-specific amplification or failed reactions.
- **DNA ladder (size standard):** A mixture of DNA fragments of known sizes run alongside samples on a gel; used to estimate the size of amplified products.
- **Ethidium bromide (EtBr) / SYBR Safe:** Fluorescent intercalating dyes added to agarose gels or post-staining baths to visualize DNA under UV light; EtBr is a potent mutagen requiring dedicated waste disposal; SYBR Safe is a lower-hazard alternative.

- **Master mix:** A pre-mixed reagent solution containing DNA polymerase, dNTPs, buffer, and $MgCl_2$ in optimized concentrations; reduces pipetting error and variability between replicate reactions.
- **Melting temperature (T_m):** The temperature at which 50% of a primer-template duplex is dissociated; calculated from primer sequence and salt concentration; the basis for selecting annealing temperature.
- **Non-specific amplification:** PCR products of unexpected size caused by primer mis-binding; detected as extra bands on a gel; remedied by increasing annealing temperature or redesigning primers.
- **Primer:** A short (18–25 bp) single-stranded oligonucleotide that binds to a complementary sequence on the template DNA and defines the start point for DNA polymerase extension.
- **TAE / TBE buffer:** Running buffers for agarose gel electrophoresis; TAE (Tris-acetate-EDTA) gives sharper bands for larger fragments; TBE (Tris-borate-EDTA) provides better resolution for smaller fragments and is more resistant to overheating.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Ethidium bromide (EtBr) and SYBR Safe are nucleic acid stains. EtBr is a suspected mutagen; handle with gloves at all times and dispose of gels and buffers as hazardous waste. SYBR Safe is a safer alternative but should still be handled with gloves.
- **Electrical:** Gel electrophoresis uses high voltage (80–120 V). Do not touch the buffer or gel while the power supply is on. Always disconnect the leads before opening the gel box.
- **UV radiation:** Transilluminators emit UV light that can cause skin burns and eye damage. Use a UV shield, wear UV-blocking safety glasses, and minimize exposure time. Blue-light transilluminators are preferred when used with SYBR Safe.
- **Biological:** Environmental DNA extracts may originate from samples containing pathogens. Handle with standard precautions.

5.1. Required PPE

☐ Safety glasses (UV-blocking if using a UV transilluminator)
☐ Lab coat
☐ Nitrile gloves (change frequently; required at all times when handling gels, stains, or PCR products)

5.2. Emergency Procedures

For EtBr contact with skin, wash immediately with soap and water. For UV exposure, flush eyes with water and seek medical attention. Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Thermal cycler (Bio-Rad C1000 Touch or equivalent)	Mol bio bench	Verify block type matches tube format
Gel electrophoresis system (tank, comb, casting tray)	Mol bio bench	Mini-gel (7 x 10 cm) or midi-gel
Power supply	Mol bio bench	Capable of 10–300 V
Gel imaging system (transilluminator + camera)	Mol bio bench	UV or blue-light; with gel documentation software
Micropipettes (2, 10, 100, 1000 uL)	Mol bio bench	Calibrated annually
Microwave	Lab	For melting agarose
Microcentrifuge	Mol bio bench	For brief spin-downs

6.2. Reagents and Consumables

Item	Notes
Taq DNA polymerase or high-fidelity polymerase	e.g., GoTaq (Promega), Q5 (NEB), or OneTaq (NEB)
5x or 10x PCR buffer (with $MgCl_2$)	Supplied with polymerase
dNTP mix (10 mM each)	Store at –20 C
Forward and reverse primers (10 uM working stocks)	Store at –20 C
Nuclease-free water	For master mix and negative controls
PCR tubes or plates (0.2 mL, thin-wall)	Matched to thermal cycler block
Agarose (molecular biology grade)	For gel preparation
1x TAE or TBE buffer	Running buffer; prepare from 50x stock
DNA stain (SYBR Safe or ethidium bromide)	SYBR Safe preferred
DNA ladder (100 bp and/or 1 kb)	e.g., NEB 100 bp or 1 kb Plus
6x gel loading dye	Often included with ladder or polymerase
Filter pipette tips (10, 100, 1000 uL)	Barrier tips mandatory to prevent cross-contamination

7. Procedure

7.1. PCR Workspace Preparation

! Contamination Prevention

PCR is extremely sensitive to contamination. Follow these rules:

- Set up reactions in a designated pre-PCR area, physically separated from post-PCR work (gel loading, product analysis).
- Never bring PCR products, gels, or gel imaging equipment into the pre-PCR area.
- Use dedicated pipettes for pre-PCR work. Use filter tips exclusively.
- Wipe bench surfaces with 10% bleach followed by 70% ethanol before setting up reactions.
- Change gloves frequently, especially after touching any surface outside the pre-PCR area.

7.2. Master Mix Preparation

Prepare all components on ice. Calculate volumes for the number of reactions plus 10% overage to account for pipetting losses.

Standard 25 uL reaction (GoTaq example):

Component	Volume per reaction	Final concentration
5x GoTaq Reaction Buffer	5.0 uL	1x
dNTP mix (10 mM each)	0.5 uL	200 uM each
Forward primer (10 uM)	1.25 uL	500 nM
Reverse primer (10 uM)	1.25 uL	500 nM
GoTaq DNA Polymerase (5 U/uL)	0.125 uL	0.625 U
Nuclease-free water	14.875 uL	–
Subtotal (master mix)	**23.0 uL**	
Template DNA	2.0 uL	Variable (1–10 ng)
Total	**25.0 uL**	

1. Thaw buffer, dNTPs, and primers on ice. Vortex briefly and spin down.
2. Prepare the master mix in a sterile 1.5 mL tube, adding polymerase last. Mix by pipetting; do not vortex.
3. Aliquot 23 uL of master mix into each labeled PCR tube.
4. Add 2 uL of template DNA to each tube. Include controls:
   - **Positive control:** DNA known to amplify with these primers
   - **Negative control (NTC):** 2 uL of nuclease-free water
   - **Extraction blank:** DNA extract from SOP-L700 extraction blank
5. Cap tubes, vortex briefly, and spin down.

7.3. Thermal Cycling

Program the thermal cycler with the following general protocol. Adjust the annealing temperature for your specific primer set.

Step	Temperature	Time	Cycles
Initial denaturation	95 C	2 min	1
Denaturation	95 C	30 s	30–35
Annealing	T_a C	30 s	30–35
Extension	72 C	1 min/kb	30–35
Final extension	72 C	5 min	1
Hold	4 C	Hold	–

where T_a is the annealing temperature, typically 3–5 C below the lower primer T_m. For primers with T_m of 55–60 C, start at 55 C. Optimize by running a gradient if needed (Lorenz, 2012).

Record the cycling parameters, primer names, and annealing temperature in the lab notebook.

7.4. Agarose Gel Preparation

1. Weigh agarose for a 1–2% gel (w/v), depending on the expected amplicon size:

Agarose %	Effective separation range
0.8%	500–10,000 bp
1.0%	250–7,000 bp
1.5%	100–3,000 bp
2.0%	50–1,500 bp

2. Add agarose to 1x TAE (or TBE) buffer in an Erlenmeyer flask.
3. Microwave in 30-second intervals, swirling between intervals, until the agarose is fully dissolved and the solution is clear.
4. Cool to approximately 55 C (comfortable to hold).
5. If using SYBR Safe, add 5 uL per 50 mL of gel. Swirl gently.
6. Pour into the casting tray with the comb in place. Allow to solidify (approximately 20–30 minutes at room temperature).

7.5. Loading and Running the Gel

1. Remove the comb carefully. Place the gel in the electrophoresis tank and fill with 1x TAE (or TBE) buffer until the gel is submerged by 2–3 mm.
2. Load 5 uL of DNA ladder into the first well.
3. Add loading dye to each PCR product (if not already included in the PCR buffer). Load 5–10 uL per well.
4. Record the loading order in the lab notebook.

5. Connect the leads: DNA migrates toward the positive (red) electrode. Confirm the orientation.
6. Run at 80–120 V for 30–45 minutes, or until the leading dye front has migrated approximately two-thirds of the gel length.
7. Turn off the power supply and disconnect the leads before removing the gel.

7.6. Gel Imaging

1. Place the gel on the transilluminator (blue light for SYBR Safe; UV for EtBr).
2. Capture an image using the gel documentation system.
3. Save the image with the naming convention: `YYYY-MM-DD_primers_project_gel.tif`
4. Evaluate the image:
   - Positive control: visible band at the expected size
   - NTC and extraction blank: no visible band
   - Samples: band at the expected size indicates successful amplification
5. Upload the image to the project GitHub repository under `results/gels/`.

8. Troubleshooting

Symptom	Probable Cause	Corrective Action
No bands in any lane	Failed reaction; wrong cycling parameters	Check primer and dNTP stocks; verify cycling program
Bands in NTC	Contamination	Discard reagents; clean workspace with bleach; use fresh aliquots
Multiple bands	Non-specific amplification	Increase annealing temperature; reduce primer concentration; redesign primers
Smear instead of band	DNA degradation or overloaded gel	Reduce template amount; check DNA quality on NanoDrop
Bands too faint	Insufficient template or cycles	Increase template to 5–10 ng; increase to 35 cycles
Bands in samples but not positive control	Positive control degraded	Prepare fresh positive control DNA

9. Quality Control

- Every PCR run must include: positive control, NTC, and extraction blank
- No bands should appear in the NTC or extraction blank. Any amplification in controls invalidates the run (Salter et al., 2014).
- Band size must match the expected amplicon size (compare to the DNA ladder)
- For quantitative downstream applications (library prep, sequencing), confirm band intensity is roughly uniform across samples

10. Waste Disposal

Waste Type	Container	Disposal Method
EtBr-stained gels and buffer	Designated EtBr waste container	EH&S hazardous waste pickup
SYBR Safe-stained gels	Standard trash (check local EH&S policy)	Non-hazardous per manufacturer
Used PCR tubes and tips	Standard trash	Non-hazardous after thermal cycling
Unused EtBr stock solution	Chemical waste	EH&S hazardous waste pickup

11. References

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12. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials	EtBr and UV safety
L700	DNA Extraction	Upstream: provides template DNA
L701	ddPCR Primers	Primer design and validation
L704	Amplicon Library Prep	Downstream: library preparation
L106	Freezers	Reagent and DNA storage

13. Training Record

Before performing PCR and gel electrophoresis independently, personnel must:

1. Read this SOP and SOP-L700 in full
2. Demonstrate master mix preparation with correct volumes and controls
3. Run a supervised PCR and gel, producing a clean positive control band with no NTC contamination
4. Demonstrate safe handling of DNA stains and proper gel disposal
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L704

Amplicon Library Preparation for Sequencing

Standard Operating Procedure

SOP Number	SOP-L704
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the preparation of amplicon sequencing libraries for Illumina platforms, following a two-step PCR approach. The first PCR amplifies the target marker gene (e.g., 16S rRNA for bacteria, ITS for fungi) with gene-specific primers tailed with Illumina adapter overhangs. The second PCR attaches unique dual-index barcodes and the full Illumina adapter sequences, enabling multiplexed sequencing of many samples per run. This protocol is based on the Illumina 16S Metagenomic Sequencing Library Preparation guide and the Earth Microbiome Project (EMP) standards (Caporaso et al., 2012; Illumina, Inc., 2013; Thompson et al., 2017).

2. Scope

- **Applies to:** Preparation of amplicon libraries targeting 16S rRNA (V4 or V3–V4 region), ITS (ITS1 or ITS2 region), 18S rRNA, or other marker genes from environmental DNA extracts for paired-end sequencing on Illumina MiSeq or NextSeq platforms
- **Does not apply to:** Shotgun metagenomic library preparation; long- read amplicon sequencing (PacBio, Nanopore); ddPCR (see SOP-L701); eDNA metabarcoding using non-Illumina workflows
- **Location:** Molecular biology bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve target region, primer choice, and sequencing facility
Trained Personnel	Prepare libraries per this SOP; submit to sequencing facility
New Students	Complete training before independent use

4. Definitions

- **Adapter:** A short double-stranded DNA sequence ligated to both ends of a library fragment; contains the sequencing primer binding site, an index sequence, and the flow cell binding sequence required for Illumina cluster generation.
- **Amplicon library:** A sequencing library prepared by PCR amplification of a target region (e.g., 16S rRNA gene V4 region or ITS2) using primers that incorporate Illumina adapter sequences; represents the diversity of that locus in a community sample.
- **Index (barcode):** A unique 6–12 bp sequence incorporated into the adapter of each sample's library; allows multiple samples to be pooled and sequenced together (multiplexed) and computationally separated (demultiplexed) after sequencing.
- **Library normalization:** Adjustment of each sample's library to the same molar concentration before pooling; ensures approximately equal sequencing depth across samples; performed by quantification (Qubit or qPCR) followed by dilution.

- **Library pooling:** Combining equimolar amounts of individually indexed libraries from multiple samples into a single tube for multiplexed sequencing; the total library pool is loaded onto the sequencer.
- **Paired-end sequencing:** A sequencing strategy in which both ends of a DNA insert are sequenced, producing two reads (R1 and R2) per cluster; paired reads overlap for amplicons shorter than twice the read length, enabling error correction by merging.
- **PCR inhibitor:** A substance carried over from DNA extraction that suppresses amplification efficiency (e.g., humic acids, tannins, residual ethanol); detected by inhibition testing and remedied by further purification or dilution of the template.
- **Size selection:** A step in library preparation that removes off-target fragments (primer dimers, non-specific products) using bead-based cleanup (e.g., AMPure XP beads) or gel excision; improves sequencing quality and reduces wasted reads.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** AMPure XP beads (or equivalent SPRI beads) contain polyethylene glycol (PEG) and carboxylated magnetic particles; low toxicity but handle with gloves. Molecular-grade ethanol is flammable.
- **Biological:** Environmental DNA extracts may contain pathogen-derived DNA. Handle with standard precautions.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (changed frequently)

5.2. Emergency Procedures

Refer to SOP-L003 Hazardous Materials Handling.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Thermal cycler	Mol bio bench	For both PCR1 and PCR2
Magnetic stand (for 96-well plates or strip tubes)	Mol bio bench	For SPRI bead cleanup
Qubit 4 fluorometer	Instrument bench	For library quantification

Item	Location	Notes
NanoDrop One	Instrument bench	Optional purity check
Gel electrophoresis system	Mol bio bench	For amplicon verification; see SOP-L703
Multichannel pipette (optional)	Mol bio bench	For high-throughput plate work
Plate vortex mixer	Mol bio bench	For mixing bead suspensions
Microcentrifuge and plate spinner	Mol bio bench	For brief spin-downs

6.2. Reagents and Consumables

Item	Notes
High-fidelity DNA polymerase	e.g., KAPA HiFi HotStart (Roche), Q5 (NEB), or Phusion (Thermo Fisher); high-fidelity polymerase is required to minimize PCR-introduced errors (Schirmer et al., 2015)
Gene-specific primers with Illumina overhangs (PCR1)	See Section 6.1
Nextera XT Index Kit v2 (or equivalent dual-index primers)	For PCR2 barcoding
AMPure XP beads (Beckman Coulter) or equivalent SPRI beads	For size selection and cleanup
Molecular-grade ethanol (80%, freshly prepared)	For bead washes
Nuclease-free water	For elution and dilutions
Qubit dsDNA HS Assay Kit	For library quantification
Agarose and gel supplies	For amplicon verification
PCR plates or strip tubes (0.2 mL)	Low-bind preferred
Plate sealing film or strip caps	
Filter pipette tips	Barrier tips mandatory

7. Procedure

7.1. Primer Design (PCR1: Gene-Specific Amplification)

Primers for the first PCR consist of the gene-specific sequence plus the Illumina adapter overhang. The overhang sequences are:

- Forward overhang: `5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[gene-specific forward]-3'`
- Reverse overhang: `5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[gene-specific reverse]-3'`

Commonly used gene-specific primers:

Target	Primers	Region	Amplicon	Reference
16S bacteria/archaea	515F / 806R	V4	~290 bp	Caporaso et al. (2012); Parada et al. (2016)
16S bacteria	341F / 785R	V3–V4	~460 bp	Klindworth et al. (2013)
ITS fungi	ITS1f / ITS2	ITS1	~250–400 bp	Smith & Peay (2014); White et al. (1990)
ITS fungi	fITS7 / ITS4	ITS2	~250–400 bp	Ihrmark et al. (2012)
18S eukaryotes	Euk1391f / EukBr	V9	~130 bp	Amaral-Zettler et al. (2009)

> **Note**
>
> The EMP standard primers for 16S V4 are 515F (Parada) / 806R (Apprill), which include degeneracies for improved archaeal coverage (Apprill et al., 2015; Parada et al., 2016). Use these unless the project specifically requires a different region.

7.2. PCR1: Target Amplification

1. Prepare the master mix on ice. Use a high-fidelity polymerase.

Component	Volume per reaction	Final concentration
2x KAPA HiFi HotStart ReadyMix	12.5 uL	1x
Forward primer with overhang (10 uM)	1.0 uL	400 nM
Reverse primer with overhang (10 uM)	1.0 uL	400 nM
Nuclease-free water	8.5 uL	–
Template DNA (1–10 ng)	2.0 uL	Variable
Total	**25.0 uL**	

2. Include controls: positive control, NTC, and extraction blank.
3. Cycling parameters (adjust annealing temperature per primer set):

Step	Temperature	Time	Cycles
Initial denaturation	95 C	3 min	1
Denaturation	95 C	30 s	25–28
Annealing	55 C	30 s	25–28
Extension	72 C	30 s	25–28
Final extension	72 C	5 min	1
Hold	4 C	Hold	–

> **!** Important
>
> Limit PCR1 to 25–28 cycles to minimize chimera formation and PCR bias (Schirmer et al., 2015). More cycles increase the proportion of artifacts in the sequencing data.

4. Verify amplification by running 2 uL on a 1.5% agarose gel per SOP-L703.

7.3. PCR1 Cleanup (SPRI Beads)

1. Allow AMPure XP beads to reach room temperature (approximately 30 minutes). Vortex well before use.
2. Add 20 uL (0.8x ratio) of beads to each 25 uL PCR product. Mix by pipetting 10 times.
3. Incubate at room temperature for 5 minutes.
4. Place on the magnetic stand for 2 minutes until the solution clears.
5. Aspirate and discard the supernatant.
6. Wash twice with 200 uL of freshly prepared 80% ethanol. Do not disturb the bead pellet. Allow 30 seconds per wash.
7. Remove all ethanol and air-dry the beads for 3–5 minutes. Do not over-dry (beads will crack and yield drops).
8. Remove from the magnetic stand. Elute in 27.5 uL of nuclease-free water. Pipette to mix.
9. Return to the magnetic stand. Transfer 25 uL of the clear eluate to a new tube.

7.4. PCR2: Index PCR (Barcoding)

1. Assign a unique dual-index combination (i7 + i5) to each sample. Record the plate map in the lab notebook and in the sample sheet.
2. Set up the index PCR:

Component	Volume per reaction
2x KAPA HiFi HotStart ReadyMix	12.5 uL
Nextera XT Index Primer 1 (i7)	2.5 uL
Nextera XT Index Primer 2 (i5)	2.5 uL
Cleaned PCR1 product	2.5 uL
Nuclease-free water	5.0 uL
Total	**25.0 uL**

3. Cycling parameters:

Step	Temperature	Time	Cycles
Initial denaturation	95 C	3 min	1
Denaturation	95 C	30 s	8
Annealing	55 C	30 s	8

Step	Temperature	Time	Cycles
Extension	72 C	30 s	8
Final extension	72 C	5 min	1
Hold	4 C	Hold	–

4. Clean the indexed PCR product with AMPure XP beads (0.8x ratio) as in Section 6.3. Elute in 27.5 uL.

7.5. Library Quantification and QC

1. Quantify each library using the Qubit dsDNA HS Assay (SOP-L700, Section 6.4).
2. Verify library size by running 1 uL on a 1.5% agarose gel. The expected size is the amplicon plus approximately 120 bp of adapter and index sequence.
3. Libraries with concentrations below 1 nM may not sequence well. Re-amplify or exclude them.

7.6. Library Pooling and Submission

1. Normalize each library to an equimolar concentration (typically 4 nM) by diluting with nuclease-free water.
2. Pool equal volumes of all normalized libraries into a single tube.
3. Quantify the pooled library by Qubit.
4. Prepare the sample sheet (Illumina Experiment Manager format or the sequencing facility's template) listing every sample with its index combination.
5. Submit the pooled library and sample sheet to the sequencing facility per their submission guidelines.
6. Record the submission date, facility, run type (e.g., MiSeq 2x250 or 2x300), and expected turnaround in the lab notebook.

8. Quality Control

- Every library prep run must include: positive control (mock community, if available), NTC, and extraction blank carried through both PCR1 and PCR2
- NTC and extraction blanks must show no visible band on the verification gel and no detectable concentration on the Qubit. If blanks are positive, the entire batch is suspect (Salter et al., 2014).
- Verify amplicon size on gel after PCR1; verify library size (amplicon
  - adapters) on gel after PCR2
- Record the number of PCR1 cycles and the index assignments for every sample; these are required for data analysis and troubleshooting

9. Waste Disposal

Waste Type	Container	Disposal Method
Used SPRI beads	Standard trash	Non-hazardous
Ethanol wash waste	Chemical waste or evaporate in fume hood	Small volumes can evaporate
Used PCR tubes, plates, tips	Standard trash	Non-hazardous
Gel waste	Per SOP-L703	Depends on stain used

10. References

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11. Related SOPs

SOP	Title	Relationship
L700	DNA Extraction	Upstream: provides template DNA
L703	PCR and Gel Electrophoresis	Amplicon verification
L705	Bioinformatics Pipeline	Downstream: sequence processing
L706	eDNA Metabarcoding	eDNA-specific workflow
F701	Soil Microbial Sampling	Field collection

12. Training Record

Before preparing amplicon libraries independently, personnel must:

1. Read this SOP, SOP-L703, and SOP-L700 in full
2. Demonstrate SPRI bead cleanup with correct bead ratios and wash steps
3. Prepare a supervised library from positive control DNA through pooling, with clean controls and correct library size on gel
4. Complete the sequencing facility's sample submission training
5. Be signed off by the PI or lab manager



Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L705

Bioinformatics Pipeline (QIIME 2 / DADA2)

Standard Operating Procedure

SOP Number	SOP-L705
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the bioinformatics workflow for processing amplicon sequencing data (16S rRNA, ITS, 18S rRNA) from raw demultiplexed FASTQ files through quality filtering, denoising, taxonomic classification, and generation of analysis-ready feature tables. The pipeline uses QIIME 2 as the primary framework with DADA2 as the denoising algorithm (Bolyen et al., 2019; Callahan et al., 2016). DADA2 resolves amplicon sequence variants (ASVs) at single-nucleotide resolution, replacing the older OTU-clustering approach with a more reproducible and sensitive method (Callahan et al., 2017).

2. Scope

- **Applies to:** Processing of Illumina paired-end amplicon sequencing data (MiSeq, NextSeq) for 16S, ITS, and 18S marker genes generated from EA Program environmental samples
- **Does not apply to:** Shotgun metagenomics; long-read sequencing (PacBio, Nanopore); transcriptomics (RNA-seq); ddPCR data analysis (see SOP-L702)
- **Location:** EA Program lab computers or personal workstations with QIIME 2 installed; high-performance computing cluster if available

3. Responsibilities

Role	Responsibility
Lab PI	Approve pipeline parameters and taxonomy database
Trained Personnel	Run the pipeline per this SOP; document all parameters
New Students	Complete training before independent analysis

4. Definitions

- **ASV (Amplicon Sequence Variant):** An exact DNA sequence resolved from amplicon sequencing data by denoising algorithms (DADA2, Deblur); a higher-resolution alternative to OTUs that retains single-nucleotide differences between sequences.
- **DADA2:** A denoising algorithm that models sequencing error rates to distinguish true biological sequences from sequencing errors; produces ASVs directly from paired-end reads without clustering.
- **Demultiplexing:** The computational step of assigning reads to their source sample based on index sequences; produces per-sample FASTQ files from a multiplexed sequencing run.
- **Feature table (OTU/ASV table):** A matrix of counts where rows are samples, columns are ASVs or OTUs, and each cell contains the number of reads from that sample assigned to that sequence variant; the primary data object in QIIME 2.
- **FASTQ:** A text-based file format storing DNA sequences and their per-base quality scores (Phred scores); the standard output format from Illumina sequencers; paired-end data produces R1 and R2 FASTQ files per sample.
- **OTU (Operational Taxonomic Unit):** A cluster of sequences grouped by a similarity threshold (typically 97% identity); a coarser representation of diversity than ASVs; largely superseded by ASV-based approaches.
- **Phred quality score (Q score):** A logarithmic measure of base-call accuracy: $Q = -10 \log_{10}(P_{error})$; Q20 = 99% accuracy; Q30 = 99.9% accuracy; used to set quality-trimming thresholds in DADA2.
- **QIIME 2:** An open-source bioinformatics platform for microbiome analysis; manages data as typed artifacts (.qza) with embedded provenance records; wraps DADA2, taxonomic classifiers, diversity analyses, and visualization tools.
- **Rarefaction:** Subsampling each sample to the same number of reads to correct for unequal sequencing depth before diversity comparisons; samples below the rarefaction depth are excluded.

- **Taxonomic classifier:** A trained machine-learning model (e.g., naive Bayes classifier trained on SILVA or UNITE) that assigns taxonomy to ASV sequences by comparing them to a reference database.

5. Software Requirements

Software	Version (minimum)	Notes
QIIME 2	2024.2 or later	Install via conda/mamba; see https://docs.qiime2.org
DADA2 (QIIME 2 plugin)	Bundled with QIIME 2	R package also available for standalone use
R and RStudio	R 4.3+	For downstream statistical analysis; see SOP-L801
phyloseq (R package)	1.44+	For importing QIIME 2 artifacts into R (McMurdie & Holmes, 2013)
FastQC	0.12+	Optional; for initial read quality inspection

Install QIIME 2 in a dedicated conda environment to avoid dependency conflicts:

```
# Example installation (check docs.qiime2.org for current commands)
conda env create -n qiime2-2024.2 \
  --file https://data.qiime2.org/distro/amplicon/qiime2-amplicon-2024.2-py39-linux-conda.yml
conda activate qiime2-2024.2
```

6. Procedure

6.1. Data Organization

1. Create a project directory with the following structure:

```
project_name/
  raw_data/          # Demultiplexed FASTQ files from sequencing facility
  qiime2/            # QIIME 2 artifacts and visualizations
  results/           # Exported tables, trees, taxonomy
  scripts/           # Shell and R scripts
  metadata/          # Sample metadata TSV file
```

2. Prepare a sample metadata file (`metadata.tsv`) in tab-separated format. The first column must be `sample-id` matching the FASTQ filenames. Include all experimental variables (site, treatment, date, depth, replicate). Validate the file at https://keemei.qiime2.org/ (Rideout et al., 2014).

6.2. Import Raw Reads

Import demultiplexed paired-end FASTQ files into a QIIME 2 artifact:

```
qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path raw_data/ \
  --input-format CasavaOneEightSingleLanePerSampleDirFmt \
  --output-path qiime2/demux.qza
```

Visualize read quality:

```
qiime demux summarize \
  --i-data qiime2/demux.qza \
  --o-visualization qiime2/demux-summary.qzv
```

Open `demux-summary.qzv` at https://view.qiime2.org to inspect quality score profiles. Use these plots to determine truncation parameters for DADA2 (Section 5.3).

6.3. Denoising with DADA2

DADA2 performs quality filtering, chimera removal, and denoising in a single step, producing an ASV feature table and representative sequences (Callahan et al., 2016).

```
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs qiime2/demux.qza \
  --p-trunc-len-f 240 \
  --p-trunc-len-r 200 \
  --p-trim-left-f 0 \
  --p-trim-left-r 0 \
  --p-n-threads 4 \
  --o-table qiime2/table.qza \
  --o-representative-sequences qiime2/rep-seqs.qza \
  --o-denoising-stats qiime2/dada2-stats.qza
```

! Setting Truncation Parameters

Choose `--p-trunc-len-f` and `--p-trunc-len-r` based on the quality plots from Section 5.2. Truncate where the median quality score drops below 25–30. The forward and reverse reads must still overlap by at least 20 bp after truncation for successful merging. For 16S V4 (515F/806R, ~290 bp amplicon) with 2x250 reads, typical values are 240 (forward) and 200 (reverse).

Review denoising statistics:

```
qiime metadata tabulate \
  --m-input-file qiime2/dada2-stats.qza \
  --o-visualization qiime2/dada2-stats.qzv
```

Check the percentage of reads passing each filter step (input, filtered, denoised, merged, non-chimeric). A healthy run retains $> 60\%$ of input reads through the full pipeline.

6.4. Taxonomic Classification

Assign taxonomy to ASVs using a pre-trained Naive Bayes classifier. Download the appropriate classifier from the QIIME 2 Data Resources page (https://docs.qiime2.org/2024.2/data-resources/).

For 16S (SILVA 138.1):

```
qiime feature-classifier classify-sklearn \
  --i-classifier silva-138.1-ssu-nr99-515f-806r-classifier.qza \
  --i-reads qiime2/rep-seqs.qza \
  --o-classification qiime2/taxonomy.qza
```

For ITS (UNITE):

```
qiime feature-classifier classify-sklearn \
  --i-classifier unite-ver9-dynamic-all-29.11.2022-classifier.qza \
  --i-reads qiime2/rep-seqs.qza \
  --o-classification qiime2/taxonomy.qza
```

Visualize taxonomy:

```
qiime taxa barplot \
  --i-table qiime2/table.qza \
  --i-taxonomy qiime2/taxonomy.qza \
  --m-metadata-file metadata/metadata.tsv \
  --o-visualization qiime2/taxa-barplot.qzv
```

6.5. Phylogenetic Tree Construction

Build a rooted phylogenetic tree for diversity analyses:

```
qiime phylogeny align-to-tree-mafft-fasttree \
  --i-sequences qiime2/rep-seqs.qza \
  --o-alignment qiime2/aligned-rep-seqs.qza \
  --o-masked-alignment qiime2/masked-aligned-rep-seqs.qza \
  --o-tree qiime2/unrooted-tree.qza \
  --o-rooted-tree qiime2/rooted-tree.qza
```

6.6. Filtering

Remove contaminant ASVs identified in negative controls, mitochondrial and chloroplast sequences, and low-frequency ASVs:

```
# Remove mitochondria and chloroplast
qiime taxa filter-table \
  --i-table qiime2/table.qza \
  --i-taxonomy qiime2/taxonomy.qza \
  --p-exclude mitochondria,chloroplast \
  --o-filtered-table qiime2/table-filtered.qza

# Remove low-frequency ASVs (e.g., singletons)
qiime feature-table filter-features \
  --i-table qiime2/table-filtered.qza \
  --p-min-frequency 2 \
  --o-filtered-table qiime2/table-final.qza
```

For decontamination using negative control data, export the feature table to R and use the `decontam` package (Davis et al., 2018).

6.7. Export for R Analysis

Export QIIME 2 artifacts for downstream analysis in R with phyloseq (McMurdie & Holmes, 2013):

```
qiime tools export \
  --input-path qiime2/table-final.qza \
  --output-path results/

qiime tools export \
  --input-path qiime2/taxonomy.qza \
  --output-path results/

qiime tools export \
  --input-path qiime2/rooted-tree.qza \
  --output-path results/
```

Alternatively, import QIIME 2 artifacts directly into R using the `qiime2R` package.

7. Quality Control

- Inspect the DADA2 denoising stats for every run. Flag samples with fewer than 1,000 reads after denoising.
- Verify that negative controls (NTC, extraction blank) contain fewer than 100 reads. If controls contain substantial reads, use `decontam` to identify and remove contaminant ASVs (Davis et al., 2018; Salter et al., 2014).

- Confirm that the positive control (mock community) recovers the expected taxa at the expected relative abundances.
- Document all QIIME 2 and DADA2 parameters in the project README and in the analysis scripts. Reproducibility requires recording the exact software versions, classifier version, and truncation lengths.
- Archive raw FASTQ files, the QIIME 2 artifacts, and the analysis scripts in the project GitHub repository per SOP-L801.

8. References

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9. Related SOPs

SOP	Title	Relationship
L700	DNA Extraction	Upstream: sample preparation
L703	PCR and Gel Electrophoresis	Amplicon verification
L704	Amplicon Library Prep	Upstream: library preparation
L706	eDNA Metabarcoding	eDNA-specific application
L801	RStudio and GitHub	Data management and version control
L802	Quality Control and QA	General QA/QC framework

10. Training Record

Before running the bioinformatics pipeline independently, personnel must:

1. Read this SOP in full
2. Complete the QIIME 2 "Moving Pictures" tutorial (https://docs.qiime2.org/2024.2/tutorials/moving-pictures/)
3. Process a test dataset under supervision, from import through taxonomy assignment and export to R
4. Demonstrate correct interpretation of DADA2 denoising statistics and quality plots
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L706

eDNA Metabarcoding Lab Workflow

Standard Operating Procedure

SOP Number	SOP-L706
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the end-to-end laboratory workflow for environmental DNA (eDNA) metabarcoding, from receipt of field-collected water filter samples through DNA extraction, PCR amplification, library preparation, sequencing, and bioinformatics analysis. eDNA metabarcoding combines

universal or group-specific primers with high-throughput sequencing to detect and characterize aquatic biodiversity from water samples without capturing organisms (Deiner et al., 2017; Taberlet et al., 2012).

This SOP serves as the integrating document that ties together the field collection procedures in SOP-F700 with the molecular and computational SOPs in the L700 series. It does not repeat detailed procedures described in other SOPs but provides the eDNA-specific modifications, QC requirements, and decision points that distinguish eDNA metabarcoding from standard amplicon sequencing of soil or tissue samples.

2. Scope

- **Applies to:** Laboratory processing of eDNA water samples for multi-species detection via amplicon sequencing on Illumina platforms; target taxa include fish (12S), amphibians (16S/12S), macroinvertebrates (COI), and general biodiversity surveys (18S, COI)
- **Does not apply to:** Species-specific eDNA detection by qPCR or ddPCR (single-species assays); soil microbial community analysis (see SOP-L704 and SOP-L705); field collection procedures (see SOP-F700)
- **Location:** Molecular biology bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve target markers, primers, and sequencing plan
Trained Personnel	Process samples per this SOP; maintain contamination controls
New Students	Complete eDNA-specific contamination training before participating

4. Definitions

- **Amplification blank (no-template control, NTC):** A PCR reaction containing all reagents except sample DNA; run with every PCR plate; any amplification in the NTC indicates contamination and invalidates the entire plate.
- **Blocking primer:** A modified oligonucleotide added to a PCR reaction to selectively suppress amplification of abundant non-target DNA (e.g., host DNA in gut or tissue eDNA samples); allows rare target sequences to be amplified competitively.
- **eDNA metabarcoding:** A method that uses universal or group-specific primers to amplify and sequence a short, taxonomically informative DNA region (barcode) from environmental DNA, yielding a community- level inventory of taxa present in a sample.
- **Environmental DNA (eDNA):** Genetic material shed into the environment by organisms through mucus, feces, skin cells, eggs, and other biological material; collected non-invasively from water, sediment, or air.

- **Extraction blank:** A tube containing only extraction reagents and no biological material, processed through the full extraction procedure; detects reagent or handling contamination; must yield no target amplification.
- **Field blank:** A filter or collection vessel processed through all field collection steps but with no environmental sample; detects airborne or handling contamination during field work.
- **Metabarcode locus:** The specific genomic region amplified for community identification; common loci include 12S rRNA (fish), 16S rRNA (bacteria, invertebrates), 18S rRNA (eukaryotes), COI (invertebrates, fish), and ITS2 (fungi, plants).
- **Occupancy model:** A statistical model that accounts for imperfect detection in eDNA surveys by estimating the probability that a species is truly present given detection/non-detection across replicate samples or PCR replicates.
- **PCR replicate:** One of multiple independent PCR reactions run from the same DNA extract; increases detection sensitivity for rare targets and allows estimation of false-negative rates; standard eDNA practice is 3–8 replicates per sample.
- **Sequence read depth (coverage):** The total number of sequencing reads assigned to a sample; directly affects the number of rare taxa detected; EA Program target is a minimum of 10,000 reads per sample after quality filtering.

5. Safety and Hazards

⚠ Hazards

- **Chemical:** Lysis buffers (guanidine salts), ethanol, SPRI beads, and DNA stains. See individual SOP safety sections for details.
- **Biological:** eDNA samples may originate from water bodies containing pathogens. Handle with standard precautions.
- **UV radiation:** If using UV transilluminator for gel imaging.

5.1. Required PPE

☐ Safety glasses
☐ Lab coat
☐ Nitrile gloves (changed frequently; mandatory at all steps)

5.2. Emergency Procedures

Refer to SOP-L003 Hazardous Materials Handling.

6. Workflow Overview

The eDNA metabarcoding workflow consists of six stages. Each stage references a detailed SOP.

Stage	Description	SOP Reference
1	Field collection and preservation	F700
2	DNA extraction from filters	L700
3	PCR amplification and verification	L703
4	Library preparation and sequencing	L704
5	Bioinformatics (QIIME 2 / DADA2)	L705
6	Taxonomic interpretation and reporting	This SOP, Section 6.6

7. Procedure: eDNA-Specific Modifications

7.1. Sample Receipt and Logging

1. Upon receipt from the field, verify that all samples match the chain of custody form per SOP-F008.
2. Inspect Sterivex capsules or filter bags for integrity (no leaks, caps intact, preservative present).
3. Log each sample in the lab notebook and Freezer Inventory Google Sheet.
4. Store preserved filters at -20 C (short-term) or -80 C (long-term) until extraction.

7.2. DNA Extraction from Filters

Follow SOP-L700 with the following eDNA-specific modifications:

- **Sterivex capsules:** Open the capsule aseptically using a sterile razor blade or the Sterivex extraction adapter. Transfer the membrane into the PowerBead Pro tube using sterile forceps. If Longmire's buffer was used as preservative, add the buffer to the bead tube as well.
- **Open membrane filters:** Transfer the folded filter directly into the bead tube using sterile forceps.
- **Extraction blanks:** Process one extraction blank per batch. For eDNA, this means running an empty bead tube through the full protocol.
- **Field blanks:** The field blank filter (from SOP-F700) is extracted alongside samples and serves as the primary contamination control for the entire field-to-lab chain.

7.3. Inhibition Testing

eDNA extracts from natural waters frequently contain PCR inhibitors (humic acids, tannins, metal ions) (McKee et al., 2015). Test for inhibition before committing to full library preparation:

1. Run an internal positive control (IPC) qPCR assay on a subset of extracts (or all extracts, if the batch is small). The IPC uses a synthetic DNA target spiked into the PCR reaction at a known concentration.
2. If the IPC C_t value shifts by more than 2 cycles compared to the uninhibited control, the extract is inhibited.
3. For inhibited samples, dilute the extract 1:5 or 1:10 with nuclease-free water and retest. Alternatively, clean the extract using a OneStep PCR Inhibitor Removal Kit (Zymo Research).

7.4. Primer Selection for eDNA Metabarcoding

Target Taxa	Marker	Primers	Amplicon	Reference
Fish	12S rRNA	MiFish-U-F / MiFish-U-R	~170 bp	Miya et al. (2015)
Amphibians	16S rRNA	16Smam / 16Smam-R (modified)	~250 bp	Vences et al. (2005)
Macroinvertebrates	COI	mlCOIintF / jgHCO2198	~313 bp	Leray et al. (2013)
General eukaryotes	18S rRNA	Euk1391f / EukBr	~130 bp	Amaral-Zettler et al. (2009)
General eukaryotes	COI	fwhF2 / fwhR2n	~205 bp	Vamos et al. (2022)

> **Note**
>
> Primer choice determines which taxa can be detected. Discuss target taxa and primer selection with the PI before beginning library preparation. Multi-marker approaches (e.g., 12S + COI) provide broader taxonomic coverage but require separate library preparations and sequencing runs (Deiner et al., 2017).

7.5. PCR and Library Preparation

Follow SOP-L703 for initial PCR screening and SOP-L704 for library preparation, with these eDNA-specific considerations:

- **Replicates:** Run 3 independent PCR replicates per sample to improve detection probability for rare species (Ficetola et al., 2015). Pool the replicates after PCR1 before cleanup and indexing.
- **Cycle number:** Use 35 cycles for PCR1 (higher than standard 16S amplicon work) because eDNA concentrations are typically very low. Be aware that additional cycles increase the risk of chimera formation and tag-jumping (Schnell et al., 2015).
- **Blocking primers (optional):** If non-target taxa (e.g., human DNA) dominate the PCR product, consider using blocking primers or PNA (peptide nucleic acid) clamps to suppress amplification of non-target sequences.
- **Controls:** In addition to the standard NTC and extraction blank, include the field blank and (if available) a positive control consisting of a mock community of known species at known concentrations.

7.6. Bioinformatics and Taxonomic Assignment

Follow SOP-L705 for denoising and taxonomic classification, with these modifications:

- **Reference database:** Use a curated database matched to the marker and target taxa:
  - 12S fish: MitoFish or MIDORI2

  - COI: BOLD / MIDORI2
  - 16S/18S: SILVA or GenBank nt

- **Species-level assignment:** For eDNA, species-level identification is the primary goal (unlike microbiome studies where genus-level is often sufficient). Use BLAST-based classification (`classify-consensus- blast` in QIIME 2) in addition to or instead of the Naive Bayes classifier for improved species-level resolution (Goldberg et al., 2016).
- **Decontamination:** Use the `decontam` R package to identify and remove contaminant ASVs using the field blank and extraction blank data (Davis et al., 2018).
- **Detection criteria:** An ASV is considered a positive detection for a species only if:

  1. It matches a reference sequence at greater than or equal to 97–99% identity (marker-dependent)
  2. It is absent from all negative controls (field blank, extraction blank, NTC), or its abundance in the sample exceeds 10x the abundance in the highest-read control
  3. It is detected in at least 2 of 3 PCR replicates (if replicates were sequenced independently) (Ficetola et al., 2015)

7.7. Reporting

eDNA metabarcoding results are reported as a species detection list per site with the following metadata:

- ASV sequence and percent identity to reference
- Number of reads per ASV per sample
- Detection across replicates (e.g., detected in 2/3 replicates)
- Negative control results
- Any taxa flagged as potential contaminants

> **!** Important
>
> eDNA metabarcoding provides presence/detection data. Quantitative interpretation of read counts as species abundance is not recommended without careful validation, because PCR amplification bias, primer affinity, and DNA shedding rates vary among taxa (Kelly et al., 2019).

8. Quality Control

- **Field blanks:** Must be negative for all target taxa. Any target taxon detected in a field blank compromises the detection claim for that taxon across all samples in the same sampling event.
- **Extraction blanks:** Must be negative. Positive extraction blanks indicate lab contamination; the entire extraction batch is suspect.
- **NTC:** Must be negative. Positive NTCs indicate PCR contamination.
- **Positive control (mock community):** Must recover all expected species. Failure to detect a known species in the mock community indicates primer bias or PCR failure.

- **Replication:** A minimum of 3 PCR replicates per sample is recommended for occupancy-based detection analyses.
- Document all control results in the project data file and report alongside species detections.

9. Waste Disposal

Refer to waste disposal tables in SOP-L700, SOP-L703, and SOP-L704.

10. References

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11. Related SOPs

SOP	Title	Relationship
F700	eDNA Field Sampling	Upstream: field collection
F008	Sample Labeling and COC	Chain of custody
L700	DNA Extraction	Stage 2: extraction
L703	PCR and Gel Electrophoresis	Stage 3: amplification
L704	Amplicon Library Prep	Stage 4: library prep
L705	Bioinformatics Pipeline	Stage 5: sequence analysis
L801	RStudio and GitHub	Data management

12. Training Record

Before processing eDNA samples independently, personnel must:

1. Read this SOP and all related SOPs (F700, L700, L703, L704, L705)
2. Demonstrate aseptic filter handling and extraction under supervision
3. Complete an eDNA-specific contamination awareness session covering pre-PCR/post-PCR separation, filter handling, and control interpretation
4. Process a supervised batch including field blank, extraction blank, and positive control with clean results
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L801

RStudio Projects and GitHub

Standard Operating Procedure

SOP Number	SOP-L801
Revision	1.07
Author(s)	Marc Los Huertos, Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP provides instructions for setting up RStudio, connecting it to GitHub, and managing collaborative research projects using version control. R (R Core Team, 2024), RStudio (Posit PBC, 2024), and GitHub (GitHub Inc., 2024) together enable collaborative, transparent, reproducible, and accessible data analysis. For a comprehensive practical guide, see (Bryan, 2023).

2. Scope

- **Applies to:** All EA Program researchers using R for data analysis and collaborative projects
- **Does not apply to:** Local-only R scripts with no version control; Python or other language workflows
- **Location:** RStudio Server at Pomona College; local installations

3. Acknowledgements

Substantial contributions: Aparna Chintapalli and Isaac Medina. We acknowledge all students who have followed these instructions and suggested improvements.

4. Definitions

- **R:** A free, open-source programming environment for statistical computing, maintained by a global community of statisticians and programmers (R Core Team, 2024).
- **RStudio:** A user interface for R that provides tools to write code, view results, manage files, and integrate with version control. Available as a desktop application or via the Pomona College server (Posit PBC, 2024).
- **GitHub:** A web-based hosting service for Git repositories that enables collaborative code and document management (GitHub Inc., 2024).
- **Repository (repo):** A GitHub project folder that contains all files, history, and collaboration settings.
- **Version control:** A system that tracks changes to files over time, allowing multiple people to collaborate while maintaining a complete history of who changed what and when.
- **SSH key:** A cryptographic key pair used to establish a secure connection between RStudio and GitHub without entering a password each time.
- **Clone:** Creating a local copy of a GitHub repository on your computer or server.
- **Branch:** A parallel line of development that lets you work on a feature or fix without affecting the main codebase until you are ready to merge.
- **Pull:** Downloading the latest changes from GitHub to your local copy.
- **Commit:** Saving a snapshot of your changes with a descriptive message.
- **Push:** Uploading committed changes from your local copy to GitHub.
- **Pull request (PR):** A request to merge changes from one branch into another; the standard review mechanism for collaborative repositories.

5. Safety and Hazards

This is a computational procedure with no physical hazards.

6. Accessing RStudio

6.1. Pomona College RStudio Server

The recommended method for EA Program work:

1. Open a browser and go to `rstudio.pomona.edu`
2. Log in with your Pomona College credentials
3. Your files are stored on the server (no local installation needed)

6.2. Local Installation

Install in this order if working offline:

1. R: cran.r-project.org
2. RStudio Desktop: posit.co/download/rstudio-desktop
3. Git: git-scm.com

7. Setting Up GitHub

7.1. Create a GitHub Account

1. Go to github.com and create a free account
2. Use your Pomona email address
3. Choose a professional username (e.g., `firstlast` or `flastname`)

7.2. Configure SSH Keys

SSH keys let RStudio communicate with GitHub securely. Set them up once per machine (or server account).

1. In RStudio, open the Terminal tab
2. Generate a key pair:

```
ssh-keygen -t ed25519 -C "your_pomona_email@pomona.edu"
```

3. Accept the default file location; leave the passphrase blank for server use
4. Print the public key:

```
cat ~/.ssh/id_ed25519.pub
```

5. Copy the output
6. On GitHub: Settings > SSH and GPG keys > New SSH key > paste and save
7. Test the connection:

   ```
   ssh -T git@github.com
   ```

   A message beginning "Hi username!" confirms success.

8. Working with Repositories

8.1. Clone an Existing Repository

1. On GitHub, navigate to the repository and click **Code > SSH**
2. Copy the SSH URL (`git@github.com:org/repo.git`)
3. In RStudio: File > New Project > Version Control > Git
4. Paste the URL; choose a local directory; click **Create Project**

8.2. The Pull-Edit-Commit-Push Workflow

Always pull before editing to avoid merge conflicts:

1. **Pull:** Click the blue down-arrow in the Git pane (or `git pull`)
2. **Edit:** Make your changes in R scripts or `.qmd` files
3. **Stage:** Check boxes next to changed files in the Git pane
4. **Commit:** Click Commit; write a clear message (e.g., `"Add soil texture analysis for sites 4-6"`)
5. **Pull again:** Pull once more before pushing to catch any concurrent changes
6. **Push:** Click the green up-arrow to push to GitHub

8.3. Branching Workflow

For experimental analyses or features that are not yet ready for the main branch:

```
git checkout -b feature/my-analysis   # create and switch to new branch
# ... make changes and commits ...
git push -u origin feature/my-analysis
# Open a Pull Request on GitHub when ready to merge
```

9. Writing Quarto Documents

9.1. Rmd vs. Qmd

Feature	Rmd	Qmd
Maintained by	RStudio/Posit	Quarto project (open source)
Requires R	Yes	No (also supports Python, Julia)
Chunk options	In header: `{r, echo=TRUE}`	On lines: `#| echo: true`
SOP system uses	Legacy	Preferred for new documents

For new EA Program documents, use `.qmd`. Existing `.Rmd` files will continue to work.

Common Chunk Options

- `#| echo: true` shows the code in the output (use for teaching)
- `#| echo: false` hides the code (use for reports)
- `#| eval: false` shows the code but does not run it
- `#| fig-cap: "Caption text"` adds a figure caption
- `#| warning: false` suppresses warning messages

10. Recommended Resources

- RStudio YouTube Introduction
- GitHub YouTube Introduction
- Understanding the GitHub Flow
- Happy Git and GitHub for the useR: (Bryan, 2023) – the most comprehensive practical guide
- Quarto Documentation

11. Advanced Topics

11.1. Protecting the Main Branch

For repositories with multiple active contributors, enable branch protection rules on GitHub:

1. Go to Settings > Branches > Add rule
2. Enter `main` as the branch name pattern
3. Check **Require a pull request before merging**
4. Optionally check **Require approvals** (set to 1)

11.2. GitHub Issues for Task Tracking

Create an issue for each task, assign it to a team member, and close it when the work is merged. This creates a record of who did what and when.

11.3. .gitignore

Add file patterns that should never be committed:

```
.Rhistory
.RData
.Rproj.user/
docs/
_freeze/
*.pdf
```

12. References

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R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

13. Training Record

Before using R/RStudio/GitHub independently for EA Program research, personnel must:

1. Read this SOP in full
2. Create a GitHub account and configure SSH keys (Sections 11.1–11.3)
3. Successfully clone, pull, edit, commit, and push to the `beginnersluck` practice repository (Sections 12–13)
4. Create and render at least one `.qmd` file (Section 17)
5. Demonstrate the Pull-Edit-Commit-Push workflow under supervision
6. Be signed off by the PI or project lead

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L802

Quality Control and Quality Assurance

Standard Operating Procedure

SOP Number	SOP-L802
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP establishes the overarching quality assurance (QA) and quality control (QC) framework for all analytical work in the EA Program laboratory. It defines the minimum QC requirements that apply across methods, describes how to calculate and interpret QC metrics, specifies detection limit procedures, and establishes the data documentation and reproducibility practices required for every analysis. Individual analytical SOPs reference this document for general QC principles and add method-specific requirements (APHA et al., 2012; U.S. Environmental Protection Agency, 2007).

Quality assurance is the system of management actions that ensures the analytical process produces results of known and documented quality. Quality control is the set of technical activities (blanks, standards, duplicates, spikes) embedded in each analytical batch to measure and control that quality in real time (Taylor, 1987).

2. Scope

- **Applies to:** All quantitative chemical, physical, and biological analyses performed in the EA Program laboratory, including those described in SOPs L002 through L999 and analyses of field-collected samples (SOPs F001 through F999)
- **Does not apply to:** Qualitative screening tests (e.g., visual soil texture by feel, SOP-F401) unless those tests include a quantitative component
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI (QA Manager)	Approve this SOP; review QC data quarterly; authorize corrective actions
Lab Manager	Maintain instrument logs, reagent inventory, and QC records; train new analysts
Analyst	Implement all QC requirements for each batch; flag QC failures; document corrective actions
Data Reviewer	Verify QC compliance before data are reported or published

4. Definitions

- **Accuracy:** The closeness of a measured value to the true or accepted reference value; assessed using certified reference materials (CRMs) and laboratory-fortified blanks (LFBs) (Taylor, 1987).
- **Calibration blank:** A solution containing only the analytical solvent or reagent matrix (no analyte); used to establish the instrument baseline (zero) for each calibration sequence.

- **Certified reference material (CRM):** A material with certified analyte concentrations traceable to a national or international metrology institute (NIST, USGS, IRMM); used to verify analytical accuracy across instruments and over time (APHA et al., 2012).
- **Control chart (Shewhart chart):** A time-series plot of QC sample results with control limits (typically ±2 warning limits and ±3 action limits); used to distinguish random analytical variation from systematic drift or instrument malfunction.
- **Continuing calibration verification (CVS):** A mid-range calibration standard analyzed at regular intervals (typically every 10 samples) during an analytical run; must recover within ±10% of the stated value; failure requires re-calibration and re-analysis of affected samples (APHA et al., 2012).
- **Detection limit – Method Detection Limit (MDL):** The minimum concentration of an analyte that can be detected with 99% confidence under stated method conditions; determined from at least 7 replicate analyses of a low-level spike following 40 CFR Part 136 Appendix B (U.S. Environmental Protection Agency, 2016).
- **Detection limit – Limit of Blank (LOB):** The highest signal expected from a blank sample; $\text{LOB} = \text{mean}_{\text{blank}} + 1.645 \times \text{SD}_{\text{blank}}$ (Armbruster & Pry, 2008).
- **Detection limit – Limit of Detection (LOD):** The lowest analyte concentration that can be reliably distinguished from the LOB; $\text{LOD} = \text{LOB} + 1.645 \times \text{SD}_{\text{low-concentration}}$; represents 95% detection probability (Armbruster & Pry, 2008).
- **Detection limit – Limit of Quantitation (LOQ):** The lowest concentration at which the analyte can be measured with acceptable precision and accuracy, typically defined as 10 × SD at the LOD spike concentration; also known as the practical quantitation limit (PQL) (Armbruster & Pry, 2008).
- **Field blank:** A sample of reagent-grade water processed through the entire field collection and preservation procedure; detects contamination introduced during sampling and transport.
- **Laboratory-fortified blank (LFB):** A reagent water blank spiked with a known concentration of target analytes and processed through the full analytical procedure; monitors ongoing accuracy and extraction efficiency.
- **Laboratory-fortified matrix (LFM):** A field sample spiked with a known concentration of target analytes; detects matrix-specific suppression or enhancement of the analytical signal.
- **Method blank:** A reagent water sample carried through the full analytical procedure alongside field samples; detects contamination introduced by reagents, equipment, or laboratory environment (APHA et al., 2012).
- **Precision:** The reproducibility of repeated measurements of the same sample; expressed as relative percent difference (RPD) for duplicates or relative standard deviation (RSD) for replicate analyses (Taylor, 1987).
- **Relative percent difference (RPD):** $|A - B| / ((A + B) / 2) \times 100$; the standard measure of duplicate precision; typical acceptance criteria are RPD 10–20% depending on method and matrix.
- **Spike recovery (%):** (Measured spiked concentration – Unspiked concentration) / Added concentration × 100; typical acceptance range is 80–120% for most environmental matrices (APHA et al., 2012).

5. Safety and Hazards

QC samples contain the same analytes as field samples and are subject to the same hazard considerations as the parent analytical method. Consult the relevant analytical SOP for safety information.

6. General QC Requirements

6.1. Every Analytical Batch

Every analytical batch must include at minimum (APHA et al., 2012; U.S. Environmental Protection Agency, 2007):

QC Element	Minimum Frequency	Purpose
Method blank	1 per batch	Detect laboratory contamination
Calibration blank	Start of each calibration	Instrument baseline
CVS (check standard)	Every 10 samples	Detect calibration drift
Duplicate	10% of samples	Assess precision
LFB	1 per batch	Assess accuracy

6.2. Additional Elements (as applicable)

QC Element	When Required
Field blank	Each sampling event
Matrix spike (LFM)	5% of samples or 1 per batch
CRM	1 per batch (where certified material is available)
Equipment blank	When non-dedicated sampling equipment is used

7. Acceptance Criteria

QC Element	Typical Acceptance Criteria	Action if Failed
Method blank	$<$ MDL (analyte absent)	Investigate contamination; do not report affected samples
CVS	$\pm$10% of stated value	Re-calibrate; re-analyze affected samples
Duplicate RPD	10–20% (method-specific)	Flag results; investigate and re-analyze if warranted
LFB recovery	80–120%	Flag results; check reagents and instrument

QC Element	Typical Acceptance Criteria	Action if Failed
LFM recovery	70–130%	Suspect matrix interference; consult PI
CRM recovery	Within certified uncertainty interval	Recalibrate; re-analyze

Acceptance criteria in individual analytical SOPs take precedence over these general values when they are stricter or when regulatory requirements apply.

8. Detection Limit Determination

8.1. Method Detection Limit (MDL)

The MDL is determined following 40 CFR Part 136 Appendix B (U.S. Environmental Protection Agency, 2016):

1. Select a spike concentration at 1–5× the estimated MDL.
2. Prepare and analyze at least 7 replicates of the spiked solution through the full analytical method.
3. Calculate the standard deviation (s) of the replicates.
4. Multiply by the one-tailed t-value at 99% confidence ($t_{n-1,0.99}$; for $n = 7$: $t = 3.143$):

$$\text{MDL} = t_{(n-1,\,0.99)} \times s$$

5. If the spike concentration is outside the range of 0.5–20× the computed MDL, repeat with an adjusted spike concentration (U.S. Environmental Protection Agency, 2016).

8.2. Limit of Detection and Limit of Quantitation

The LOB, LOD, and LOQ hierarchy follows Armbruster & Pry (2008):

$$\text{LOB} = \mu_{\text{blank}} + 1.645\,\sigma_{\text{blank}}$$

$$\text{LOD} = \text{LOB} + 1.645\,\sigma_{\text{low}}$$

$$\text{LOQ} = 10\,\sigma_{\text{low}}$$

where σ_{low} is the standard deviation of replicate analyses at the low-level spike concentration. For right-censored environmental data, apply the non-parametric methods described in Helsel (2012).

9. Control Charts

Maintain control charts for each analyte/method combination to track long-term analytical performance:

1. Plot CRM, LFB, or CVS recovery for each batch on a time-series chart.
2. Calculate and draw lines for:
   - **Center line:** Mean recovery over the last 20+ batches
   - **Warning limits:** ±2 standard deviations from the mean
   - **Action limits:** ±3 standard deviations from the mean
3. A result between warning and action limits is a warning; investigate but do not necessarily re-analyze.
4. A result beyond the action limits requires corrective action and re-analysis.
5. Systematic trends (7+ consecutive results on one side of the center line, or a steady drift) require investigation even if individual points are within limits (Taylor, 1987).

10. Corrective Actions

When a QC failure occurs:

1. Stop the analytical run; do not report affected samples.
2. Identify the probable cause (contaminated blank, degraded standard, instrument malfunction, matrix interference).
3. Correct the problem (replace reagents, recalibrate, perform maintenance).
4. Re-run the affected samples with fresh QC elements.
5. Verify that the repeated run passes all QC criteria.
6. Document the failure, root cause, corrective action, and re-analysis results in the QC log and lab notebook.

11. Metadata Documentation and Reproducibility

11.1. Laboratory Notebook Requirements

Every analytical session must be documented with at minimum:

- Date, analyst name, SOP number and revision
- Instrument ID and calibration status
- Reagent lot numbers and preparation dates
- Sample IDs, matrix type, and collection dates
- Calibration data (standards, equation, r^2)
- QC results (blanks, duplicates, LFBs, spikes, CRMs) with pass/fail
- Raw data file names and storage location
- Any deviations from the SOP, with justification
- Corrective actions taken for QC failures

11.2. Electronic Data Management

1. Store all raw instrument data files on the lab shared drive in a folder structure organized by project, year, and instrument.
2. Never modify raw data files. All processing must be done programmatically in R with version-controlled scripts.
3. Commit all analysis scripts to the lab GitHub repository per SOP-L801 (Gandrud, 2015; Xie, 2015).

11.3. R-Based QC Tracking System

The following R framework is the EA Program standard for QC documentation and reproducibility (Gandrud, 2015; Xie, 2015):

```r
library(tidyverse)
library(lubridate)
library(knitr)

session_meta <- list(
  analyst    = "Your Name",
  date       = Sys.Date(),
  sop        = "SOP-L802 v1.1",
  method_sop = "SOP-LXX vX.X",
  instrument = "Instrument ID",
  project    = "Project Name"
)

cat(sprintf("QC session: %s | Analyst: %s | Instrument: %s\n",
            session_meta$date, session_meta$analyst,
            session_meta$instrument))
```

```r
calc_mdl <- function(reps, alpha = 0.01) {
  n <- length(reps); s <- sd(reps)
  t_val <- qt(1 - alpha, df = n - 1)
  list(n = n, sd = s, t_value = round(t_val, 3),
       MDL = round(t_val * s, 4), LOQ = round(10 * s, 4))
}

calc_rpd <- function(a, b) abs(a - b) / ((a + b) / 2) * 100

calc_recovery <- function(measured, expected) (measured / expected) * 100
```

Each analytical R script should:

1. Read the raw data file
2. Run all QC checks programmatically

3. Produce a formatted QC summary table
4. Generate calibration plots and control charts
5. Export a clean, QC-verified dataset as CSV
6. Render to HTML or PDF for archiving

Store templates in the project GitHub repository under `analysis/templates/` (Gandrud, 2015; Xie, 2015).

12. QC Requirements by Method Category

QC Element	Water Chemistry	Soil Extraction	Digestion (Metals)	Biological (Colilert)	Instrumental (TOC, ICP)
Calibration blank	Each cal	Each cal	Each cal	N/A	Each cal
Method blank	1/batch	1/batch	1/batch	1/batch	1/batch
Field blank	1/event	N/A	N/A	N/A	N/A
Duplicate	10%	10%	10%	10%	10%
LFB	1/batch	1/batch	1/batch	N/A	1/batch
Matrix spike	5% (or 1/batch)	5%	5%	N/A	5%
CRM	1/batch (if avail.)	1/batch	1/batch	Positive control	1/batch
CVS	Every 10 samples	Every 10	Every 10	N/A	Every 10

13. Waste Disposal

QC samples generate the same waste streams as routine samples. Dispose of QC waste (blanks, standards, spikes) according to the parent method's waste disposal procedure.

14. References

- APHA et al. (2012)
- Taylor (1987)
- U.S. Environmental Protection Agency (2007)
- U.S. Environmental Protection Agency (2016)
- Armbruster & Pry (2008)
- Helsel (2012)
- Gandrud (2015)
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15. Related SOPs

SOP	Title	Relationship
L801	RStudio and GitHub	Version control for analysis scripts
L803	Data Entry and Database	Data recording standards
F300	Surface Water Sampling	Field blank requirements
F008	Sample Labeling and COC	Chain of custody

16. Training Record

Before applying QC frameworks independently, personnel must:

1. Read this SOP in full
2. Demonstrate correct MDL calculation using the calc_mdl() function on a test dataset
3. Prepare a complete QC summary table for one analytical batch under supervision
4. Set up and interpret a control chart for one analyte/method combination
5. Be signed off by the PI or lab manager

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L803

Data Entry and Database Management

Standard Operating Procedure

SOP Number	SOP-L803
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes standardized procedures for entering, organizing, validating, and managing research data in the EA Program. It covers the full data lifecycle from initial recording through archival storage, ensuring that all datasets are consistent, traceable, and reproducible (Broman & Woo, 2018; Wickham, 2014).

2. Scope

- **Applies to:** All tabular data generated in the EA Program laboratory and field campaigns, including instrument outputs, field observations, and manually recorded measurements
- **Does not apply to:** Spatial/GIS data (see SOP-L804), bioinformatics sequence data (see SOP-L705), or instrument-specific export procedures (see SOP-L807)
- **Location:** EA Program laboratory (SGM 133) and RStudio Server

3. Responsibilities

Role	Responsibility
Lab PI	Approve data management plan; ensure compliance with funder requirements
Lab Manager	Maintain database templates; perform quarterly backups
Analyst / Student	Enter data following this SOP; run validation checks; document metadata
Data Reviewer	Verify data entries against lab notebooks before analysis

4. Definitions

- **Data dictionary:** A companion metadata file that describes each variable in a dataset, including the column name, description, units, data type, expected range, and source SOP; required for every EA Program dataset.
- **ISO 8601:** An international standard for representing dates and times as YYYY-MM-DD; the required date format for all EA Program data files because it sorts chronologically and is unambiguous across locales.
- **Missing value:** A data cell for which no measurement was made or the measurement was invalid; represented as blank or NA in EA Program datasets; never coded as 0 (which is a valid measurement).
- **Primary key:** A unique identifier for each row in a data table (e.g., sample_id); enables unambiguous linking between related tables and detection of duplicate entries.
- **Tidy data:** A data structure in which each variable has its own column, each observation has its own row, and each type of observational unit forms its own table (Wickham, 2014); the standard format for EA Program analytical datasets.

- **Version control:** A system that records changes to files over time so that specific versions can be recalled; GitHub/Git is the EA Program version control platform for all data, scripts, and SOPs.

5. Safety and Hazards

Data entry is a computational activity with no physical hazards. Ergonomic best practices (regular breaks, proper screen height) are encouraged for extended data entry sessions.

6. Equipment and Materials

6.1. Software

Item	Access	Notes
RStudio Server	rstudio.pomona.edu	Primary data processing tool
R (version 4.x)	Pre-installed on server	Required for validation scripts
Git / GitHub	github.com/marclos	Version control for all data files
Microsoft Excel / Google Sheets	Campus license	Acceptable for initial entry only
Text editor (VS Code, nano)	Various	For editing CSV files directly

6.2. File Storage

Location	Purpose	Backup Frequency
GitHub repository	Active project data	Every commit
College network drive	Archival storage	Semester end
External hard drive (lab)	Physical backup	Semester end

7. Procedure

7.1. File Naming Conventions

All data files must follow this naming pattern:

```
[project]_[datatype]_[YYYY-MM-DD].[ext]
```

For example: `claremont_soilpH_2026-05-21.csv`

Rules for file names:

1. Use lowercase letters only
2. Separate words with underscores (not spaces or hyphens)

3. Include the date of collection or generation in ISO 8601 format (YYYY-MM-DD)
4. Use `.csv` as the default file format for tabular data

> **Tip**
>
> Never use spaces, special characters, or accented letters in file names. These cause problems across operating systems and with R's file-reading functions.

7.2. Directory Structure

Organize project data using a consistent directory tree:

```
project_name/
  data/
    raw/          # Unmodified instrument or field data
    processed/    # Cleaned, validated datasets
    metadata/     # Data dictionaries, codebooks
  analysis/
    scripts/      # R scripts and Quarto documents
    output/       # Figures, tables, reports
  README.md        # Project overview and data description
```

Raw data files in `data/raw/` must never be modified. All cleaning and transformation steps should produce new files in `data/processed/` with scripts that document every change (Wilson et al., 2017).

7.3. Data Entry from Lab Notebooks

1. Open the lab notebook to the relevant page
2. Create a new CSV file (or open the project template) in `data/raw/`
3. Enter column headers in the first row following the variable naming conventions below
4. Enter each observation as a separate row
5. Record the notebook page number, date, and analyst initials in the metadata file
6. Save and commit to Git with a descriptive message

7.4. Variable Naming Conventions

Column headers (variable names) must be:

1. Lowercase, with underscores separating words (e.g., `soil_ph`, `no3_mg_l`)
2. Free of spaces, special characters, and units in the name itself
3. Documented in an accompanying data dictionary (see Section 6.7)

Use consistent naming across related datasets. For example, always use `site_id` (not `Site`, `site`, `SiteID`, or `location`) when referring to the same sampling locations.

7.5. Data Types and Formatting

Data Type	Convention	Example
Dates	ISO 8601 (YYYY-MM-DD)	`2026-05-21`
Times	24-hour (HH:MM)	`14:30`
Missing values	Leave cell empty or use `NA`	`NA`
Numeric	No commas; use period for decimal	`1234.56`
Categorical	Consistent spelling; lowercase	`upstream`
Below detection	Flag column; numeric = MDL/2	See QC SOP

Common Mistakes

Never use `0` to represent a missing value. A zero is a valid measurement. Use `NA` or leave the cell blank. Never merge cells in a spreadsheet, and never use color coding as the sole carrier of information (Broman & Woo, 2018).

7.6. Data Validation in R

After entry, run the validation script to check for common errors. A minimal validation template:

```
library(tidyverse)

# Read the raw data
dat <- read_csv("data/raw/project_variable_2026-05-21.csv")

# --- Structure checks ---
glimpse(dat)                          # Column types correct?
stopifnot(nrow(dat) > 0)             # File is not empty

# --- Missing data summary ---
dat %>%
  summarize(across(everything(), ~ sum(is.na(.)))) %>%
  pivot_longer(everything(), names_to = "variable",
               values_to = "n_missing") %>%
  filter(n_missing > 0)

# --- Range checks ---
dat %>%
  summarize(
    ph_min = min(soil_ph, na.rm = TRUE),
    ph_max = max(soil_ph, na.rm = TRUE)
  )
# Are values within physically plausible ranges?

# --- Duplicate check ---
```

```r
dat %>%
  group_by(site_id, date) %>%
  filter(n() > 1)
# Flag unexpected duplicates for review

# --- Categorical consistency ---
dat %>% count(site_id, sort = TRUE)
# Look for typos (e.g., "upstrream" vs "upstream")
```

7.7. Data Dictionaries (Metadata)

Every dataset must have a companion data dictionary saved as `data/metadata/[project]_datadictionary.csv` with the following columns:

Column	Description
`variable`	Column name in the data file
`description`	Plain-language description
`units`	Units of measurement (or "categorical", "text")
`type`	Data type: numeric, integer, character, date, logical
`range`	Expected range or valid categories
`source_sop`	SOP number for the method that generated the data

7.8. Version Control with Git

All data files and scripts must be tracked in a Git repository hosted on GitHub. Follow the procedures in SOP-L801 for committing and pushing changes. Key rules:

1. Commit raw data files when they are first created (and never modify them after the initial commit)
2. Commit processed data files after each cleaning or transformation step
3. Write informative commit messages (e.g., "Add soil pH data from Apr 15 sampling" rather than "update")
4. Use branches for experimental analyses; merge to `main` when finalized

7.9. Database Management for Multi-Year Projects

For projects spanning multiple semesters or involving multiple analysts, maintain a relational data structure:

1. Create a **sites table** (`data/metadata/sites.csv`) with location identifiers, coordinates, and site descriptions
2. Create a **samples table** (`data/metadata/samples.csv`) linking each sample to a site, date, and collector
3. Link analytical results to samples using unique `sample_id` values

This structure prevents duplication, supports merging across semesters, and enables relational joins in R (Wickham, 2014):

```
sites <- read_csv("data/metadata/sites.csv")
samples <- read_csv("data/metadata/samples.csv")
results <- read_csv("data/processed/chemistry_results.csv")

# Join all tables
full_data <- results %>%
  left_join(samples, by = "sample_id") %>%
  left_join(sites, by = "site_id")
```

8. Quality Control and Acceptance Criteria

- All data entries must be verified against the original lab notebook by a second person (or by the analyst after a 24-hour waiting period)
- Validation scripts must pass with zero unexpected errors before data are used in analysis
- The data dictionary must be complete for every variable in the dataset
- Refer to SOP-L802 for analytical QC requirements that apply to the underlying measurements

9. Waste Disposal

Not applicable (computational procedure).

10. References

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11. Training Record

Training is documented in the EA Program training log. Before working independently with project data, personnel must:

1. Read this SOP in full
2. Complete the RStudio and GitHub orientation (SOP-L801)
3. Successfully enter, validate, and commit a practice dataset under supervision
4. Be signed off by the PI or lab manager

12. Appendix A: CSV Template

A minimal CSV template for a typical water chemistry dataset:

```
sample_id,site_id,date,time,analyst,ph,conductivity_us_cm,temp_c,notes
W001,site_a,2026-05-21,09:30,MLH,7.2,345,18.5,
W002,site_b,2026-05-21,10:15,MLH,6.8,412,19.1,slight turbidity
```

13. Appendix B: Recommended R Packages for Data Management

Package	Purpose
`tidyverse`	Data import, cleaning, and transformation
`janitor`	Consistent column names, tabulation
`validate`	Rule-based data validation
`pointblank`	Automated data quality reporting
`here`	Portable file paths across systems
`skimr`	Quick summary statistics

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L804

GIS and Spatial Data Management

Standard Operating Procedure

SOP Number	SOP-L804
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Author(s)	Marc Los Huertos
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12 Appendix A: Recommended R Packages for Spatial Analysis **8**

1. Purpose

This SOP establishes procedures for acquiring, organizing, projecting, and archiving geospatial data used in EA Program research. It covers coordinate reference systems, file formats, metadata standards, and reproducible mapping workflows in R, ensuring that spatial data are interoperable across projects and analysts (Lovelace et al., 2019; Pebesma & Bivand, 2023).

2. Scope

- **Applies to:** All vector (point, line, polygon) and raster (satellite imagery, DEMs, interpolated surfaces) data used in EA Program projects
- **Does not apply to:** Drone image acquisition or photogrammetric processing (see SOP-L805); GPS receiver operation in the field (see relevant field SOPs)
- **Location:** EA Program laboratory (SGM 133) and RStudio Server

3. Responsibilities

Role	Responsibility
Lab PI	Approve spatial data management plan; ensure data-sharing compliance
GIS Lead	Maintain spatial data library; enforce projection and metadata standards
Analyst / Student	Follow naming conventions; document coordinate systems; archive final layers
Data Reviewer	Verify spatial metadata completeness before publication

4. Definitions

- **Coordinate reference system (CRS):** A framework that associates coordinates with locations on Earth; consists of a datum (reference ellipsoid), a projection (for 2D maps), and a coordinate system (e.g., latitude/longitude or meters); data layers must share the same CRS for spatial operations.
- **Geodatabase:** A structured container for storing multiple spatial datasets, attribute tables, and topological rules in a single file; the preferred format for complex GIS projects with many related layers.
- **GeoTIFF:** A TIFF image file with embedded geospatial metadata (CRS, extent, resolution); the standard raster format for spatial data exchange in remote sensing and GIS.
- **Raster:** A spatial data model representing continuous surfaces as a grid of equally sized cells (pixels), each with one or more attribute values; used for elevation, imagery, and interpolated surfaces.
- **Shapefile (.shp):** A common vector spatial data format introduced by Esri; stores geometry (points, lines, polygons) and attribute data in multiple associated files (.shp, .dbf, .shx,

.prj minimum); widely supported but limited by 2 GB file size and 10-character field name constraints.
- **Vector:** A spatial data model representing discrete features as points, lines, or polygons defined by coordinate pairs; used for sample locations, stream networks, and land parcel boundaries.
- **WGS84 (World Geodetic System 1984):** The global geodetic datum used by GPS; EPSG code 4326; the required CRS for all EA Program GPS-collected point data before projection into a local coordinate system.

5. Safety and Hazards

GIS work is a computational activity with no physical hazards. When using GPS receivers or conducting ground-truth fieldwork, follow SOP-F001 Field Safety.

6. Equipment and Materials

6.1. Software

Item	Access	Notes
R (version 4.x)	RStudio Server	Primary analysis platform
`sf` package	CRAN	Vector data handling
`terra` package	CRAN	Raster data handling
`tmap` / `ggplot2`	CRAN	Map production
QGIS (3.x)	Free download	Visual inspection and digitizing
Google Earth Engine	earthengine.google.com	Satellite imagery processing

6.2. Hardware

Item	Location	Notes
GPS receivers (Garmin)	SGM 133 equipment cabinet	Field coordinate collection
High-resolution monitor	SGM 133	For digitizing and map review

7. Procedure

7.1. Coordinate Reference Systems

All EA Program spatial data must use one of the following coordinate reference systems (CRS). Document the CRS in every file, script, and metadata record.

CRS Name	EPSG Code	Use Case
WGS 84 (geographic)	4326	GPS data, web maps, data exchange
NAD83 / UTM Zone 11N	26911	Local analysis in Southern California
NAD83 / California Zone 5	2229	County and municipal data

> **Tip**
>
> When in doubt, store data in EPSG:4326 (latitude/longitude, WGS 84). Transform to a projected CRS for distance and area calculations.

When loading spatial data in R, always verify and set the CRS explicitly:

```
library(sf)

# Read a shapefile
sites <- st_read("data/spatial/sampling_sites.shp")

# Check CRS
st_crs(sites)

# Transform to UTM Zone 11N for area calculations
sites_utm <- st_transform(sites, crs = 26911)
```

7.2. File Formats

Format	Extension	Use Case	Notes
GeoPackage	`.gpkg`	Preferred for all vector data	Single file, open standard
Shapefile	`.shp` + `.dbf` + `.shx` + `.prj`	Legacy compatibility	Keep all component files together
GeoTIFF	`.tif`	Raster data	Include CRS in header
CSV with XY	`.csv`	Simple point data	Requires separate CRS documentation
GeoJSON	`.geojson`	Web applications	Always WGS 84

GeoPackage (`.gpkg`) is the preferred format for new projects because it stores geometry, attributes, and CRS in a single file without the limitations of shapefiles (10-character field names, 2 GB size limit) (Pebesma & Bivand, 2023).

7.3. File Naming and Directory Structure

Follow the conventions in SOP-L803 with the addition of a `data/spatial/` subdirectory:

```
project_name/
  data/
     raw/
     processed/
     spatial/
        raw/          # Downloaded or collected spatial data
        processed/    # Clipped, projected, joined layers
        metadata/     # FGDC or ISO metadata files
     metadata/
  analysis/
     scripts/
     maps/             # Exported map figures
  README.md
```

7.4. Spatial Data Acquisition

Common data sources for EA Program research:

Source	URL	Data Types
USGS National Map	nationalmap.gov	DEMs, hydrography, boundaries
USGS EarthExplorer	earthexplorer.usgs.gov	Landsat, NAIP imagery
CA State Geoportal	gis.data.ca.gov	State and county layers
NRCS Web Soil Survey	websoilsurvey.sc.egov.usda.gov	SSURGO soil data
OpenStreetMap	openstreetmap.org	Roads, buildings, land use
Google Earth Engine	earthengine.google.com	Multi-temporal satellite data

When downloading external data, record the source URL, download date, and any subsetting parameters in the metadata file.

7.5. Spatial Data Processing in R

A standard workflow for processing spatial data:

```r
library(sf)
library(terra)
library(tidyverse)

# --- 1. Load vector data ---
sites <- st_read("data/spatial/raw/sampling_sites.gpkg")
watershed <- st_read("data/spatial/raw/claremont_watershed.gpkg")
```

```
# --- 2. Verify and harmonize CRS ---
st_crs(sites) == st_crs(watershed)
sites <- st_transform(sites, crs = st_crs(watershed))

# --- 3. Spatial operations ---
# Clip sites to watershed boundary
sites_in_watershed <- st_intersection(sites, watershed)

# Buffer sites by 100 m
site_buffers <- st_buffer(sites_in_watershed, dist = 100)

# --- 4. Load and extract raster data ---
dem <- rast("data/spatial/raw/dem_10m.tif")
sites_in_watershed$elevation_m <- extract(dem, sites_in_watershed)$value

# --- 5. Save processed outputs ---
st_write(sites_in_watershed,
         "data/spatial/processed/sites_with_elevation.gpkg",
         delete_dsn = TRUE)
```

7.6. Map Production

All maps for reports and publications must include:

1. Title and date
2. Scale bar (use `tmap::tm_scale_bar()` or `ggspatial::annotation_scale()`)
3. North arrow
4. Legend with clear labels and units
5. Data source attribution
6. CRS/projection noted in the caption or margin

```
library(tmap)

tm_shape(watershed) +
  tm_polygons(col = "lightblue", alpha = 0.3) +
  tm_shape(sites_in_watershed) +
  tm_dots(col = "elevation_m", size = 0.5,
          palette = "viridis",
          title = "Elevation (m)") +
  tm_scale_bar(position = c("left", "bottom")) +
  tm_compass(position = c("right", "top")) +
  tm_layout(
    title = "Sampling Sites: Claremont Watershed",
    title.size = 1.2,
    legend.outside = TRUE
  )
```

7.7. Metadata Documentation

Each spatial dataset must have a metadata record. At minimum, include:

Field	Description
Title	Descriptive name of the layer
Abstract	Brief description of content and purpose
CRS	EPSG code and full name
Extent	Bounding box coordinates
Source	Origin of data (URL, agency, field GPS)
Date created/acquired	When the data were generated or downloaded
Processing steps	Summary of transformations applied
Contact	Analyst name and email

For formal publications, use FGDC or ISO 19115 metadata standards. For internal projects, the table above saved as a text file alongside the data is sufficient.

7.8. GPS Data Import

When importing GPS waypoints or tracks:

1. Download data from the GPS unit to `data/spatial/raw/` in GPX format
2. Import into R using `sf::st_read("file.gpx", layer = "waypoints")`
3. Verify coordinates visually by plotting over a basemap
4. Assign the correct CRS (typically EPSG:4326 for consumer GPS units)
5. Document the GPS model, estimated accuracy, and collection conditions

8. Quality Control and Acceptance Criteria

- Verify CRS for every imported dataset before analysis
- Check that point locations fall within expected geographic bounds (e.g., all sites should be within the study area polygon)
- Validate topology for polygon layers (no gaps, overlaps, or self-intersections) using `sf::st_is_valid()`
- Compare GPS coordinates with known reference points when available
- Document the spatial accuracy and precision of each data source

9. Waste Disposal

Not applicable (computational procedure).

10. References

Lovelace, R., Nowosad, J., & Muenchow, J. (2019). *Geocomputation with R*. Chapman; Hall/CRC. https://doi.org/10.1201/9780203730058

Pebesma, E., & Bivand, R. (2023). *Spatial data science: With applications in R*. Chapman; Hall/CRC. https://doi.org/10.1201/9780429459016

11. Training Record

Training is documented in the EA Program training log. Before working independently with spatial data, personnel must:

1. Read this SOP in full
2. Complete the RStudio and GitHub orientation (SOP-L801)
3. Demonstrate proficiency with `sf` and basic spatial operations in R under supervision
4. Produce a correctly formatted map with all required elements
5. Be signed off by the PI or GIS lead

12. Appendix A: Recommended R Packages for Spatial Analysis

Package	Purpose
`sf`	Vector data (points, lines, polygons)
`terra`	Raster data analysis
`stars`	Spatiotemporal raster/vector data cubes
`tmap`	Thematic maps (static and interactive)
`leaflet`	Interactive web maps
`ggspatial`	Spatial layers in ggplot2
`mapview`	Quick interactive map previews
`exactextractr`	Fast raster extraction by polygons
`geodata`	Download climate, soil, and elevation data
`tidyterra`	Tidy methods for terra objects

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L805

Drone Image Processing (Photogrammetry)

Standard Operating Procedure

SOP Number	SOP-L805
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP describes the post-processing workflow for aerial imagery collected by uncrewed aerial systems (UAS/drones). It covers image organization, photogrammetric reconstruction (orthomosaics, digital surface models, and point clouds), ground control point integration, accuracy assessment, and data archiving (Anderson & Gaston, 2013; Iglhaut et al., 2019).

2. Scope

- **Applies to:** Photogrammetric processing of RGB, multispectral, and thermal drone imagery collected for EA Program research
- **Does not apply to:** Drone flight planning, airspace authorization, or FAA Part 107 compliance (covered in the drone operations field SOP); GIS analysis of processed outputs (see SOP-L804)
- **Location:** EA Program laboratory (SGM 133) and processing workstations

3. Responsibilities

Role	Responsibility
Lab PI	Approve processing workflows; ensure data storage compliance
Drone Pilot / Operator	Deliver organized imagery with flight logs; place and survey GCPs
Processing Analyst	Run photogrammetric software; assess quality; archive outputs
Data Reviewer	Verify accuracy metrics before outputs are used in analysis

4. Definitions

- **Digital elevation model (DEM):** A raster representing bare-ground surface elevation; derived by removing vegetation and structures from a digital surface model using filtering algorithms.
- **Digital surface model (DSM):** A raster representing the elevation of all surfaces in the scene, including vegetation canopy and structures; the direct output of photogrammetric point cloud processing.
- **Ground control point (GCP):** A precisely surveyed point on the ground (surveyed with RTK-GPS or total station) that appears in multiple overlapping images; used to georeference the photogrammetric model to a known coordinate system and improve absolute accuracy.
- **Orthomosaic:** A geometrically corrected aerial image composed of many overlapping individual photos, in which scale is uniform and features can be measured accurately; produced by photogrammetric processing.
- **Photogrammetry:** The science of making measurements from photographs; in drone operations, the process of generating 3D models, DEMs, and orthomosaics from overlapping aerial images using structure-from-motion algorithms.

- **Point cloud:** A set of data points in 3D space (X, Y, Z coordinates) representing the surface of objects; generated by photogrammetric processing of overlapping images; the intermediate product from which DEMs and DSMs are derived.
- **Structure from Motion (SfM):** A photogrammetric algorithm that reconstructs 3D geometry from 2D overlapping images by identifying and matching common features across multiple images; the computational core of drone photogrammetry software (Agisoft Metashape, Pix4D).

5. Safety and Hazards

> ⚠ Hazards
>
> Photogrammetric processing is computationally intensive but poses no physical hazards. However, extended processing sessions may generate significant heat from workstation components. Ensure adequate ventilation around processing workstations.

For field-related hazards during drone operations and GCP placement, refer to SOP-F001 Field Safety.

6. Equipment and Materials

6.1. Software

Item	Access	Notes
Agisoft Metashape Professional	Campus license	Primary photogrammetry tool
OpenDroneMap (ODM)	Free / open source	Alternative processing
WebODM	Self-hosted	Browser interface for ODM
QGIS (3.x)	Free download	Visualization and QC
R with `terra` / `sf`	RStudio Server	Post-processing analysis
CloudCompare	Free download	Point cloud inspection

6.2. Hardware

Item	Minimum Specs	Notes
Processing workstation	32 GB RAM, dedicated GPU, SSD	More RAM for large datasets
External storage	2 TB+ portable SSD	Raw image archive

6.3. Drone Platforms

Platform	Sensor	Resolution
DJI Mavic 3 Enterprise	RGB (Hasselblad)	20 MP; 0.7 cm/px at 100 m
DJI Matrice 350 RTK	Zenmuse P1 / L2	High-res RGB or LiDAR
Custom multispectral rig	MicaSense RedEdge-P	5-band multispectral

7. Procedure

7.1. Image Organization

Before processing, organize raw imagery following a consistent directory structure:

```
project_name/
  drone/
      raw_images/
          flight_2026-05-21_rgb/
              DJI_0001.JPG
              DJI_0002.JPG
              ...
          flight_2026-05-21_multi/
      flight_logs/
          flight_2026-05-21.csv
      gcp/
          gcp_coordinates.csv
      processed/
          orthomosaic/
          dsm/
          point_cloud/
          report/
      README.md
```

Check that all images have valid EXIF data (GPS coordinates, camera parameters). Remove obviously corrupted or blurry images before processing.

```r
library(exifr)

# Read EXIF from all images in a flight folder
exif_data <- read_exif(
  list.files("drone/raw_images/flight_2026-05-21_rgb/",
             pattern = "\\.JPG$", full.names = TRUE),
  tags = c("FileName", "GPSLatitude", "GPSLongitude",
           "GPSAltitude", "FocalLength", "ImageWidth",
           "ImageHeight", "DateTimeOriginal")
)

# Check for missing GPS
```

```
missing_gps <- exif_data %>%
  filter(is.na(GPSLatitude) | is.na(GPSLongitude))
cat(sprintf("%d images missing GPS coordinates\n", nrow(missing_gps)))

# Summary
cat(sprintf("Total images: %d\n", nrow(exif_data)))
cat(sprintf("Date range: %s to %s\n",
            min(exif_data$DateTimeOriginal),
            max(exif_data$DateTimeOriginal)))
```

7.2. Ground Control Points (GCPs)

GCPs are surveyed reference points used to improve the geometric accuracy of photogrammetric outputs.

1. Place GCPs before the flight. Use high-contrast targets (black and white checkerboard, minimum 30 cm) visible from flight altitude
2. Survey GCP positions using a differential GPS or RTK receiver. Record coordinates in WGS 84 (EPSG:4326) and a local projected CRS (e.g., EPSG:26911)
3. Format GCP data as a CSV file:

```
gcp_id,longitude,latitude,elevation_m,crs
GCP01,-117.7123,34.0966,398.2,EPSG:4326
GCP02,-117.7118,34.0971,401.5,EPSG:4326
```

4. Use a minimum of 5 GCPs distributed across the study area. Reserve 2 or more as independent check points for accuracy assessment

7.3. Photogrammetric Processing (Agisoft Metashape)

The following workflow applies to Agisoft Metashape Professional. Adapt parameters for your specific project needs.

Step 1: Create project and import photos

1. Create a new Metashape project and save to `drone/processed/`
2. Add photos from the flight folder
3. Verify camera positions on the reference pane

Step 2: Align photos

1. Workflow > Align Photos
2. Settings: Accuracy = High; Key point limit = 40,000; Tie point limit = 10,000; Adaptive camera model fitting = enabled
3. Review the sparse point cloud and camera positions

Step 3: Import and assign GCPs

1. Import GCP coordinates from the CSV file
2. Assign each GCP to its corresponding target in at least 3 photos
3. Uncheck GCPs designated as check points
4. Run Optimize Cameras

Step 4: Build dense point cloud

1. Workflow > Build Point Cloud
2. Quality = High (or Medium for initial assessment); Depth filtering = Moderate
3. Inspect the dense cloud for artifacts; remove obvious noise manually or with CloudCompare

Step 5: Build DEM and orthomosaic

1. Workflow > Build DEM (Source: Point Cloud)
2. Workflow > Build Orthomosaic (Surface: DEM; Blending mode: Mosaic)

Step 6: Export products

1. Export DEM as GeoTIFF to `drone/processed/dsm/`
2. Export orthomosaic as GeoTIFF to `drone/processed/orthomosaic/`
3. Export point cloud as `.laz` to `drone/processed/point_cloud/`
4. Export processing report as PDF to `drone/processed/report/`

> **Tip**
>
> For large datasets (more than 1,000 images), process on the lab workstation rather than a laptop. Expect processing times of several hours for dense cloud generation at High quality.

7.4. OpenDroneMap (Alternative Workflow)

For projects using the open-source pipeline:

```
# Run ODM with standard settings
docker run -ti --rm \
  -v /path/to/project/drone:/datasets \
  opendronemap/odm \
  --project-path /datasets \
  --dsm \
  --orthophoto-resolution 2.0 \
  --dem-resolution 5.0 \
  --gcp /datasets/gcp/gcp_list.txt
```

7.5. Accuracy Assessment

Evaluate positional accuracy using the reserved check points:

```
library(terra)
library(sf)

# Load the DSM
dsm <- rast("drone/processed/dsm/dsm_2026-05-21.tif")

# Load check points
check_pts <- read_csv("drone/gcp/check_points.csv") %>%
  st_as_sf(coords = c("longitude", "latitude"), crs = 4326) %>%
  st_transform(crs = crs(dsm))

# Extract DSM elevation at check points
check_pts$dsm_elev <- extract(dsm, check_pts)$value

# Calculate vertical RMSE
check_pts <- check_pts %>%
  mutate(error_m = dsm_elev - elevation_m)

rmse_z <- sqrt(mean(check_pts$error_m^2, na.rm = TRUE))
mae_z <- mean(abs(check_pts$error_m), na.rm = TRUE)

cat(sprintf("Vertical RMSE: %.3f m\n", rmse_z))
cat(sprintf("Vertical MAE:  %.3f m\n", mae_z))
cat(sprintf("n check points: %d\n", nrow(check_pts)))
```

Report accuracy metrics in all publications and project reports. Typical targets for environmental applications: horizontal RMSE less than 2 times the ground sampling distance (GSD); vertical RMSE less than 3 times the GSD (James et al., 2017).

7.6. Multispectral Processing

For multispectral imagery (e.g., MicaSense RedEdge):

1. Include reflectance calibration panel images (taken before and after each flight)
2. Process each band alignment in Metashape or use MicaSense's Atlas or `micasense` Python library
3. Export calibrated reflectance orthomosaics (not raw DN values)
4. Calculate vegetation indices in R:

```
library(terra)

# Load calibrated reflectance bands
```

```
red <- rast("drone/processed/orthomosaic/red_reflectance.tif")
nir <- rast("drone/processed/orthomosaic/nir_reflectance.tif")

# Calculate NDVI
ndvi <- (nir - red) / (nir + red)
names(ndvi) <- "NDVI"

writeRaster(ndvi, "drone/processed/orthomosaic/ndvi_2026-05-21.tif",
            overwrite = TRUE)
```

8. Quality Control and Acceptance Criteria

- Photo alignment: at least 90% of images must align successfully
- Reprojection error: mean below 1.0 pixel after GCP optimization
- Check point RMSE: document and compare against project requirements
- Visual inspection: no visible seam lines, ghosting, or geometric distortion in the orthomosaic
- File integrity: verify that all exported GeoTIFFs have valid CRS headers and correct extent

9. Waste Disposal

Not applicable (computational procedure).

10. References

Anderson, K., & Gaston, K. J. (2013). Lightweight unmanned aerial vehicles will revolutionize spatial ecology. *Frontiers in Ecology and the Environment*, *11*(3), 138–146. https://doi.org/10.1890/120150

Iglhaut, J., Cabo, C., Puliti, S., Piermattei, L., O'Connor, J., & Rosette, J. (2019). Structure from motion photogrammetry in forestry: A review. *Current Forestry Reports*, *5*(3), 155–168. https://doi.org/10.1007/s40725-019-00094-3

James, M. R., Robson, S., d'Oleire-Oltmanns, S., & Niethammer, U. (2017). Optimising UAV topographic surveys processed with structure-from-motion: Ground control quality, quantity and bundle adjustment. *Geomorphology*, *280*, 51–66. https://doi.org/10.1016/j.geomorph.2016.11.021

11. Training Record

Training is documented in the EA Program training log. Before processing drone imagery independently, personnel must:

1. Read this SOP in full
2. Complete Agisoft Metashape (or ODM) training with a supervised practice dataset

3. Demonstrate a complete processing workflow from raw images to accuracy-assessed outputs
4. Be signed off by the PI or drone operations lead

12. Appendix A: Processing Parameter Reference

Parameter	Low Quality	Medium	High (Recommended)
Photo alignment	Low accuracy	Medium	High
Dense cloud	Low	Medium	High
DEM resolution	10x GSD	5x GSD	2x GSD
Orthomosaic	10x GSD	5x GSD	Native GSD
Approx. processing time (500 images)	30 min	2 hr	6 hr+

13. Appendix B: Troubleshooting

Symptom	Probable Cause	Corrective Action
Low alignment rate (<80%)	Insufficient overlap	Re-fly with 80%+ front/side overlap
High reprojection error	Incorrect GCP placement	Re-identify GCP markers in photos
Artifacts in orthomosaic	Moving objects (water, cars)	Mask affected areas; adjust blending
Out-of-memory error	Dataset too large for RAM	Reduce quality; split into chunks
DSM shows "doming" distortion	No GCPs; insufficient geometry	Add GCPs; increase flight line diversity

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L806

Statistical Analysis Templates (R)

Standard Operating Procedure

SOP Number	SOP-L806
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP provides standardized R analysis templates for common statistical procedures used in EA Program research. It establishes reproducible workflows for exploratory data analysis, hypothesis testing, regression modeling, and reporting, ensuring that all analyses are transparent, well-documented, and consistent across projects (Wickham et al., 2023; Zuur et al., 2010).

2. Scope

- **Applies to:** All quantitative statistical analyses performed for EA Program research, coursework, and publications
- **Does not apply to:** Bioinformatics sequence analysis (see SOP-L705); GIS-specific spatial statistics (see SOP-L804); instrument calibration curve fitting (see method-specific SOPs)
- **Location:** RStudio Server (rstudio.pomona.edu)

3. Responsibilities

Role	Responsibility
Lab PI	Approve analytical approach; review statistical methods in manuscripts
Analyst / Student	Select appropriate methods; follow templates; document all decisions
Data Reviewer	Verify that assumptions are checked and reported
Statistical Advisor	Consult on complex designs (mixed models, multivariate methods)

4. Definitions

- **ANOVA (Analysis of Variance):** A statistical test that partitions total variance in a response variable into components attributable to one or more categorical factors; tests whether group means differ significantly; assumes normality and homogeneity of variance.
- **Confidence interval:** A range of values computed from sample data that, under repeated sampling, contains the true population parameter with a stated probability (e.g., 95%); wider intervals reflect greater uncertainty.
- **Effect size:** A standardized measure of the magnitude of a difference or relationship, independent of sample size; examples include Cohen's d (mean differences), r^2 (explained variance), and Hedge's g; should always accompany p-values.
- **Mixed model:** A statistical model containing both fixed effects (factors whose levels are deliberately chosen) and random effects (factors whose levels represent a random sample from a larger population); appropriate when data have hierarchical or repeated-measures structure.
- **p-value:** The probability of observing a test statistic as extreme as the one obtained (or more extreme) if the null hypothesis is true; does not measure the probability that the null hypothesis is true; must be interpreted with effect size.

- **Power:** The probability of correctly rejecting the null hypothesis when it is false; increases with sample size, effect size, and significance level; should be considered in study design to ensure adequate sample sizes.
- **Residual:** The difference between an observed value and the value predicted by a statistical model; examination of residuals is the primary diagnostic for checking model assumptions.

5. Safety and Hazards

Statistical analysis is a computational activity with no physical hazards.

6. Equipment and Materials

6.1. Software

Item	Access	Notes
R (version 4.x)	RStudio Server	Primary analysis platform
RStudio IDE	rstudio.pomona.edu	Integrated development environment
Quarto	Pre-installed	Report generation
Git / GitHub	github.com/marclos	Version control

6.2. Core R Packages

Package	Purpose
`tidyverse`	Data wrangling and visualization
`broom`	Tidy model output
`car`	Diagnostic tests (Levene, VIF)
`lme4`	Mixed-effects models
`emmeans`	Estimated marginal means and contrasts
`vegan`	Community ecology and ordination
`MuMIn`	Model selection and multi-model inference
`performance`	Model diagnostics and assumption checks
`report`	Automated statistical reporting
`ggplot2`	Publication-quality figures
`knitr` / `kableExtra`	Formatted tables

7. Procedure

7.1. Analysis Workflow Overview

Every statistical analysis should follow this sequence:

1. State the research question and hypotheses
2. Import and validate data (see SOP-L803)
3. Conduct exploratory data analysis (EDA)
4. Check assumptions for the intended statistical test
5. Fit the model
6. Evaluate model diagnostics
7. Interpret results and generate figures/tables
8. Write up methods and results in a Quarto document
9. Commit the complete analysis to Git

7.2. Quarto Document Header

Begin every analysis with a standard Quarto header:

```
---
title: "Analysis: [Project Name]"
author: "[Your Name]"
date: today
format:
  html:
    toc: true
    code-fold: true
    embed-resources: true
bibliography: references.bib
---
```

7.3. Exploratory Data Analysis Template

```
library(tidyverse)

# --- 1. Load and inspect data ---
dat <- read_csv("data/processed/project_data.csv")
glimpse(dat)

# --- 2. Summary statistics ---
dat %>%
  group_by(treatment) %>%
  summarize(
    n = n(),
    mean = mean(response, na.rm = TRUE),
    sd = sd(response, na.rm = TRUE),
    median = median(response, na.rm = TRUE),
    min = min(response, na.rm = TRUE),
    max = max(response, na.rm = TRUE)
  )
```

```r
# --- 3. Distributional plots ---
ggplot(dat, aes(x = response)) +
  geom_histogram(bins = 20, fill = "steelblue", color = "white") +
  facet_wrap(~ treatment) +
  labs(x = "Response Variable (units)", y = "Count") +
  theme_minimal()

ggplot(dat, aes(x = treatment, y = response)) +
  geom_boxplot(fill = "steelblue", alpha = 0.5) +
  geom_jitter(width = 0.1, alpha = 0.4) +
  labs(x = "Treatment", y = "Response Variable (units)") +
  theme_minimal()

# --- 4. Missing data assessment ---
dat %>%
  summarize(across(everything(), ~ sum(is.na(.)))) %>%
  pivot_longer(everything(), names_to = "variable",
               values_to = "n_missing") %>%
  filter(n_missing > 0)
```

7.4. Two-Sample Comparisons

```r
# --- Check normality within groups ---
dat %>%
  group_by(treatment) %>%
  summarize(
    shapiro_p = shapiro.test(response)$p.value
  )

# --- Check equal variances ---
car::leveneTest(response ~ treatment, data = dat)

# --- Parametric: two-sample t-test ---
t_result <- t.test(response ~ treatment, data = dat, var.equal = FALSE)
t_result

# --- Nonparametric alternative: Wilcoxon rank-sum ---
wilcox.test(response ~ treatment, data = dat)

# --- Effect size ---
library(effectsize)
cohens_d(response ~ treatment, data = dat)
```

7.5. One-Way ANOVA Template

```r
# --- Fit ANOVA ---
aov_model <- aov(response ~ treatment, data = dat)

# --- Check assumptions ---
# Normality of residuals
shapiro.test(residuals(aov_model))
qqnorm(residuals(aov_model)); qqline(residuals(aov_model))

# Homogeneity of variance
car::leveneTest(response ~ treatment, data = dat)

# Residual vs. fitted plot
plot(aov_model, which = 1)

# --- ANOVA table ---
summary(aov_model)

# --- Post-hoc pairwise comparisons ---
library(emmeans)
emm <- emmeans(aov_model, ~ treatment)
pairs(emm, adjust = "tukey")

# --- Nonparametric alternative: Kruskal-Wallis ---
kruskal.test(response ~ treatment, data = dat)
```

> **Tip**
>
> Always check assumptions before interpreting p-values. If residuals are non-normal or variances are unequal, consider a transformation (log, square root) or switch to a nonparametric test. Document the reasoning in your analysis script (Zuur et al., 2010).

7.6. Linear Regression Template

```r
# --- Fit linear model ---
lm_model <- lm(response ~ predictor1 + predictor2, data = dat)

# --- Model summary ---
summary(lm_model)

# --- Tidy output ---
library(broom)
tidy(lm_model, conf.int = TRUE)
```

```r
glance(lm_model)

# --- Diagnostics ---
par(mfrow = c(2, 2))
plot(lm_model)
par(mfrow = c(1, 1))

# Check multicollinearity
car::vif(lm_model)

# --- Model performance ---
library(performance)
check_model(lm_model)

# --- Visualization ---
ggplot(dat, aes(x = predictor1, y = response)) +
  geom_point(alpha = 0.6) +
  geom_smooth(method = "lm", se = TRUE, color = "steelblue") +
  labs(x = "Predictor (units)", y = "Response (units)",
       caption = sprintf("R-squared = %.3f, p = %.4f",
                         summary(lm_model)$r.squared,
                         glance(lm_model)$p.value)) +
  theme_minimal()
```

7.7. Mixed-Effects Model Template

For data with nested or repeated-measures structure:

```r
library(lme4)
library(lmerTest)  # For p-values via Satterthwaite

# --- Fit mixed model ---
# Fixed effect: treatment
# Random effect: site (multiple samples per site)
mixed_model <- lmer(response ~ treatment + (1 | site_id), data = dat)

# --- Summary with p-values ---
summary(mixed_model)

# --- Marginal and conditional R-squared ---
MuMIn::r.squaredGLMM(mixed_model)

# --- Post-hoc comparisons ---
library(emmeans)
emm <- emmeans(mixed_model, ~ treatment)
pairs(emm, adjust = "tukey")
```

```r
# --- Residual diagnostics ---
plot(mixed_model)
qqnorm(resid(mixed_model)); qqline(resid(mixed_model))
```

7.8. Multivariate / Community Analysis Template

```r
library(vegan)

# --- Prepare community matrix ---
# Rows = sites, columns = species/variables
comm_matrix <- dat %>%
  select(site_id, species, abundance) %>%
  pivot_wider(names_from = species, values_from = abundance,
              values_fill = 0) %>%
  column_to_rownames("site_id")

# --- Diversity indices ---
diversity_h <- diversity(comm_matrix, index = "shannon")
richness <- specnumber(comm_matrix)

# --- Ordination (NMDS) ---
nmds <- metaMDS(comm_matrix, distance = "bray", k = 2,
                trymax = 100)
stressplot(nmds)

# Plot
ordiplot(nmds, type = "n")
points(nmds, col = as.factor(env_data$treatment), pch = 16)
legend("topright", legend = levels(as.factor(env_data$treatment)),
       col = 1:nlevels(as.factor(env_data$treatment)), pch = 16)

# --- PERMANOVA ---
adonis2(comm_matrix ~ treatment, data = env_data,
        method = "bray", permutations = 999)
```

7.9. Figure Formatting Standards

All figures for reports and publications must follow these conventions:

1. Use `theme_minimal()` or a consistent custom theme
2. Label axes with variable names and units
3. Use colorblind-friendly palettes (e.g., `viridis`, `RColorBrewer` "Set2")
4. Include figure captions using the `#| fig-cap:` chunk option
5. Save publication figures at 300 DPI as both PDF and PNG:

```
ggsave("analysis/output/fig_treatment_response.pdf",
       width = 6, height = 4, units = "in")
ggsave("analysis/output/fig_treatment_response.png",
       width = 6, height = 4, units = "in", dpi = 300)
```

7.10. Reporting Statistical Results

When writing results in a Quarto document, follow APA conventions:

- Report test statistics, degrees of freedom, and p-values: "Treatment had a significant effect on soil pH ($F(2, 27) = 4.53$, $p = 0.020$)"
- Report effect sizes alongside p-values
- Include confidence intervals for parameter estimates
- Round to a consistent number of decimal places (typically 2 for test statistics, 3 for p-values)
- Use `broom::tidy()` and `knitr::kable()` for formatted tables

```
library(broom)
library(knitr)

tidy(aov_model) %>%
  kable(digits = 3, caption = "ANOVA results for soil pH by treatment")
```

8. Quality Control and Acceptance Criteria

- Every analysis must include explicit assumption checks (normality, homoscedasticity, independence) documented in the script
- All models must include diagnostic plots
- Results must be reproducible: running the script from start to finish on the same data must produce identical output
- Analyses must be committed to Git with clear commit messages
- The PI or a designated reviewer must approve the analytical approach before results are included in publications

9. Waste Disposal

Not applicable (computational procedure).

10. References

Wickham, H., cCetinkaya-Rundel, M., & Grolemund, G. (2023). *R for data science* (2nd ed.). O'Reilly Media. https://r4ds.hadley.nz/

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

11. Training Record

Training is documented in the EA Program training log. Before conducting statistical analyses independently, personnel must:

1. Read this SOP in full
2. Complete the RStudio and GitHub orientation (SOP-L801)
3. Complete the introductory R statistics module (see RTricks)
4. Demonstrate a complete analysis workflow (EDA through reporting) on a practice dataset under supervision
5. Be signed off by the PI

12. Appendix A: Decision Guide for Choosing a Statistical Test

Research Question	Data Type	Groups	Recommended Test
Compare 2 group means	Continuous, normal	2	Two-sample t-test
Compare 2 group medians	Non-normal	2	Wilcoxon rank-sum
Compare 3+ group means	Continuous, normal	3+	One-way ANOVA
Compare 3+ group medians	Non-normal	3+	Kruskal-Wallis
Relationship between 2 variables	Both continuous	N/A	Linear regression / correlation
Multiple predictors	Continuous response	N/A	Multiple regression
Nested / repeated measures	Continuous	Any	Mixed-effects model
Community composition	Abundance matrix	Any	NMDS + PERMANOVA
Categorical association	Counts	Any	Chi-squared / Fisher's exact

13. Appendix B: Common Data Transformations

Transformation	R Code	When to Use
Log (natural)	`log(x)`	Right-skewed data; multiplicative relationships
Log (base 10)	`log10(x)`	Concentration data spanning orders of magnitude
Square root	`sqrt(x)`	Count data; moderate skew
Arcsine square root	`asin(sqrt(x))`	Proportions (0 to 1)

Transformation	R Code	When to Use
Box-Cox	`MASS::boxcox(model)`	Let the data determine the best transformation

> **Caution**
>
> Always report whether data were transformed and provide results on both the transformed and original scales when possible. Transformations change the hypothesis being tested (e.g., log-transformed ANOVA compares geometric means, not arithmetic means).

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-L807

Instrument Data Export and File Management

Standard Operating Procedure

SOP Number	SOP-L807
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This SOP standardizes the procedures for exporting raw data from laboratory instruments, transferring files to the data management system, and organizing instrument outputs for downstream analysis. It ensures that raw instrument data are preserved without modification, properly documented, and accessible for reproducible analysis (Gandrud, 2015).

2. Scope

- **Applies to:** All analytical instruments in the EA Program laboratory that produce digital data files, including spectrophotometers, ICP-OES, TOC analyzers, ion chromatographs, balances with data output, pH/conductivity meters with logging, and field sensors that store data internally
- **Does not apply to:** Manual readings recorded directly in lab notebooks (see SOP-L010); drone imagery (see SOP-L805); bioinformatics sequencing data (see SOP-L705)
- **Location:** EA Program laboratory (SGM 133)

3. Responsibilities

Role	Responsibility
Lab PI	Approve file management protocols; ensure backup compliance
Lab Manager	Maintain instrument computers; manage shared storage; train users on export procedures
Analyst / Student	Export data following this SOP; verify file integrity; commit to version control
Data Reviewer	Confirm that raw files match lab notebook entries and are properly archived

4. Definitions

- **Comma-separated values (CSV):** A plain-text file format in which values are separated by commas and rows by line breaks; universally readable; the preferred format for sharing tabular data between instruments and analysis software.
- **Data provenance:** The documented history of how data were collected, processed, transformed, and stored; enables reproduction of results and detection of errors; captured through lab notebooks, metadata files, and Git commit history.
- **File naming convention:** A standardized pattern for naming files that encodes date, instrument, project, and analyst information without spaces or special characters (e.g., `YYYY-MM-DD_InstrumentID_ProjectCode_UserInitials_raw.csv`); required for all EA Program instrument export files.

- **Instrument export file:** The raw data file generated directly by instrument software (e.g., Shimadzu LabSolutions .lcd or .txt export, Isodat .dxf export, HOBOware .csv export); must be saved unmodified as the archival primary record.
- **Metadata:** Data describing other data; includes instrument settings, calibration records, sample identifiers, units, processing steps, and analyst notes; stored alongside raw data in a companion metadata file.
- **Raw data:** The unprocessed data as produced by the instrument or analyst at the time of measurement; must never be altered; all subsequent processing produces derived data files that preserve the original.

5. Safety and Hazards

Data export is a computational activity with no physical hazards. When interacting with instruments (connecting USB drives, accessing instrument PCs), follow the safety protocols in the relevant instrument SOP.

6. Equipment and Materials

6.1. Instruments and Export Formats

Instrument	Native Format	Preferred Export	Notes
Shimadzu UV-Vis (UV-1900i)	`.spc` / `.csv`	CSV	Export from UVProbe software
Thermo iCAP ICP-OES	`.rst` / `.csv`	CSV	Export from Qtegra software
Shimadzu TOC-L	`.toa` / `.csv`	CSV	Export from TOC-Control
Dionex ICS Ion Chromatograph	`.cdf` / `.csv`	CSV	Export from Chromeleon
Mettler Toledo balance	Direct serial output	CSV via terminal log	Use logging script
YSI ProDSS multiparameter	`.csv` / `.dat`	CSV	Download via KorDSS software
Hach DR3900 spectrophotometer	Internal memory	CSV via USB	Export from instrument menu
Bio-Rad QX200 ddPCR	`.qlp` / `.csv`	CSV	Export from QuantaSoft
PANalytical XRF	`.csv` / `.xls`	CSV	Export from SuperQ

6.2. Transfer Media

Item	Purpose	Notes
USB flash drive (lab)	Sneakernet transfer	Dedicated drive; virus-scanned

Item	Purpose	Notes
Network shared folder	Direct instrument PC transfer	If instrument PC has network access
RStudio Server upload	Upload to analysis environment	Via Files pane or `scp`

7. Procedure

7.1. General Export Workflow

The same sequence applies to all instruments:

1. **Complete the analytical run** and verify results on the instrument software
2. **Export raw data** from the instrument software to CSV format (or the most open/portable format available)
3. **Name the file** following the convention: `[instrument]_[method]_[YYYY-MM-DD]_[batch].[ext]` (e.g., `icpoes_metals_2026-05-21_batch01.csv`)
4. **Transfer the file** to the project's `data/raw/` directory using USB or network transfer
5. **Verify the transfer** by opening the file and checking row counts, column headers, and a few spot-checked values against the instrument display
6. **Record the export** in the lab notebook: file name, instrument, date, number of samples, and any export settings
7. **Commit to Git** with a descriptive message

> ⚠ Critical Rule
>
> Never modify raw instrument files after export. If corrections are needed, create a new file in `data/processed/` and document the changes in your processing script. The raw file is your permanent record.

7.2. Instrument-Specific Export Instructions

7.2.1. Shimadzu UV-Vis (UV-1900i / UVProbe)

1. In UVProbe, open the data file (`.spc`)
2. File > Save As > CSV
3. Select "Wavelength and Absorbance" columns
4. Save to the USB drive as `uvvis_[method]_[YYYY-MM-DD].csv`

7.2.2. Thermo iCAP ICP-OES (Qtegra)

1. In Qtegra, navigate to the completed sequence
2. Right-click the sequence > Export Results
3. Select CSV format; include sample IDs, element concentrations, and QC flags
4. Save to the USB drive as `icpoes_[method]_[YYYY-MM-DD]_[batch].csv`

7.2.3. Shimadzu TOC-L (TOC-Control)

1. In TOC-Control, open the sample table for the completed run
2. File > Export > CSV
3. Include sample name, injection number, area, concentration, and CV
4. Save as `toc_[method]_[YYYY-MM-DD].csv`

7.2.4. YSI ProDSS Field Sensor

1. Connect the ProDSS to a computer via USB
2. Open KorDSS software
3. Select the data log for the sampling event
4. Export as CSV, including date/time, site ID, and all parameters
5. Save as `ysi_[project]_[YYYY-MM-DD].csv`

7.2.5. Bio-Rad QX200 ddPCR (QuantaSoft)

1. In QuantaSoft, open the completed experiment
2. Options > Export CSV (Well Data or Cluster Data)
3. Include well ID, sample name, target, concentration, positive/negative droplet counts
4. Save as `ddpcr_[target]_[YYYY-MM-DD].csv`
5. Also export the amplitude data for gating verification

7.3. File Transfer to RStudio Server

After exporting to USB or local machine:

```
# Option 1: scp from local machine to RStudio Server
scp icpoes_metals_2026-05-21_batch01.csv \
  username@rstudio.pomona.edu:~/project/data/raw/

# Option 2: Use the RStudio Files pane
# Click Upload > Browse > select file > OK
```

7.4. Automated Import Scripts in R

Create a standardized import script for each instrument type. This ensures consistent parsing across analysts and batches:

```
#' Import ICP-OES data from Qtegra CSV export
#'
#' @param file_path Path to the exported CSV file
#' @return A tibble with standardized column names
import_icpoes <- function(file_path) {
  raw <- read_csv(file_path, skip = 2)  # Skip Qtegra header rows
```

```
  raw %>%
    rename_with(tolower) %>%
    rename_with(~ gsub(" ", "_", .)) %>%
    mutate(
      date_analyzed = as.Date(date_analyzed, format = "%m/%d/%Y"),
      across(where(is.character) & !c(sample_id, sample_type),
             as.numeric)
    ) %>%
    # Flag values below detection
    mutate(across(
      where(is.numeric),
      ~ if_else(. < 0, NA_real_, .)
    ))
}

# Usage
icp_data <- import_icpoes("data/raw/icpoes_metals_2026-05-21_batch01.csv")
glimpse(icp_data)
```

```
#' Import TOC data from TOC-Control CSV export
#'
#' @param file_path Path to the exported CSV file
#' @return A tibble with standardized column names
import_toc <- function(file_path) {
  raw <- read_csv(file_path)

  raw %>%
    rename_with(tolower) %>%
    rename_with(~ gsub("[^a-z0-9]", "_", .)) %>%
    select(sample_name, injection, area, concentration_mg_l = conc,
           cv_percent = cv) %>%
    filter(!is.na(sample_name))
}
```

7.5. Data Integrity Checks

After importing, run basic integrity checks:

```
# --- Row count matches instrument ---
cat(sprintf("Rows in file: %d\n", nrow(icp_data)))
# Compare with number of samples in lab notebook

# --- No fully blank rows ---
stopifnot(!any(rowSums(is.na(icp_data)) == ncol(icp_data)))

# --- Sample IDs match the run sequence ---
```

```
expected_ids <- c("Blank", "Std1", "Std2", "Std3",
                  paste0("S", sprintf("%03d", 1:20)),
                  "CRM", "Dup")
setdiff(expected_ids, icp_data$sample_id)
# Should be empty if all expected samples are present

# --- Calibration standards in expected range ---
icp_data %>%
  filter(grepl("^Std", sample_id)) %>%
  select(sample_id, starts_with("fe_")) %>%
  print()
```

7.6. Archival and Backup

All raw instrument files must be archived in three locations:

Location	When	Responsible
GitHub repository (`data/raw/`)	Immediately after export	Analyst
Lab instrument PC	Retained on instrument	Lab Manager
External backup drive	End of each semester	Lab Manager

> **Tip**
>
> Large binary files (e.g., raw `.spc` or `.qlp` files that exceed GitHub's file size recommendations) should be stored on the external backup drive and the network share, not in Git. Only the exported CSV goes into the repository.

7.7. Instrument Log Cross-Reference

Every data export event must be recorded in the instrument log (see SOP-L007). The log entry should include:

1. Date and time of the analytical run
2. Analyst name
3. File name(s) exported
4. Number of samples (including QC samples)
5. Any instrument warnings or anomalies noted during the run
6. Cross-reference to the lab notebook page number

8. Quality Control and Acceptance Criteria

- Raw files must be unmodified after export (verify with file hash if needed: `md5sum filename.csv`)

- File names must follow the naming convention in Section 6.1
- Row counts and sample IDs must match the run sequence recorded in the lab notebook
- Calibration data in the export must match the calibration curve approved during the run
- Refer to SOP-L802 for analytical QC criteria (blanks, duplicates, recoveries)

9. Waste Disposal

Not applicable (computational procedure).

10. References

Gandrud, C. (2015). *Reproducible research with R and RStudio* (2nd ed.). CRC Press.

11. Training Record

Training is documented in the EA Program training log. Before exporting instrument data independently, personnel must:

1. Read this SOP in full
2. Complete training on the specific instrument (see the instrument-specific SOP)
3. Demonstrate a complete export, transfer, verification, and commit workflow under supervision
4. Be signed off by the PI or lab manager

12. Appendix A: File Hash Verification

To confirm that a file has not been modified after export, compute and record an MD5 hash:

```
# On Linux / Mac / RStudio Server terminal
md5sum data/raw/icpoes_metals_2026-05-21_batch01.csv
# Record this hash in the lab notebook alongside the file name
```

```
# In R
tools::md5sum("data/raw/icpoes_metals_2026-05-21_batch01.csv")
```

13. Appendix B: Instrument Data Format Quick Reference

Instrument	Header Rows to Skip	Delimiter	Encoding	Date Format
Shimadzu UV-Vis	1	Comma	UTF-8	MM/DD/YYYY

Instrument	Header Rows to Skip	Delimiter	Encoding	Date Format
Thermo iCAP	2	Comma	UTF-8	MM/DD/YYYY HH:MM
Shimadzu TOC-L	0	Comma	Shift-JIS (convert to UTF-8)	YYYY/MM/DD
Dionex ICS	3	Comma	UTF-8	MM/DD/YYYY HH:MM
YSI ProDSS	0	Comma	UTF-8	MM/DD/YYYY HH:MM
Bio-Rad QX200	0	Comma	UTF-8	YYYY-MM-DD
PANalytical XRF	Variable	Tab or comma	UTF-8	DD/MM/YYYY

Environmental Analysis Program
Pomona College
Biogeochemistry Laboratory

SOP-XXX

SOP Under Development

Standard Operating Procedure

SOP Number	SOP-XXX
Revision	–
Author(s)	
Date	2026-06-26
Organization	EA Program, Pomona College

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Environmental Analysis Program
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SOP-XXX

Title of Procedure

Standard Operating Procedure

SOP Number	SOP-XXX
Revision	0.1
Author(s)	Your Name
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

[State the objective of this SOP. What does this procedure accomplish, and why does it matter? Cite at least one key reference.]

2. Scope

- **Applies to:** [equipment, materials, processes, or sites covered]
- **Does not apply to:** [exclusions; link to the SOP that does cover them, e.g., "quantitative cover estimation (see SOP-F603)"]
- **Location:** [room number, building, field site, or "All EA Program field sites"]

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **[Term 1]:** [Definition, with units and citation if applicable]
- **[Term 2]:** [Definition]
- **[Term 3]:** [Definition]

5. Safety and Hazards

⚠ Hazards

- **Physical:** [e.g., heat, pressure, sharp objects]
- **Chemical:** [e.g., corrosive reagents, toxic fumes]
- **Biological:** [e.g., soil-borne pathogens, ticks, poison oak]

5.1. Required PPE

☐ Safety glasses / goggles
☐ Lab coat or field-appropriate clothing
☐ Nitrile gloves
☐ [Other PPE as needed]

5.2. Required Training

- SOP-F001 Field Safety *(field SOPs)*
- SOP-L002 Laboratory Safety *(lab SOPs)*

5.3. Emergency Procedures

Refer to SOP-F001 or SOP-L004 as appropriate.

6. Equipment and Materials

Item	Location	Notes
[Equipment name]	[Room / bench]	[Model number, if relevant]

7. Estimated Time

Task	Time
[Setup / preparation]	[XX minutes]
Main procedure	[XX minutes]
[Cleanup / data entry]	[XX minutes]

8. Procedure

8.1. Preparation

1. [First preparatory step]
2. [Second preparatory step]

8.2. Main Procedure

1. [Step-by-step instructions]
2. [Be specific: settings, volumes, durations]
3. [Include decision points as needed]

> **Tip**
>
> Include practical tips or common mistakes here. These callout boxes render nicely in both HTML and PDF.

8.3. Shutdown and Cleanup

1. [Post-procedure steps]
2. [Equipment shutdown sequence]

9. Quality Control and Acceptance Criteria

- [Expected result, measurement, or visual indicator]
- [Acceptable range or pass/fail criteria]
- [What to do if results are outside the acceptable range]

10. Data Management

1. Enter data from field sheets into the project spreadsheet within 48 hours
2. Required fields: [list the minimum set]
3. Version-control analysis scripts per SOP-L801

11. Waste Disposal

Waste Type	Container	Disposal Method
[e.g., solvent]	[e.g., red jug]	[EH&S pickup schedule]

12. References

13. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
XXXX	[Title]	[Relationship]

14. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. [Additional skill-specific requirements]
5. Be signed off by the PI or designated trainer

15. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
[Symptom]	[Cause]	[Fix]