

Biology and Wildlife
STANDARD OPERATING PROCEDURE
Autoclaving

Location(s): Murie 215

Chemical(s): None

Specific Hazards:

- o steam – improper use of autoclave can expose user to dangerous steam burns
- o extremely hot materials – materials that have been autoclaved are very hot; liquids can be superheated and boil violently if jostled when removed from the autoclave

Contact Information:

Laboratory Supervisor: Denise Kind dmkind@alaska.edu 474-6298

Laboratory Manager: Patrick Knavel pdknavel@alaska.edu 474-5622

1. Purchasing:

Any autoclave bags, ties, tape, trays will be ordered by the Laboratory Manager. If supplies are running low, it is the responsibility of the users to notify the Laboratory Manager in a timely fashion.

2. Storage:

Autoclave trays and gloves are stored in 215 in the cabinet nearest the autoclave and on the counter so that they are readily visible.

Autoclave tape and bags are stored in 215 in the cabinet and drawers nearest the autoclave.

Paper and ink ribbons for the autoclave are handled by the Laboratory Manager. If the autoclave printer is not working, users shall notify the Laboratory Manager.

3. Authorized personnel:

- All authorized personnel must have completed all required employee and laboratory safety training.
- The Instructor is authorized to train their TAs on the proper use of the autoclave for the materials to be autoclaved. The instructor may delegate training to the B&W Laboratory Supervisor by making arrangements at least two (2) weeks in advance.
- TAs, once trained, are authorized to use the autoclave.
- Students shall not use the autoclave.

4. Training requirements:

The user must demonstrate competency and familiarity regarding the safe handling and use of these materials prior to using them. Training shall include the following:

- Review of this SOP and autoclave manual.

5. Use location:

- Murie 215

6. Personal protective equipment (PPE):

All personnel are required to wear the following personal protective equipment (PPE) whenever conducting this procedure:

- Heat-protective autoclave gloves must be worn when taking materials out of the autoclave.
- Lab coat
- Safety glasses

PPE must be inspected prior to use and replaced if damaged. Notify the Laboratory Manager of any PPE needs.

7. Spill equipment:

In the event of an autoclave malfunction or leak, the user may activate the emergency autoclave shut-off if it is safe to do so. If there is any question of safety in the room, the user shall notify the Laboratory Manager and Laboratory Supervisor immediately. If they cannot be reached, Facilities Services shall be contacted directly at 474-7000 and the malfunction reported. After hours, 474-7000 redirects to University Dispatch. An autoclave malfunction may require that they contact the Facilities on-call person to handle the problem immediately, as a malfunctioning autoclave can pose significant risk to people and the building.

8. Procedure:

Instructors shall provide TAs and students with detailed, written lab procedures to follow. Instructors shall train TAs on each procedure before TAs instruct students in the procedure.

Materials needed:

- autoclave bags & bag ties (for processing waste)
- autoclave-safe glassware (Type I borosilicate glass) or autoclave-safe plastic containers, loosely capped with autoclave-safe caps, aluminum foil, or other suitable tops
- autoclave tape (or heat-sensitive strip on bag)
- autoclave tray
- heat-protective gloves
- nitrile gloves for loading waste into autoclave bags

Procedure Notes:

PPE must be used appropriately throughout the procedures. Only TAs or instructors should run the autoclave.

Procedure Steps:

1. Wear nitrile gloves while handling items contaminated with microbes (e.g. Petri dishes that are to be disposed of). Nitrile gloves are not otherwise needed, as hazardous chemicals should not be autoclaved.
2. Place material to be autoclaved in autoclave tray. Be sure it is an autoclave tray! Other trays melt and ruin the autoclave. A tray must ALWAYS be used as secondary containment for the items being autoclaved.
 - For autoclave bags, add 100-200 mL water to the bag, then loosely close it. This will help generate steam inside the bag, which will greatly aid in destroying microorganisms.
 - Don't fill bottles or tubes more than 2/3 full to prevent boil-overs and loss of media.
 - Keep lids and caps loosely closed. Tightly sealed containers may explode.
 - Use only Type I borosilicate glass (Pyrex) or autoclavable plastic containers. Do not use old food jars to autoclave materials; they will break under autoclave conditions and create a hazard.
3. Determine the length of time required.
 - The media or object must reach and be held at a temperature of 121°C for a minimum of 15 minutes.
 - Small volumes of liquid or glassware reach this temperature very quickly. A 20 minute cycle allows sufficient time for the temperature of these to reach 121°C and remain there for at least 15 minutes.

- Large volumes of liquid (>500 mL) will NOT reach 121°C as quickly as smaller volumes. A longer cycle (35+ minutes) is needed to allow extra time for the liquids to reach 121°C. Remember, the entire volume of liquid has to be at 121°C for 15 minutes in order to achieve sterilization.
4. Determine the cycle required. Consult the manual for the autoclave you are using. Remember that when autoclaving liquids, the exhaust rate must be carefully controlled in order to keep the liquids from boiling over. Don't waste your media by bypassing the slow exhaust.
 5. Wearing heat-resistant autoclave gloves, place the tray in the autoclave. Even though the tray you are loading may be cool, the inside surfaces of the autoclave chamber may be hot enough to cause a burn.
 6. Close the autoclave door securely.
 7. Enter the correct cycle. **Fill out the autoclave log immediately.**
 8. Wait 10 minutes after the cycle ends before unloading. The autoclave should tell you when it is safe to be unloaded. Waiting will help keep liquids from violently boiling over when moved.
 9. Before unloading the autoclave, don heat-resistant autoclave gloves. Exercise care when removing hot items from the autoclave. Hot liquids can be superheated and can boil over if jostled.
 10. Place the tray on a heat-proof surface (such as a heat-proof cart or laboratory bench).
 11. Bags of autoclaved waste shall be disposed of in the autoclave waste bin in Murie 215. This bin is labeled and located directly across from the autoclave.
 12. Allow materials to cool sufficiently before handling. Media to be poured should be allowed to cool to the specified temperature.
 13. Return autoclave trays and gloves to the autoclave room promptly so that they are available to other users. If you need to keep an autoclave tray in your lab to collect materials to be autoclaved, contact the Laboratory Manager to make arrangements.

9. Waste disposal and clean up:

The authorized person(s) using this material is (are) responsible for the safe collection, preparation and proper disposal of waste unless otherwise stated below. Waste shall be disposed of as soon as possible and in accordance with all laboratory and University procedures.

When autoclaving waste for disposal, refer to the Waste Handling Flowchart (Appendix 3 of the Biology and Wildlife CHP) to be sure that autoclaving is the appropriate disposal method for the waste in question. Consult the Laboratory Supervisor if you have questions.

Students and TAs shall dispose of used materials properly.

- Wastes to be autoclaved will be placed appropriately in a biohazard bag (Petri dishes, contaminated plastic pipet tips, etc.) when produced. The biohazard bag should be in a biohazard bucket for secondary containment.
- The biohazard bucket lid must always close completely. When full, the bag should be removed and autoclaved following the above procedure.
- Autoclaved waste must be placed in the autoclave waste bin in 215 after autoclaving. Never place contaminated materials in this bin.

10. Decontamination:

If something breaks, shatters or melts in the autoclave, notify the Laboratory Supervisor and Laboratory Manager immediately. Post a sign on the autoclave warning others not to use it and why. Do NOT brush broken glass or clumps of agar into the drain; this will clog the autoclave and prevent steam from draining properly from the autoclave.

11. Exposures: Emergency procedures to be followed (from SDS):

Exposure to heated material from an autoclave can cause severe burns. Chemicals being autoclaved and broken glassware are additional hazards individuals may be exposed to. The list of exposure precautions listed below is for the heated materials in an autoclave. If chemicals are being autoclaved, the relevant Safety Data Sheets for the chemicals must also be consulted to determine the appropriate response to an exposure.

General advice

Consult a physician. Move out of dangerous area.

Eye contact

Flush eyes with water. Consult a physician.

Skin contact

Skin contact with autoclaved materials can cause burns. If the skin is intact, cool the burn with cool water. Do NOT scrub or apply any soaps or lotions. If the skin is broken, blistered, blackened, or charred (2nd or 3rd degree burns) or if any burn (1st, 2nd or 3rd degree) covers a large area of the body, immediately consult a physician. Call 911 for emergency medical assistance.

Ingestion of materials

In the event autoclaved solutions are ingested, consult the Safety Data Sheet for relevant exposure information and procedures. Never give anything by mouth to an unconscious person. Immediately consult a physician.

Inhalation

If vapors from autoclaved solutions or steam are breathed in, move the person immediately to fresh air. Call 911; possible burns in the lungs require medical attention. If not breathing, give artificial respiration. Consult Safety Data Sheet for the material that was autoclaved for more information on exposure procedures.

12. Spills:

- If a spill occurs, personal safety should come first.
- Alert everyone in the area where the spill occurred so that they can avoid contact with spilled material.
- Broken glass should be placed in a broken glass container.
- Spilled liquid media should be cleaned up with absorbent material such as paper towels. Disposal should be in accordance with the precautions listed in the Safety Data Sheet for the media in question; note that the majority of media is non-hazardous from a waste standpoint. Caution must be used when cleaning up hot media; it may be preferable to allow it to cool before clean-up.
- Hot agar should NEVER be disposed of in a sink drain; it will solidify and clog the sink. Hot agar-based solutions may be allowed to cool and then cleaned up in their solid state and disposed of appropriately.
- Clean the area where the spill occurred with a standard laboratory cleaner and water.
- Spills inside the autoclave: use absorbent material such as paper towels to absorb the spilled material if it is safe to do so. Place absorbent material as a barrier to spilled materials entering the autoclave drain (paper towels are acceptable).

- If the spill cannot be cleaned up by the user, or if there is any possibility that material may have entered and clogged the autoclave drain, contact the Laboratory Supervisor and Laboratory Manager immediately. Place a sign on the autoclave warning others not to use it and stating the reason. You will NOT get into any trouble for reporting that you had an accident in the autoclave. People will be very irritated if you cause a problem and fail to report it, though. Report any accidents or mishaps so that they can be dealt with promptly. This will keep the autoclave in working order and available for use.

13. Phone numbers:

Biology and Wildlife Laboratory Supervisor	474-6298
Biology and Wildlife Laboratory Manager	474-5622
EHSRM Hazardous Materials (if Lab Supervisor not available, assistance with a spill)	474-5617
EHSRM Industrial Hygiene (if HazMat not available; assistance with exposure)	474-6771
EHSRM office (if HazMat or Industrial Hygiene not available)	474-5413
University of Alaska Fairbanks Emergency Response (serious accidents, fire)	911

14. Other important information:

Users must also follow the specified procedures for the materials they are autoclaving. NEVER autoclave corrosive chemicals (e.g. strong acids or bases), solvents, volatile compounds, chlorinated compounds (e.g. bleach), flammable substances, or highly reactive compounds. Follow the Waste Handling Flowchart (Appendix 3) and Autoclave Flowchart (Appendix 4) in the Biology and Wildlife CHP.