

WATER AND AQUATIC METHODS

Field Sampling and Laboratory Analysis for Water Quality

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

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Environmental Analysis Program Coordinator



STANDARD OPERATING PROCEDURE (SOP) FOR WATER SCIENCE: FIELD AND LABORATORY WORK



Accurate Measurement. Proper Handling. Reliable Data.

1. PURPOSE	2. WATER SCIENCE PARAMETERS	3. ROLES & RESPONSIBILITIES
To provide standardized procedures for collecting, measuring, analyzing, and documenting water quality and quantity data in the field and laboratory to ensure accuracy, consistency, and data integrity.	Temperature (°C) pH Electrical Conductivity (µS/cm) Dissolved Oxygen (mg/L) Turbidity (NTU) Nutrients (N, P) Hardness (mg/L as CaCO ₃) Other Parameters (TSS, BOD, COD, Alkalinity, etc.)	Field Team Leader: Planning, site selection, safety, quality assurance. Field Technician: Sampling, in-situ measurements, documentation. Laboratory Analyst: Sample analysis, calibration, data recording. Data Manager: Data validation, storage, reporting.

4. EQUIPMENT OVERVIEW			
FIELD MEASUREMENT INSTRUMENTS		SAMPLING EQUIPMENT	FIELD SUPPORT ITEMS
			
			
			
			
• Multi-parameter Water Quality Meter (pH, EC, TDS, Temp.)	• Dissolved Oxygen (DO) Meter	• Turbidimeter	• GPS
• Flow Meter / Current Meter	• Van Dorn Sampler	• Grab Sampler	• Peristaltic Pump
• Sample Bottles & Containers	• Cooler / Ice	• Gloves	• Labels & Marker
• Field Notebook	• pH/EC Calibration Solutions		

5. FIELD PROCEDURES				
5.1 SITE SELECTION	5.2 IN-SITU MEASUREMENTS	5.3 WATER SAMPLING	5.4 SAMPLE PRESERVATION	5.5 FIELD DOCUMENTATION
				
• Define objective of sampling. • Select representative locations. • Consider land use, access, and safety. • Record GPS coordinates and site description.	1. Calibrate instruments (see Sec. 6). 2. Rinse probes with sample water. 3. Measure parameters: Temp, pH, EC, DO, Turbidity, etc. 4. Allow readings to stabilize. 5. Record values in field sheet.	1. Wear clean gloves. 2. Rinse sample bottle 2-3 times with sample (if appropriate). 3. Collect sample at mid-depth (avoid surface film and bottom). 4. Leave minimal headspace. 5. Label immediately. 6. Preserve if required (see Sec. 7).	• Follow preservation requirements (see table below). • Use proper reagents and volumes. • Keep samples cool (≤4°C). • Transport to lab within holding time.	• Date, time, weather conditions. Location (GPS), site code. • Sample ID, depth, method. • Instruments used & calibration. • Observations and notes.

6. INSTRUMENT CALIBRATION (FIELD)	7. SAMPLE PRESERVATION & HOLDING TIMES	8. SAMPLE TRANSPORT																																																
<table border="1"> <thead> <tr> <th>PARAMETER</th><th>CALIBRATION SOLUTIONS / METHOD</th><th>FREQUENCY</th></tr> </thead> <tbody> <tr> <td>pH</td><td>pH 4.00, 7.00, 10.00 buffers</td><td>Daily / Before use</td></tr> <tr> <td>EC</td><td>1413 µS/cm standard solution</td><td>Daily / Before use</td></tr> <tr> <td>DO</td><td>Air saturation or zero DO solution</td><td>Daily / Before use</td></tr> <tr> <td>Turbidity</td><td>0, 20, 100, 800 NTU standards</td><td>Daily / Before use</td></tr> <tr> <td>Others</td><td>Follow manufacturer guidelines</td><td>As required</td></tr> </tbody> </table> - Record calibration results in field log. - Recalibrate if readings drift or after extreme conditions.	PARAMETER	CALIBRATION SOLUTIONS / METHOD	FREQUENCY	pH	pH 4.00, 7.00, 10.00 buffers	Daily / Before use	EC	1413 µS/cm standard solution	Daily / Before use	DO	Air saturation or zero DO solution	Daily / Before use	Turbidity	0, 20, 100, 800 NTU standards	Daily / Before use	Others	Follow manufacturer guidelines	As required	<table border="1"> <thead> <tr> <th>PARAMETER</th><th>PRESERVATION</th><th>HOLDING TIME</th></tr> </thead> <tbody> <tr> <td>pH, EC, DO, Temp.</td><td>None (on-site measurement)</td><td>Measure on-site</td></tr> <tr> <td>Turbidity</td><td>None</td><td>48 hours</td></tr> <tr> <td>TSS</td><td>Cool ≤4°C</td><td>7 days</td></tr> <tr> <td>Nutrients (Nitrate/Nitrite)</td><td>Cool ≤4°C</td><td>48 hours</td></tr> <tr> <td>Nutrients (Total N, Total P)</td><td>H₂SO₄ to pH=2, Cool ≤4°C</td><td>28 days</td></tr> <tr> <td>BOD</td><td>Cool ≤4°C</td><td>48 hours</td></tr> <tr> <td>COD</td><td>H₂SO₄ to pH=2, Cool ≤4°C</td><td>7 days</td></tr> <tr> <td>Metals</td><td>HNO₃ to pH=2, Cool ≤4°C</td><td>6 months</td></tr> <tr> <td>Microbiological</td><td>Cool ≤4°C</td><td>6-24 hours</td></tr> </tbody> </table>	PARAMETER	PRESERVATION	HOLDING TIME	pH, EC, DO, Temp.	None (on-site measurement)	Measure on-site	Turbidity	None	48 hours	TSS	Cool ≤4°C	7 days	Nutrients (Nitrate/Nitrite)	Cool ≤4°C	48 hours	Nutrients (Total N, Total P)	H ₂ SO ₄ to pH=2, Cool ≤4°C	28 days	BOD	Cool ≤4°C	48 hours	COD	H ₂ SO ₄ to pH=2, Cool ≤4°C	7 days	Metals	HNO ₃ to pH=2, Cool ≤4°C	6 months	Microbiological	Cool ≤4°C	6-24 hours	- Check labels and sealing. - Store in cooler with ice packs. - Transport to laboratory ASAP. - Avoid direct sunlight and extreme temperatures. - Submit chain-of-custody form with samples. - Log samples upon arrival in the laboratory.
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9. LABORATORY PROCEDURES					
9.1 SAMPLE RECEIPT	9.2 SAMPLE PREPARATION	9.3 ANALYSIS	9.4 QUALITY CONTROL	9.5 DATA MANAGEMENT	9.6 REPORTING
 • Check sample IDs. • Verify chain-of-custody. • Inspect condition. • Record date/time of receipt. • Store appropriately.	 • Mix gently (do not shake). • Filter if required (0.45 µm). • Dilute if necessary. • Prepare blanks and standards.	 • Follow Standard Methods. • Run blanks, duplicates, and standards. • Use calibrated instruments. • Record raw data.	 • Check blanks (should be low). • Verify standards (recovery 90-110%). • Run duplicates (RPD < 20%). • Document all QC results.	 • Enter data correctly. • Verify and validate. • Backup data regularly. • Maintain clear records.	 • Summarize results. • Compare with guidelines. • Provide interpretation. • Archive all records.

10. COMMON WATER SAMPLING LOCATIONS	11. BEST PRACTICES	12. SAFETY IN WATER WORK
River (Surface) Stream (Midstream) Lake (Mid-depth) Groundwater (Well)	- Use clean equipment and bottles. - Calibrate instruments regularly. - Follow standard methods and SOPs. - Maintain sample integrity. - Document everything.	- Wear appropriate PPE. - Be cautious near deep or fast-flowing water. - Do not work alone. - Avoid contamination of samples. - Follow local health and safety regulations.



GOOD DATA. CLEAN WATER. HEALTHY ENVIRONMENT.



Pomona College
Claremont, California

WATER AND AQUATIC METHODS

Water and Aquatic Methods

Field sampling and laboratory analysis for water quality and aquatic systems

EA Program – Biogeochemistry Lab, Pomona College

Compiled: June 26, 2026

SOP Number	Title
SOP-F300	Surface Water Sampling
SOP-F301	Stream Discharge Measurement – Wadable Streams
SOP-F302	Stormwater Sampling
SOP-F303	Groundwater Monitoring Well Sampling
SOP-F304	Stream Physical Habitat Assessment – SWAMP Protocol
SOP-F305	Sediment Sampling – Streams and Lakes
SOP-F306	Wetland Hydrology Monitoring
SOP-F307	Lake and Reservoir Profiling
SOP-F307	Wetland Delineation
SOP-F308	eDNA Water Sample Collection
SOP-F315	Bird Point Count Surveys
SOP-L300	pH and Conductivity – Bench-Top Measurements
SOP-L301	Alkalinity – Titration Method
SOP-L303	Hach Colorimetric Analysis Spectrophotometer / Colorimeter
SOP-L305	IDEXX Quanti-Tray 2000 for *E. coli*/Total Coliform Enumeration
SOP-L306	Biochemical Oxygen Demand (BOD~5~)
SOP-L307	ELISA: Bisphenol A, Bisphenol B, and Pesticides in Water
SOP-L308	Microplastics in Water – Extraction and Enumeration
SOP-L309	Pesticide Analysis via Gas Chromatography
SOP-L310	Chlorophyll *a* Extraction and Fluorometric Analysis
SOP-L311	Dissolved Organic Carbon in Water – Shimadzu TOC-L (NPOC Mode)
SOP-L312	Total Suspended Solids (TSS) and Total Dissolved Solids (TDS)
SOP-L313	Metals in Water (ICP-MS Prep and Acidification)
SOP-L315	eDNA Laboratory Processing



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, $\text{Na}_2\text{S}_2\text{O}_3$ 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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15 Appendix A: Field Data Sheet

8

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	$0.6 \times$ depth from the surface	Depth ≤ 2.5 ft or Pygmy
Two-point	0.2 and $0.8 \times$ depth (average the two)	Depth > 2.5 ft
Surface	$0.2 \times$ 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)	Revolu- tions	Time (s)	Velocity (ft/s)	Width (ft)	Q_sub (cfs)
-----------------	---------------	-----------------------------------	------------------	----------	--------------------	---------------	----------------

Station (ft)	Depth (ft)	Meter depth setting (ft)	Revolu- tions	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
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 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 ₂ SO ₄ to pH < 2; 4 °C	28 days
Ammonia	Poly, 250 mL	H ₂ SO ₄ to pH < 2; 4 °C	28 days
Total phosphorus	Poly, 250 mL	H ₂ SO 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 / <i>E. coli</i>	Sterile, 100 mL	Na ₂ S ₂ O 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.

California State Water Resources Control Board. (2017). *Quality assurance program plan*. Surface Water Ambient Monitoring Program (SWAMP). https://www.waterboards.ca.gov/water_issues/programs/swamp/

California State Water Resources Control Board. (2018). *Storm water sampling guidance document for compliance with California construction and industrial general permits*. https://www.waterboards.ca.gov/water_issues/programs/stormwater/docs/draft_cnst_sample_guidance.pdf

U.S. Environmental Protection Agency. (2002). *Urban stormwater BMP performance monitoring: A guidance manual for meeting the national stormwater BMP database requirements* (EPA-821-B-02-001). Office of Water, U.S. EPA. https://www.epa.gov/sites/default/files/2015-10/documents/2002_10_24_npdes_swbmp_bmpguide.pdf

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
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 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 CO₂ and O₂ 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 dewateres 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 ₂ SO 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)
- 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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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

Elapsed Time (min)	Cum. Vol. (L)	Flow (L/min)	Draw- down (cm)	pH	Temp (°C)	SpC (µS/cm)	DO (mg/L)	ORP (mV)	Turbidity (NTU)
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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
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 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 $37.5\times$ 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 ($37.5 \times$ 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)

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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	Densimeter 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 densimeter 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% HNO₃) 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

i 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

- Mudroch, A., Azcue, J. M., & Mudroch, P. (1999). *Manual of physico-chemical analysis of aquatic sediments*. CRC Press. <https://doi.org/10.1201/9781003069823>
- US Environmental Protection Agency. (2001). *Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: Technical manual* (EPA-823-B-01-002). Office of Water, US EPA. <https://www.epa.gov/sites/default/files/2015-09/documents/collectionmanual.pdf>

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 Levellogger, 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

i 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
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 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 $1\text{ }^{\circ}\text{C m}^{-1}$; 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
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 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)	<i>Jepson Manual</i> 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.

i 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).

- Record depth to surface water or saturated soil (standing water in the pit).
- 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)

- 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](#)).

i 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.csv)

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
Organization	EA Program, Pomona College

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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 m²), 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-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	<i>Sibley Guide to Birds</i> or <i>Kaufman Field Guide</i>
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
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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, ground-water, 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 $\mu\text{S}/\text{cm}$ 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: $\text{pH} = -\log_{10}[H^+]$; 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	<i>TBD – confirm model (e.g., Accumet AB150, Orion Star, or equivalent)</i>
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 $\mu\text{S}/\text{cm}$ standard after every 10 samples. If the reading has drifted by more than $\pm 5\%$, recalibrate.
- Run duplicate measurements on at least 10% of samples. Acceptable precision: ± 0.1 pH units and $\pm 5\%$ 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.
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- 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 CaCO₃ (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 CaCO₃; in natural waters, primarily contributed by bicarbonate (HCO₃⁻), carbonate (CO₃²⁻), 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
Bromocresol green	4.5	Total (T) alkalinity
Mixed bromocresol 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: $\pm 5\%$ 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 ₂ CO 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 H₂SO₄ 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 H₂SO₄ from Concentrated H₂SO₄

Concentrated H₂SO₄ 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 H₂SO₄ 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 Na₂CO₃ (Section 6.1)

12.2. N H₂SO₄ from 0.1 N Stock

1. Transfer 200.00 mL of standardized 0.1000 N H₂SO₄ 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
Revision	1.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the 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 $\text{NO}_3^-/\text{NH}_4^+$ 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: $A = \epsilon cl$, 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 $\pm 10\%$ 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 $5 \times$ 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

Ana-lyte	Hach Method	Pro-gram No.	Reagent / Cat. No.	Instru-ment	Sample Range Vol.	Reac-tion Time	Key Inter-ferences	Sta-tus
Re-ac-tive P (ortho-P)	PhosVer 3 (Ascorbic Acid)	490	2106069 (pillows)	DR3900, DR900	0.02–2.50 mL mg/L PO ₄ ⁻ P	2 min	Silica above 200 mg/L; arsenate	Ac-tive
To-tal Phos-pho-rus	Acid persul-fate diges-tion + PhosVer 3	536	TNT843	DR3900 + DRB200	0.06–3.50 mL mg/L PO ₄ ⁻ P	30 min dig. + 2 min	High turbidity	TBD
Ni-trate (NO ₃ ⁻)	Cad-mium reduc-tion	355	2106169 (pillows)	DR3900, DR900	0.1–30.0 mL mg/L NO ₃ ⁻ N	5 min	NO ₂ ⁻ above 2 mg/L; Cl ⁻ above 100 mg/L	Ac-tive
Ni-trate (NO ₃ ⁻) HR	Chro-motropic acid	410	TNT835/TNT836	DR3900	0.2–30.0 mL mg/L NO ₃ ⁻ N	15 min	Cl ⁻ above 500 mg/L	TBD
Ni-trite (NO ₂ ⁻)	Diazo-tiza-tion	371	2107169 (pillows)	DR3900, DR900	0.002–0.300 mL mg/L NO ₂ ⁻ N	20 min	Strong oxidizers	TBD
Am-mo-nia LR	Salicy-late	385	2668000 (pillows)	DR3900, DR900	0.01–0.50 mL mg/L NH ₃ ⁻ N	20 min	Ca above 1000 mg/L CaCO ₃	Ac-tive
Am-mo-nia HR	Salicy-late HR	343	TNT832	DR3900	0.4–50.0 mL mg/L NH ₃ ⁻ N	15 min	Same as LR	TBD
To-tal Ni-tro-gen	Persul-fate dig. + chro-motropic acid	350	TNT826/TNT827	DR3900 + DRB200	1–25 mL mg/L N	30 min dig. + 15 min	–	TBD

8.2. Metals

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Sample Range Vol.	Reaction Time	Key Interferences	Status
Iron (total)	FerroVer	265	2105669 (pillows)	DR3900, DR900	0.02–3.100 mL mg/L Fe	3 min	Cu above 5 mg/L	Active
Iron (Fe ²⁺)	1,10-Phenanthroline	255	2105769 (pillows)	DR3900, DR900	0.02–3.100 mL mg/L Fe	3 min	Strong oxidizers	TBD
Manganese	PAN	290	Check specific kit	DR3900	0.006–0.700 mL mg/L Mn	2 min	Fe above 5 mg/L	TBD
Copper	Bicinchoninate	135	2105969 (pillows)	DR3900, DR900	0.04–5.100 mL mg/L Cu	2 min	Fe above 10 mg/L	TBD
Zinc	Zincon	780	Check specific kit	DR3900	0.02–3.100 mL mg/L Zn	Immediate	Cu, Pb, Co above 5 mg/L	TBD
Lead	Lead-Trak (dithionite zone)	277	2877200 (AccuVac)	DR3900	5–150 25 mL µg/L Pb	1 min	Cu, Hg, Bi, Sn	TBD
Chromium (Cr ⁶⁺)	DPC	090	Check specific kit	DR3900, DR900	0.010–0.700 mL mg/L Cr	5 min	Fe above 1 mg/L	TBD

8.3. Other Parameters

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Sample Range Vol.	Reaction Time	Key Interferences	Status
Hardness (total)	Cal-MagVer 3	105	2429800 (pillows)	DR3900, DR900	10–4000 mL mg/L as CaCO ₃	Immediate	Mn above 4 mg/L	TBD
Chloride	Mercuric thiocyanate	070	2268500 (pillows)	DR3900, DR900	0.1–25.10 mL mg/L Cl ⁻	Immediate	High turbidity	TBD
Silica	Silicomolybdate	656	2429600 (pillows)	DR3900	1–100 10 mL mg/L SiO ₂	10 min	PO ₄ ; sulfide	TBD
Sulfate	SulfateVer 4	680	2106769 (pillows)	DR3900, DR900	2–70 10 mL mg/L SO ₄	5 min	Silica	TBD

Ana-lyte	Hach Method	Pro-gram No.	Reagent / Cat. No.	Instru-ment	Sample Range Vol.	Reac-tion Time	Key Inter-ferences	Sta-tus
COD	Dichro-mate reactor	430	TNT821/822/823	DR3900 + DRB200	3–15002 mL mg/L	2 hr dig.	Cl ⁻ above 2000 mg/L	<i>TBD</i>

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](https://www.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 $\pm 10\%$ 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: $\pm 10\%$ of the stated value (Hach Company, 2024a).
- **Duplicates:** Analyze at least 10% of samples in duplicate; acceptable RPD less than 20% above $5 \times$ 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
Date	2026-06-26
Organization	EA Program, Pomona College

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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 <i>E. coli</i> 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
<i>Escherichia coli</i>	Yellow wells, fluorescence
<i>Klebsiella pneumoniae</i>	Yellow wells, no fluorescence
<i>Pseudomonas aeruginosa</i>	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 (BOD₅)

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 (BOD₅) in water and wastewater samples using the manometric method (WTW OxiTop system). BOD₅ 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 BOD₅ 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).
- **BOD₅:** 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 BOD₅ 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 BOD₅ 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 O₂ consumption is tracked by measuring the resulting decrease in headspace pressure; CO₂ 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 (CO₂ 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 KH₂PO₄, 21.75 g K₂HPO₄, 33.4 g Na₂HPO₄ · 7H₂O, and 1.7 g NH₄Cl in ~500 mL deionized water and dilute to 1 L (pH 7.2).
2. **Magnesium sulfate:** Dissolve 22.5 g MgSO₄ · 7H₂O in deionized water; dilute to 1 L.
3. **Calcium chloride:** Dissolve 27.5 g CaCl₂ in deionized water; dilute to 1 L.
4. **Ferric chloride:** Dissolve 0.25 g FeCl₃ · 6H₂O 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 BOD₅ 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 BOD₅ 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 BOD₅ 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 BOD₅ internally from the headspace pressure decrease (WTW (Xylem Analytics), 2019). Record the displayed mg/L value directly.

9.2. Blank Correction

$$\text{BOD}_{5,\text{corrected}} = \text{BOD}_{5,\text{sample}} - \text{BOD}_{5,\text{blank}}$$

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:

$$\text{BOD}_{5,\text{final}} = \text{BOD}_{5,\text{corrected}} \times \frac{V_{\text{total}}}{V_{\text{sample}}}$$

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 BOD₅ = 198 ±30.5 mg/L (APHA et al., 2012, Method 5210 B).
- Blank BOD₅ 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; reseal 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 BOD₅ 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 H₂SO₄), 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 $\mu\text{g/L}$	50 μL	Active
Bisphenol B	Abraxis	<i>TBD</i>	<i>TBD</i>	50 μL	<i>TBD</i>
Atrazine	Abraxis	500001	0.04–5.0 $\mu\text{g/L}$	50 μL	Active
Glyphosate	Abraxis	500086	0.075–4.0 $\mu\text{g/L}$	50 μL	<i>TBD</i>
2,4-D	Abraxis	500201	0.05–5.0 $\mu\text{g/L}$	50 μL	<i>TBD</i>

i 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:

```
# 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 $\mu\text{g/L}$ (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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- Voller, A., Bartlett, A., & Bidwell, D. E. (1978). The enzyme-linked immunosorbent assay (ELISA). *Journal of Clinical Pathology*, 31(6), 507–520. <https://doi.org/10.1136/jcp.31.6.507>

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 $-20\text{ }^{\circ}\text{C}$) 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	<i>TBD catalog number</i>
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 ₂ O 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.

California State Water Resources Control Board. (2022). *Policy handbook establishing a standard method of testing and reporting of microplastics in drinking water*. California State Water Resources Control Board. https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/microplastics.html

Erni-Cassola, G., Gibson, M. I., Thompson, R. C., & Christie-Oleza, J. A. (2017). Lost, but found with Nile Red: A novel method for detecting and quantifying small microplastics and nanoplastics in environmental samples. *Environmental Science & Technology*, 51(23), 13641–13648. <https://doi.org/10.1021/acs.est.7b04512>

Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports*, 7, 44501. <https://doi.org/10.1038/srep44501>

Thornton Hampton, L. M., Mehinto, A. C., & Weisberg, S. B. (2025). *Standard operating procedures for the collection of samples for microplastics analysis part 1: Surface sediment and aquatic biota* (Technical Report #1410.A). Southern California Coastal Water Research Project (SCCWRP). <https://www.sccwrp.org/>

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	<i>TBD</i>	Critical for quantitative results
Organic matter interference assessment	<i>TBD</i>	Needed for high-organic samples
Polymer identity confirmation (FTIR access)	<i>TBD</i>	Lab does not currently have FTIR
Microplastic size class definition	<i>TBD</i>	Original SOP noted “DEFINE SIZE”
Nile Red and acetone catalog numbers	<i>TBD</i>	Add to materials table
Hydrogen peroxide digestion protocol	<i>TBD</i>	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 $\times$ 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 $\pm 15\%$ 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):** $|A - B| / ((A + B) / 2) \times 100$; used to assess duplicate precision; acceptance criterion is RPD $\leq 20\%$ 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 $\pm 15\%$; 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)
- McNair et al. (2019)

McNair, H. M., Miller, J. M., & Snow, N. H. (2019). *Basic gas chromatography* (3rd ed.). John Wiley; Sons. <https://doi.org/10.1002/9781119450795>

United States Environmental Protection Agency. (2007). *Method 8081B: Organochlorine pesticides by gas chromatography* [SW-846]. U.S. EPA, Office of Solid Waste. <https://www.epa.gov/hw-sw846/sw-846-test-method-8081b-organochlorine-pesticides-gas-chromatography>

United States Environmental Protection Agency. (2018). *Method 8270E: Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS)* [SW-846]. U.S. EPA, Office of Resource Conservation; Recovery. <https://www.epa.gov/hw-sw846/sw-846-test-method-8270e-semivolatile-organic-compounds-gas-chromatographymass>

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



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L310

Chlorophyll *a* Extraction and Fluorometric Analysis

Standard Operating Procedure

SOP Number	SOP-L310
Revision	0.1
Author(s)	Kristy Morris, Virginia Paschal
Date	2026-06-26
Organization	EA Program, Pomona College

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13 Appendix B: Interferences

9

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 <i>SOP-L109</i>
Fume hood	Required for all acetone and HCl work

6.2. Consumables

Item	Notes
Whatman GF/F glass fiber filters (47 mm, 0.7 μ m)	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 <i>a</i> standard (free of chlorophyll <i>b</i>)	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)

i 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 $\pm 20\%$ of the known value
- Relative standard deviation (RSD) of replicate measurements should be within $\pm 20\%$
- Field duplicates should agree within $\pm 20\%$ 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.

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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 <i>b</i>	Overestimates chlorophyll <i>a</i>	Optical filters in the Chlor-A module minimize this; if severe, use HPLC
Pheophytin <i>a</i>	Overestimates chlorophyll <i>a</i> 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
Revision	0.2
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

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9

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 ($18.2 \text{ M}\Omega \cdot \text{cm}$) 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, $\text{KC}_8\text{H}_5\text{O}_4$):** The primary calibration standard for TOC analysis; a pure, non-hygroscopic organic acid weighed accurately to prepare standards of known carbon concentration; $1.0000 \text{ g KHP} = 0.4711 \text{ g C}$.
- **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 $^\circ\text{C}$ 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 TNM-L (optional)	Operated per SOP-L204 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 CO₂ 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 CO₂ 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

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

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: $RPD (\%) = |A - B| / ((A + B) / 2) \times 100$; 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 $\pm 10\%$ 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 $\text{RPD} \leq 20\%$ 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 HNO₃ dropwise to the sample container to achieve a final matrix concentration of 2% (v/v). For example, add 1.0 mL concentrated HNO₃ 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 HNO₃ 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) HNO₃ 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](#)
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 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