

Autoclave Guidance

Purpose: An autoclave provides a physical method for decontaminating waste prior to disposal and sterilizing equipment or media prior to use. This guidance document is intended for laboratory personnel, facility managers, and researchers to ensure safe and effective autoclave use.

Best Practices for Autoclave Use

- Do not leave an autoclave operating unattended for long periods of time; monitor operation periodically during a cycle in case of failure.
- The exterior surfaces of autoclaves are typically very hot, particularly on models 15 years or older which have little or no heat shielding. Attach conspicuous warning signs to remind people of the heat hazard.
- Do not stack or store combustible materials next to an autoclave (e.g., cardboard, plastic, volatile or flammable liquids).
- Do not overload an autoclave because it will prevent efficient steam distribution. Longer sterilization times may be required to achieve decontamination if an autoclave is tightly packed.
- Place medical (e.g., needles, razor blades) and non-medical (e.g., plastic pipettes, serological pipets, glass slides) sharps in an appropriate rigid disposal container prior to autoclaving.
- Do not overfill a biohazard bag. Steam and heat cannot penetrate as easily to the interior of a densely packed biohazard bag and may affect disinfection of its contents.
- Do not autoclave contaminated items and clean items together during the same autoclave cycle.
 - Clean items generally require shorter sterilization times (15-20 minutes) while a bag of infectious waste (approximately 24" x 36") typically requires 45 minutes to an hour to be effectively decontaminated throughout.
- Before using the autoclave, check for any items left inside by previous users that could pose a hazard.
- Be sure to clean the drain strainer before loading the autoclave in accordance with manufacturer's recommendations.

Autoclave Supplies

- Use autoclave-specific hard-sided containers or plastic biohazard bags.
 - After autoclaving, only clear or orange biohazard bags may be disposed in regular trash.
 - Red biohazard bags must be picked up by MERI for disposal.
- Color-changing indicator tape is imprinted with a dye that changes color when the correct temperature is reached. Autoclave indicator tape can be useful to visually differentiate autoclaved from non-autoclaved materials.

- Autoclave indicator tape only indicates that a relatively high temperature has been reached and does not indicate that the time or temperature of the cycle was sufficient to inactivate biohazards or that steam penetration has occurred.
- Some biohazard bags are also imprinted with a color-changing dye which changes color upon exposure to steam or high temperatures. This is not an indication of a successful decontamination cycle.
- Heat-resistant gloves.
- Cycle validation supplies such as chemical and biological indicators (see **Efficacy Testing**).

Material that Can be Autoclaved

- Reusable plastics that will be autoclaved MUST be comprised of material that can withstand autoclaving. Non-autoclavable plastic materials will melt and could plug the drain line.
- Waste bags to be autoclaved must be loosely packed and not more than 2/3 full. Steam must be able to penetrate to all contents of the bag.
- Do not place sharps or pointed hard objects directly into a biohazard bag; a thicker or rigid container must be used such as a sharps container or cardboard box.

Materials that Cannot be Autoclaved

Never autoclave solvents, combustible, volatile, flammable, radioactive, or corrosive materials (e.g., bleach, ethanol, methanol, acids, bases, phenol, etc.)



Autoclavable Materials: Materials loaded into an autoclave must be made to withstand autoclaving. Autoclaving materials that are not marketed as such will result in damage to the item as well as the autoclave.

Handling and Transporting Waste

The necessity to transport materials to and from autoclaves increases the potential for accidents in laboratory buildings. Take measures to avoid spills of potentially infectious biological materials:

- Transport materials on carts.
- Always handle waste bags from the top—never from the bottom.
- All contaminated or potentially contaminated material transported for decontamination must have secondary, leakproof containment on the cart to minimize loss of containment during a spill event.
- Decontaminate surfaces with an approved disinfectant prior to transport to autoclave area.
- Secure all containers filled with biological/biohazardous material (liquid or solid).
- In case an accidental spill does occur during transport, ensure a spill kit is available for cleanup. Review the [Campus Transport of Biologicals](#) guidance document and [Biological Waste Disposal](#) on the [Environment, Health and Safety website](#).

Autoclave Loading

- Wear proper PPE for handling items for autoclaving (e.g., disposable gloves, lab coat, eye protection or face shield).
- Load the autoclave per the manufacturer recommendations.
- Do not place items directly on the autoclave chamber floor, either place items on a rack or inside of an autoclavable tray.
- Avoid overfilling an autoclave or allowing a load to contact the chamber walls.
- Do not transfer contents from an overfilled bag to another bag. This practice can lead to injury and/or exposure to infectious agents.
- Review guidance for autoclaving specific items:
 - **Liquids**
 - To improve heat distribution around bottles, add 1-2 cm of water to a tray.
 - Loosen caps of containers of liquids prior to loading into the autoclave; sealed bottles may shatter or explode during pressurization. Never seal a container with liquid with a cork or stopper.
 - **Bags with solid waste**
 - Loosely close to allow steam to penetrate the contents and maximize sterilization. Add water (approximately 250 ml) to a bag if the load is dry.
 - **Medical and non-Medical Sharps**
 - Refer to the Sharps Disposal Poster and the Lab Pipette and Tips Disposal Poster found on the [Sharps Disposal webpage](#).
 - **Clean item/Container Sterilization**
 - Place individual glassware pieces, tools, or other clean items inside a heat resistant plastic or metal tray that sits on a shelf or rack.

A.



B.



Autoclave Loading: A. Ensure that bags are loosely closed. Use a cart for heavy items and use a secondary container. Always wear proper PPE and closed toe shoes when handling biohazardous waste, and do not carry bags from the bottom. **B.** Caps must be loosened for any bottles containing liquid and add 1-2 cm of water to the secondary container. Do not overfill bottles.

Example Cycle Settings for Autoclaves

Load Type	Temperature	Pressure	Time (min.)
Sterilization of clean materials or liquids	≥ 121°C (249°F)	~ 15-30 psi	15 - 20
Decontamination of Waste	≥ 121°C (249°F)	~ 15-30 psi	45 - 60

Autoclave Unloading

- Wear proper PPE for handling items for autoclaving (e.g., disposable gloves, lab coat, eye protection or face shield). In addition, use heat-resistant gloves as items coming out of the autoclave are extremely hot.
- Do not open the autoclave until the cycle is finished.
- Check the gauges to ensure the pressure has reduced fully.
- After the completion of a run, slightly crack open the door to allow steam to vent. Wait a full five minutes if the autoclave load contains only dry glassware, and no less than ten minutes when autoclaving liquids before removing the items.
- Slowly remove trays of items from the autoclave; take care to not touch hot surfaces or spill liquid from pans or containers.
- Place items on a heat tolerant surface and allow to cool before touching without heat protection.
- **Solid materials:** Apply an “OK to Trash” sticker complete with your name and room number prior to disposing with regular waste.

Spills, Breakage, and Injuries

- Notify a supervisor or department safety officer and follow what is described in the approved biosafety protocol for a spill or exposure event or injury incident.
 - Regardless of the degree of severity, report any injury to your supervisor or principal investigator.
 - If a known or potential biological exposure or release occurs during an incident, a [First Report of Biological Exposure or Release Event form](#) must be completed and submitted to the Office of Biological Safety.
 - You can treat minor burns yourself using standard first aid. Burns to the face, third-degree burns, or burns over large areas of the body are emergencies – seek immediate help and medical treatment (call 911).
- Clean up immediately with appropriate absorbent and disinfectant/cleanser for material. Take care if material is hot from autoclave.
- If a spill occurs inside a hot autoclave, allow autoclave to cool sufficiently prior to clean up.
- Post signs warning others in area of spill during clean up, or while allowing area to cool prior to clean up.
- For solid material spill or breakage:
 - Clean up immediately. Take care if material is hot from autoclave.
 - Do not pick up broken glass with hands; use dustbin or forceps and dispose in proper receptacle.
 - Allow autoclaved items to cool sufficiently prior to clean up.
 - Post signs warning others in area of spill during clean up, or while allowing area to cool prior to clean up.

Routine Maintenance

The best way to ensure that an autoclave is performing properly is to conduct routine maintenance. Follow the manufacturer's recommendations for routine preventative maintenance and cleaning the chamber. Record all maintenance activities in the appropriate log.

In absence of manufacturer guidelines, the following cleaning procedure may be used:

- Wash wetted portion of the chamber thoroughly using a mild diluted detergent and a soft cloth or pad. A nylon soap pad may be used for surface film not easily removed. Never use a wire brush, abrasives, or steel wool on door and chamber assembly.
- Ensure drains or screens on the floor of the autoclave are not blocked with debris.
- After washing, thoroughly rinse with clean, soft water.

Efficacy Testing

The purpose of efficacy testing is to ensure the inactivation of biological materials by autoclaving. The BMBL (Biosafety in Microbiological and Biomedical Laboratories) recommends routine efficacy monitoring via the use of biological indicators. In addition, the [CDC's disinfection guidance](#) recommends weekly inclusion of biological indicators along with inclusion of chemical indicators in every load. Perform efficacy testing **monthly**, at a minimum, using biological indicators. An autoclave printout has a positive value as a general day-to-day log of usage and cycle settings but provides no evidence of the inactivation of biologicals or steam penetration through the innermost parts of a load. Annual manufacturer's maintenance checks of the devices would be inadequate to ensure sterilization efficacy of a frequently used autoclave.

Regular testing by use of a sterility indicator serves to verify that:

- Sufficient temperature was reached to kill microorganisms.
- The duration of the cycle was sufficient to kill microorganisms.
- Adequate steam penetration occurred throughout a load to kill microorganisms.
- Efficacy test results must be recorded, retained, and available to all parties using the autoclave.

Efficacy testing must be described in the Disinfection/Inactivation section of your Biosafety Protocol. Laboratory personnel must follow efficacy testing procedures described in their approved Biosafety Protocol. There are several different types of indicators that can be used to verify proper autoclave operation. Use indicators according to manufacturer/supplier instructions. Supplies can be found on ShopUW+ from vendors such as Mesa Labs, Steris, 3M Attest, Croxtex, and Propper Manufacturing.

A.



B.



Embedding Indicators: These examples demonstrate how to use biological or chemical indicators when autoclaving. **A.** Place a biological or chemical indicator in a conical tube. Attach the tube to an autoclavable string and place the conical tube inside of the bag of waste. Loosely tie the string around the outside of the bag so the indicator can be retrieved and read after the cycle has completed. **B.** Place a biological or chemical indicator inside a conical tube. Using autoclave safe tape, secure the tube to one end of a long wooden dowel rod. Place the dowel rod, indicator side down, into the middle of the bag of waste, and loosely close the bag opening around the dowel rod using autoclave tape.

Indicator Types

- **Biological Indicators (BIs)**

- Contain difficult-to-kill spores (e.g., *Geobacillus stearothermophilus*) which require a temperature of 121°C for 20 minutes to be destroyed.
- Place in the most difficult to sterilize area in the autoclave such as the center of a load.
- Provide a true indicator of decontamination.
- Types of BIs:

- Bacterial Spore Strips are spores contained on a paper carrier within envelopes which, after sterilization, are aseptically transferred to culture media and incubated under defined growth conditions, typically 24-72 hours for results.
- Self-contained BIs are vials which contain bacterial spores, culture media, and everything required to verify the cycle after sterilization and requiring no need for aseptic transfer of material. May require 24-72 hours for results.
- Rapid Read BIs are a special type of self-contained BI which can provide results in minutes up to a few hours.
- Fluorescence Technology BIs require a special device to read the BI results.

A.



B.



Biological Indicator Examples: **A.** Spore ampule. **B.** Fully enclosed, self-contained spore vial composed of a sealed glass ampoule inside of a thermoplastic tube. In the presence of spore growth, the liquid will turn a bright yellow within 24 hours indicating the sterilization failed.

- **Chemical Indicator Strips**

- A quick read of steam penetration but are not dependent upon time exposed.
- Quickly identify that a cycle has failed.
- Do NOT verify the inactivation of biologicals.
- Are small and fit in tight spaces or on the outside of loads for quick review.
- There are 6 different types of chemical indicators with different performance indicators and intended uses. It is recommended to use Class 5 or Class 6 chemical indicators.



A.



B.



Chemical Indicator Examples: A. Class 5. Comply SteriGage Integrators are a Class 5 chemical indicator that is easy to read to verify if critical parameters of the sterilization cycle are met. **B. Class 6.** The PMS Steri-test emulating indicator can be used in every pack and will provide assurance of steam penetration and presence of all critical parameters of steam sterilization cycle.

Efficacy Testing Failure

Autoclave failure is usually due to operator/user error or mechanical failure. Examples include:

- Containers blocking access of steam to the load
- Improperly venting bags or containers prior to autoclaving
- Not adding water to a dry load before autoclaving
- Autoclaving a bag of waste that is too large for the autoclave
- Over-filling a biohazard bag

If efficacy test results indicate that the autoclave failed, first check the autoclave log to ensure the correct temperature and cycle time were used. If not, adjust settings to the correct time/temperature and rerun the load with new indicators.

- If the correct time and temperature were achieved during the efficacy test, and the autoclave did not pass, change one or more of the following parameters and rerun the load with a new indicator.
 - **Density:** Load the bag 2/3 of the holding capacity or less. Do not compress the waste to fit more in the bag. Steam cannot penetrate completely through densely packed waste bags.
 - **Loading:** Change how materials are loaded into the autoclave to allow steam to better move from the top of the chamber to the bottom and penetrate the load:
 - Avoid crowding or stacking.

- Make sure bags are not touching the top or sides of the autoclave.
 - Try using shallower trays; make sure they will still contain any spills.
- **Steam:** Add approximately 250ml of water to dry waste loads to facilitate steam generation.
- **Time:** Increase the cycle time by 15 minutes. If the cycle fails again, try adjusting another parameter. Cycle time may be increased by 15-minute increments until the cycle passes; however, this indicates that cycle times will need to be longer for each regular load run.
- Record the parameters changed for the re-test on the autoclave record/log.
 - The changes will become the new parameters for autoclaving waste in this specific autoclave, so be sure to make whatever changes are necessary to protocols and instructions for autoclave use; inform others who use the autoclave.
- If changing parameters is ineffective, call the appropriate autoclave service provider as indicated in the manufacturer's manual or laboratory records. Mechanical failure of the autoclave must be addressed by a trained technician. Contact the service company responsible for maintenance, the department or building manager, or [Physical Plant Customer Service](#).
- Post a sign on the autoclave stating that it is "Not in Use" until maintenance has been performed and the autoclave has been verified to be operating properly via efficacy testing.

Training Requirements

All users must undergo documented training for autoclave operation which will:

- Minimize the risk for injury to staff and damage to equipment or facilities.
- Ensure sufficient sterilization of laboratory materials.
- Ensure decontamination of biohazardous materials is performed in accordance with regulatory requirements.
- Allow users to become familiar with the manufacturer's operations manual of available autoclave model(s).
 - If training will take place in the lab by an experience lab member, the lab member must cover PPE, spill prevention and cleanup, safety precautions, and proper loading and unloading of the autoclave.

Training Courses

- [Biosafety 106: Autoclave Use](#) is a recommended course for operating an autoclave.
- [Cage Wash and Autoclave Safety](#) is recommended for animal care staff who will be using autoclaves.

References

- Centers for Disease Control and Prevention (CDC) Sterilization: Monitoring: <https://www.cdc.gov/oralhealth/infectioncontrol/fags/monitoring.html>
- World Health Organization (WHO) Quality Assurance in Bacteriology and Immunology, No. 47: <https://apps.who.int/iris/handle/10665/205730>

- BMBL (Biosafety in Microbiological and Biomedical Laboratories), Appendix B: Decontamination and Disinfection of Laboratory Surfaces and Items:
https://www.cdc.gov/labs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf
- Steris Life Sciences:
https://www.sterislifesciences.com/resources/documents/articles/steris_6-iso-types-of-chemical-indicators-for-steam-sterilization_infographic_lsfc-br4166