



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L103

Using Micropipettes

Standard Operating Procedure

SOP Number	SOP-L103
Revision	0.3
Author(s)	Isaac Medina
Date	2026-06-26
Organization	EA Program, Pomona College

FOR INTERNAL USE ONLY

This document is the property of the Environmental Analysis Program at Pomona College.
Unauthorized distribution is not permitted.

Table of contents

1 Purpose	2
2 Scope	2
3 Responsibilities	2
4 Definitions	2
5 Safety and Hazards	3
5.1 Required PPE	3
5.2 Emergency Procedures	3
6 Equipment and Materials	3
6.1 Equipment	3
6.2 Consumables	3
7 Procedure	4
7.1 Selecting the Correct Pipette	4
7.2 Setting the Volume	4
7.3 Loading a Tip	4
7.4 Aspirating (Loading) a Sample	5
7.5 Dispensing a Sample	5
7.6 Ejecting the Tip	5
7.7 Important Precautions	5
8 Quality Control and Acceptance Criteria	6
8.1 Gravimetric Accuracy Check	6
8.2 Calibration Schedule	6
9 Waste Disposal	6
10 References	7
11 Training Record	7
12 Appendix A: Volume Display Reading Guide	7

13 Appendix B: Troubleshooting

7

1. Purpose

This procedure describes the proper use, volume setting, tip loading, sample aspiration and dispensing, and care of Gilson Pipetman micropipettes. Micropipettes are precision instruments used to transfer small volumes of liquid (less than 1 mL) with high accuracy. Correct technique is essential for reproducible results in chemical, biological, and environmental analyses.

2. Scope

- **Applies to:** All Gilson Pipetman micropipettes (P10, P20, P200, P1000) in the EA Program laboratory
- **Does not apply to:** Serological pipettes, Pasteur pipettes, bottle-top dispensers, or Repipet-type dispensers
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure annual calibration
Lab Manager	Schedule calibration, maintain tip inventory
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Aerosol-resistant (filter) tip:** A pipette tip containing a hydrophobic filter that prevents aerosols and liquid from entering the pipette barrel; required when pipetting volatile, radioactive, infectious, or PCR-sensitive samples.
- **Air-displacement pipette:** The standard laboratory micropipette in which a piston displaces air in the tip, which in turn displaces liquid; the most common type; accuracy depends on tip seal quality and pipetting technique.
- **Dead volume:** The small volume of liquid remaining in the tip after dispensing; varies by tip type and affects accuracy at low volumes.
- **Forward pipetting:** The standard technique in which the plunger is depressed to the first stop before immersing the tip, then liquid is aspirated by releasing the plunger; used for aqueous solutions.
- **Reverse pipetting:** A technique in which the plunger is depressed past the second stop before aspirating; liquid is dispensed only to the first stop, leaving excess in the tip; used for viscous or volatile liquids to improve accuracy.

- **Systematic error:** A consistent, directional bias in pipette delivery; detected by gravimetric calibration; caused by worn seals, incorrect technique, or using tips not matched to the pipette model.
- **Volume range:** The minimum and maximum volume a given pipette model is designed to deliver accurately; pipetting below 10% of the maximum volume significantly increases relative error.

5. Safety and Hazards

Hazards

- **Chemical:** Liquids being pipetted may be hazardous. Consult the relevant SOP and Safety Data Sheet for the material being transferred.
- **Physical:** Repetitive pipetting can cause repetitive strain injuries. Take breaks during extended pipetting sessions.
- **Biological:** When pipetting biological samples, use filter tips to prevent aerosol contamination of the pipette barrel.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

If hazardous liquid enters the pipette barrel, stop immediately. Decontaminate the pipette per the manufacturer's instructions and refer to [SOP-L003 Hazardous Materials Handling](#).

6. Equipment and Materials

6.1. Equipment

Item	Volume Range	Notes
Pipetman P10	1.0 – 10.0 μL	Yellow or white tip
Pipetman P20	2.0 – 20.0 μL	Yellow tip
Pipetman P200	20 – 200 μL	Yellow tip
Pipetman P1000	200 – 1000 μL	Blue tip

6.2. Consumables

Item	Notes
Disposable pipette tips	Match tip size to pipette; see Section 6.3
Filter pipette tips	Required for biological or volatile samples
Tip waste container	Benchtop receptacle for used tips

7. Procedure

7.1. Selecting the Correct Pipette

Always select the smallest pipette that covers the target volume. Using an unnecessarily large pipette for a small volume reduces accuracy. The volume ranges are:

Pipette	Range	Smallest Graduation
P10	1.0 – 10.0 μL	0.02 μL
P20	2.0 – 20.0 μL	0.02 μL
P200	20 – 200 μL	0.2 μL
P1000	200 – 1000 μL	2 μL

! Important

Never set the pipette below the minimum or above the maximum of its range. Exceeding these limits will damage the piston seal and put the pipette out of calibration.

7.2. Setting the Volume

1. Identify the volume display window on the front face of the pipette. The three-digit display reads differently for each pipette size:
 - **P10:** digits represent ones, tenths, and hundredths of μL
 - **P20:** digits represent tens, ones, and tenths of μL
 - **P200:** digits represent hundreds, tens, and ones of μL
 - **P1000:** digits represent thousands, hundreds, and tens of μL
2. Turn the volume adjustment ring clockwise to increase volume or counterclockwise to decrease volume
3. If you overshoot the target while dialing down, dial below the desired setting and then dial back up to the correct value. This avoids mechanical backlash in the piston mechanism.

7.3. Loading a Tip

1. Select the correct tip size for the pipette being used. Tip boxes are labeled with the compatible pipette sizes. When in doubt, confirm with the lab manager.
2. Press the pipette shaft firmly into a tip in the tip box. A slight twist while pressing helps ensure a secure, airtight seal.

3. Do not touch the tip with your hands.
4. Always use a fresh tip for each different reagent or sample to prevent cross-contamination.

7.4. Aspirating (Loading) a Sample

1. Depress the plunger slowly to the **first stop** (the point of initial resistance). This is the calibrated load volume.
2. While holding the plunger at the first stop, immerse the tip into the liquid just enough to cover the opening (3–4 mm). Do not plunge the tip to the bottom of the vessel.
3. Slowly and steadily release the plunger to draw liquid into the tip. Do not let the plunger snap back.
4. Wait one to two seconds before removing the tip from the liquid, especially with viscous solutions. This allows the full volume to enter the tip and prevents air bubbles.
5. Visually inspect the tip: there should be no air gap or bubble at the distal end. If there is, dispense the liquid back and repeat.

Tip

For viscous liquids (glycerol, concentrated DNA solutions), use the “reverse pipetting” technique: depress the plunger to the **second stop** before immersing the tip, draw up the liquid by releasing to the rest position, and then dispense by pressing only to the **first stop**. The small amount of liquid remaining in the tip is discarded.

7.5. Dispensing a Sample

1. Touch the tip to the inner wall of the receiving vessel at a slight angle
2. Depress the plunger slowly to the **first stop** to dispense most of the liquid
3. Continue pressing to the **second stop** to expel the final drop
4. Hold the plunger at the second stop while withdrawing the tip from the vessel
5. Release the plunger only after the tip is clear of the liquid

7.6. Ejecting the Tip

1. Hold the pipette over the tip waste container
2. Press the tip ejector button on the back of the pipette
3. Never reuse a tip between different solutions

7.7. Important Precautions

- Never point a pipette upward with liquid in the tip. Liquid can flow back into the barrel and damage the piston and seals.
- Never lay a pipette down with a loaded tip.
- When not in use, set the pipette to its maximum volume and hang it vertically on the pipette stand. This relaxes the piston spring and extends the instrument’s life.
- Do not use a pipette as a stirring rod.

- If the plunger action feels rough, sticky, or inconsistent, stop using the pipette and report the issue to the lab manager.

8. Quality Control and Acceptance Criteria

8.1. Gravimetric Accuracy Check

Verify pipette accuracy by weighing dispensed volumes of distilled water on the analytical balance (see [SOP-L200](#)):

1. Tare a clean microcentrifuge tube on the analytical balance
2. Pipette the test volume of distilled water into the tube
3. Record the mass. At 20 C, 1 μL of water weighs approximately 1.00 mg.
4. Repeat for a total of 10 replicates
5. Calculate the mean and coefficient of variation (CV)

Pipette	Test Volume	Acceptable CV
P10	10 μL	less than 3%
P20	20 μL	less than 2%
P200	200 μL	less than 1%
P1000	1000 μL	less than 0.5%

If the CV exceeds the acceptable threshold, the pipette should be removed from service and sent for professional calibration.

8.2. Calibration Schedule

All micropipettes must be professionally calibrated at least once per year. Calibration stickers on each pipette show the date of last calibration and the next due date. Do not use a pipette with an expired calibration.

9. Waste Disposal

Waste Type	Disposal Method
used pipette tips (non-hazardous)	Standard tip waste box; dispose in regular trash when full
used tips contaminated with hazardous chemicals	Chemical waste per SOP-L003
used tips contaminated with biological material	Biohazard bag; autoclave before disposal

10. References

- Gilson. *Pipetman Classic: user's Guide*. Gilson, Inc., Middleton, WI.
- APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater*. 22nd ed. American Public Health Association (Eds.). Washington. 1360 pp.

11. Training Record

Before using micropipettes independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Demonstrate correct volume setting, tip loading, aspiration, and dispensing technique under supervision
4. Perform a gravimetric accuracy check (Section 7.1) with acceptable results for at least two pipette sizes
5. Be signed off by the PI or lab manager

12. Appendix A: Volume Display Reading Guide

The three-digit volume display reads differently for each Pipetman size. The table below shows the maximum display setting and the place value of each digit:

Pipette	Max Display	Digit 1 (top)	Digit 2 (middle)	Digit 3 (bottom)
P10	1 0 . 0 0	10 μL	1 μL	0.1 μL
P20	2 0 . 0	10 μL	1 μL	0.1 μL
P200	2 0 0	100 μL	10 μL	1 μL
P1000	1 0 0 0	1000 μL	100 μL	10 μL

13. Appendix B: Troubleshooting

Symptom	Probable Cause	Corrective Action
Liquid drips from tip after aspiration	Tip not seated properly; damaged tip	Reseat or replace tip
Air bubble in tip after aspiration	Plunger released too quickly; tip not immersed deep enough	Re-aspirate slowly with tip 3–4 mm below surface
Dispensed volume too low	Pipette out of calibration; wrong tip size	Perform gravimetric check; verify tip compatibility
Plunger feels sticky or rough	Liquid entered barrel; worn piston seal	Clean barrel; send for service

Symptom	Probable Cause	Corrective Action
Volume display will not turn	Mechanical jam	Do not force; contact lab manager
