



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L112

Autoclave and Steam Sterilization

Standard Operating Procedure

SOP Number	SOP-L112
Revision	0.1
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Date	2026-06-26
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1. Purpose

This procedure describes the safe operation and maintenance of the laboratory autoclave for steam sterilization of glassware, media, reagent water, and biohazardous waste. Autoclaving uses saturated steam under pressure (typically 121 C at 15 psi for 15–30 minutes) to achieve sterilization

by denaturing proteins and nucleic acids in microorganisms. In the EA Program, the autoclave is used to sterilize equipment for microbiological analyses (e.g., [SOP-L305 Colilert](#)), to decontaminate foreign soil samples ([SOP-L015](#)), and to sterilize waste before disposal.

2. Scope

- **Applies to:** All use of the laboratory autoclave in EA Program laboratories for sterilization of liquids, solids, glassware, and biohazardous waste
- **Does not apply to:** Dry heat sterilization (use muffle furnace or drying oven); chemical sterilization (e.g., bleach, ethylene oxide); filter sterilization (see [SOP-L114](#))
- **Location:** *TBD* (confirm autoclave location; shared facility or SGM)

3. Definitions

- **Autoclave:** A pressurized vessel that uses saturated steam to achieve sterilization by heating materials to 121 °C at 15 psi (103 kPa) for a defined exposure time.
- **Biological indicator:** A standardized preparation of resistant bacterial spores (typically *Geobacillus stearothermophilus*) used to verify that the autoclave cycle achieved sterilization; must be run periodically to validate cycle effectiveness.
- **Chemical indicator (autoclave tape):** Tape printed with a dye that changes color when exposed to steam and heat; indicates that the item was exposed to the cycle but does not confirm sterilization.
- **Gravity displacement cycle:** A standard autoclave cycle in which steam enters from the top, displaces cooler air downward and out through a drain; used for wrapped instruments, glassware, and liquid media.
- **Liquid cycle:** An autoclave cycle with a slow exhaust phase to prevent superheated liquids from boiling violently when pressure is released; required for all liquid loads.
- **Sterilization:** The complete elimination of all viable microorganisms, including bacterial endospores; achieved by saturated steam at 121 °C for at least 15 minutes at 15 psi.
- **Wrapped load:** Items wrapped in autoclave paper or placed in autoclave pouches before sterilization; wrapping maintains sterility after the cycle.

4. Responsibilities

Role	Responsibility
Lab PI	Authorize autoclave use, approve waste protocols
Lab Manager	Schedule maintenance, verify biological indicators
Trained Personnel	Operate the autoclave per this SOP
New Students	Complete training before independent use

5. Safety and Hazards

Hazards

- **Thermal/Pressure:** The autoclave operates at 121 C and 15 psi. Pressurized steam causes severe burns. Never attempt to open the door while the chamber is pressurized.
- **Scalding liquids:** Autoclaved liquids are superheated and can boil violently (bumping) when disturbed. Allow liquids to cool inside the chamber for at least 10 minutes after the cycle completes, and open the door slowly.
- **Glassware failure:** Cracked or starred glassware may shatter under pressure. Inspect all glassware before loading.
- **Biological:** Biohazardous waste must be contained in autoclave bags. Assume the exterior of bags is contaminated until the cycle is verified.

5.1. Required PPE

- ☐ Heat-resistant gloves (autoclave gloves, rated for steam)
- ☐ Safety glasses or face shield
- ☐ Lab coat
- ☐ Closed-toed shoes

5.2. Emergency Procedures

For steam burns, flush with cool running water for at least 20 minutes and seek medical attention. If the autoclave fails to depressurize, do not force the door; shut off power and contact the lab manager. Refer to [SOP-L002 Laboratory Safety](#).

6. Equipment and Materials

Item	Notes
Laboratory autoclave (gravity or pre-vacuum)	Confirm model and location with lab manager
Autoclave-safe trays (stainless steel or polypropylene)	For containing loads
Autoclave bags (clear or orange, printed with biohazard symbol)	For waste sterilization
Autoclave tape (indicator tape)	External indicator; changes color at 121 C
Biological indicator ampoules (e.g., <i>Geobacillus stearothermophilus</i> spore strips)	For periodic verification
Chemical integrator strips (Class 5)	Internal indicator in each load
Aluminum foil	For covering flask openings
Heat-resistant secondary containers	For liquids (to contain boil-over)

7. Procedure

7.1. Pre-Use Inspection

1. Check the water level in the autoclave reservoir; refill with distilled or deionized water if below the minimum mark
2. Inspect the door gasket for cracks, brittleness, or debris; clean if needed
3. Verify that the drain screen/strainer is clear of debris
4. Ensure the pressure gauge reads zero and the chamber is at ambient temperature

7.2. Loading

1. Select the appropriate cycle for the load type:

Load Type	Cycle	Temperature	Time	Notes
Wrapped instruments, glassware	Gravity	121 C	30 min	Loosely pack for steam penetration
Liquids (media, water)	Liquid / Slow exhaust	121 C	15–30 min	Leave caps loose or cover with foil; use slow exhaust to prevent boil-over
Biohazardous waste	Gravity	121 C	60 min	Double-bag in autoclave bags; do not seal bags tightly
Foreign soil (SOP-L015)	Gravity	121 C	30 min	Per SOP-L015 requirements

2. Place items in autoclave-safe trays. Do not overload; leave space for steam circulation.
3. For liquids: loosen caps or cover with foil. Place containers in a secondary tray to catch boil-over.
4. For waste bags: leave bags slightly open or use bags with steam-permeable patches. Place bags upright in a tray.
5. Place a chemical integrator strip inside the center of the load (the coldest point)
6. Apply autoclave indicator tape to the outside of each package or bag

7.3. Running the Cycle

1. Close and latch the autoclave door securely
2. Select the appropriate cycle (see table above)
3. Start the cycle and record the start time in the autoclave logbook
4. Monitor the temperature and pressure gauges during the initial pressurization to confirm normal operation
5. Do not leave the area until the autoclave has reached operating temperature and pressure

7.4. Unloading

1. When the cycle is complete, verify that the chamber pressure has returned to zero before opening the door
2. Crack the door open 1–2 cm and wait 2–5 minutes to allow residual steam to vent (prevents steam flash and liquid bumping)
3. Wearing autoclave gloves and face shield, open the door fully
4. Allow liquids to sit in the chamber for an additional 10 minutes after opening the door before removing
5. Remove trays using autoclave gloves; place on a heat-resistant surface away from foot traffic
6. Check the chemical integrator strip: it should show a complete color change indicating adequate steam exposure. If incomplete, the load must be re-processed.
7. Record the cycle completion time, temperature, pressure, and integrator result in the autoclave logbook

7.5. Waste Handling After Sterilization

1. After a successful waste cycle, seal the autoclave bags
2. Sterilized biohazardous waste can be disposed in the regular trash **only if** the institution's waste policy permits this and the biological indicator program confirms efficacy
3. Label the sealed bag with "Autoclaved" and the date
4. If the chemical integrator did not pass, do not dispose; re-run the cycle or contact the lab manager

7.6. Shutdown

1. After the final cycle of the day, drain the chamber if the manufacturer recommends it
2. Leave the door slightly ajar to allow the chamber to dry and prevent odor buildup
3. Wipe the door gasket with a damp cloth to remove debris

8. Verification and Quality Control

- **Chemical indicators:** Include a Class 5 integrator strip in every load. This confirms that the load reached the target temperature for the required duration.
- **Biological indicators:** Run a *Geobacillus stearothermophilus* spore test at least monthly (or per campus EH&S policy). Incubate the exposed ampoule per the manufacturer's instructions. A positive (growth) result means the autoclave failed; quarantine all loads processed since the last successful test and contact the lab manager.
- **Autoclave logbook:** Record every cycle: date, operator, load description, cycle type, temperature, pressure, duration, and indicator results.
- **Annual maintenance:** Schedule a professional inspection and calibration of the pressure gauge, safety valve, and temperature sensor at least annually.

9. Maintenance

Task	Frequency
Check water level in reservoir	Before each use
Clean door gasket	Weekly
Clean drain screen/strainer	Weekly
Run biological indicator test	Monthly
Professional inspection and calibration	Annually
Replace door gasket	As needed (when cracked or leaking)

10. Waste Disposal

Waste Type	Disposal Method
Successfully autoclaved biohazardous waste	Standard trash (if permitted by campus policy)
Failed autoclave loads (indicator did not pass)	Re-process or contact EH&S
Used chemical/biological indicator strips	Standard trash
Autoclave water (drain)	Drain (non-hazardous unless contaminated)

11. References

- CDC/HICPAC. (2008). *Guideline for Disinfection and Sterilization in Healthcare Facilities*. Centers for Disease Control and Prevention.
- Autoclave manufacturer's operating manual (consult for model- specific instructions, cycle parameters, and maintenance schedules)

12. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L006	Waste Disposal	Waste classification and pickup
L015	Foreign Soil Handling	Autoclave requirement for imported soil
L101	Glassware Cleaning	Pre-sterilization cleaning
L305	Colilert Quanti-Tray	Sterilized glassware requirement

13. Training Record

Before operating the autoclave independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Observe a trained operator run a complete cycle (loading through unloading and indicator verification)
4. Demonstrate proper loading for liquids and for waste (bag preparation, indicator placement, cycle selection)
5. Run one complete cycle under supervision with a passing chemical integrator result
6. Be signed off by the PI or lab manager