

Requirements for Decontamination by Autoclaving

Summary: Steam sterilization (autoclaving) is one of the most effective methods for decontaminating biohazardous material. Autoclaves use saturated steam under high pressure to decontaminate infectious materials (i.e., cultures, cells, contaminated glassware, pipettes, etc.) and to sterilize media, lab ware and other items. Biohazardous material and full sharps containers generated within research laboratories are placed in Contaminated Materials Containers (CMCs) and treated at the hospital autoclave. However, some laboratories that generate biohazardous material may use local laboratory (or departmental) autoclaves. When using laboratory or departmental autoclaves to decontaminate biohazardous materials, **the Principal Investigator (or Department Head) is responsible for meeting the following mandatory requirements:**

1. Maintaining a log book in accordance with Section 4a below.
2. Autoclaving for a minimum of 30 minutes (see Section 4b) unless validation data demonstrates that less time is sufficient for decontamination.
3. Documenting proper validation techniques when decontaminating materials (see Section 8).

The recommendations below serve as guidelines to help autoclave operators ensure a safe and effective process. For questions concerning autoclave requirements or guidelines and safety, contact the Office of Environmental Health and Safety Office at 434-982-4911.

What to do

- 1 **Select appropriate containers/bags for autoclaving.**



How to do it

For biohazardous dry materials:

- a. **Use polypropylene (clear) bags or reusable autoclavable (Nalgene) containers.** Make sure your plastic bag or container is suitable, since not all plastics can be autoclaved (i.e., polyethylene or HDPE). They can melt and ruin the autoclave chamber.
- b. **Ensure that contaminated materials are free of sharp objects** that may puncture bags. Autoclave bags are tear resistant, but can be punctured or burst in the autoclave.
- c. **Fill bags only 2/3 full.** Ensure adequate steam penetration by closing bags loosely, leaving a small opening and closing with autoclave indicator tape.
- d. **Sharps containers** – EHS recommends that these be picked up by EHS.

For biohazardous liquids (or non-biohazardous liquids):

- e. **Fill liquid containers only 1/2 full.** Loosen caps or use vented closures.
- f. **Never put sealed containers in an autoclave.** They can explode. Large bottles with narrow necks may also explode if filled too full of liquid.

For non-biohazardous glassware/labware:

- g. Cap Pyrex bottles loosely – whether empty or filled to prevent explosions due to expansion.
- h. Cover bottles that are not made of safety glass (e.g., not Pyrex) with aluminum foil.

- 2 Place clear autoclave bag or other container in a **SECONDARY** container labeled with a biohazard symbol/sticker.



- Make sure your plastic secondary container** is suitable for autoclaving. Not all plastics can be autoclaved (i.e., polyethylene or HDPE).
- Polypropylene, polycarbonate or stainless steel tubs** are typically used for secondary containment. See Nalgene Labware's [Autoclaving](#) Web page for additional plastic considerations.
- Select a container with the lowest sides and widest diameter** possible for the autoclave. This will promote penetration of steam and will collect any leakage or overflow of liquids.
- Leave space between items/bags** to allow steam circulation. Water can also be added to plastic pans at a depth of 2.5 cm or into bags with solid materials.
- Ensure secondary container is labeled with the universal Biohazard symbol so that BSL2 waste can be easily identified.**

- 3 Select the correct autoclave cycle for your materials and check the drain screen.



- Review the manufacturers operating manual and any laboratory Standard Operating Procedures (SOP) for the autoclave unit. Training should be provided for any new autoclave operators.
- “Slow Exhaust”** is the recommended cycle for biohazardous dry goods and liquids.
- Check the drain screen** at the bottom of the chamber before using the autoclave. Clean out any debris.
- For efficient heat transfer**, steam must flush the air out of the autoclave chamber. If the drain screen is blocked with debris, a layer of air may form at the bottom of the autoclave and prevent proper operation.
- Autoclave the load immediately after preparation.** Do not leave unprocessed items in the autoclave overnight.

- 4 Fill out the autoclave logbook and be aware of required cycle times.



PLEASE NOTE THE FOLLOWING REQUIREMENTS (a-b):

- Each unit used to decontaminate must have an autoclave log** where the operator records the date, name, cycle time, and monthly biological indicator results.

For both dry and liquid biohazardous materials:

- Cycle time must be set for a minimum of **30 minutes @ 121°C, 15 psi¹** unless decontamination in less time can be demonstrated (see section 8 for more information on validation procedures).
- Liquids and large loads containing many bags may require more time.

For sterilization of non-biohazardous dry goods (i.e., glassware) or liquids (i.e., broth or media):

- Cycle time for sterilization will vary and does not require

validation. For larger volumes allow extra time and for dry goods, allow approximately 20 minutes of drying time. Dry time may need to be increased for enclosed items such as pipette tips or bottles with lids.

5 Follow these precautions when the autoclave cycle is finished.



- a. **Wear personal protection equipment:**
 - a. Lab coat
 - b. Eye protection
 - c. Closed-toe shoes
 - d. Heat-resistant gloves to remove items, especially hot glassware
- b. **Never open an autoclave set for "slow exhaust" until the cycle is complete.** Superheated liquids can boil over and damage the autoclave and injure the operator.
- c. **Open the door cautiously.** Stand behind the door and slowly open it. Allow all steam to escape before reaching inside.
- d. **Let liquids stand another 10–20 minutes** after the autoclave is opened to avoid any movement that could cause them to boil.
- e. **Do not override autoclave's built-in safety control features** under any circumstance (contact your autoclave repair representative for assistance).

6 Properly dispose of materials.



- a. **Biohazardous materials that are autoclaved** should be discarded as non-contaminated laboratory waste and placed in the regular trash or outside dumpster.
- b. **Biohazardous materials not autoclaved** (that either cannot be autoclaved or when the laboratory chooses not to autoclave themselves) must be placed in a Contaminated Materials Container (CMC).

7 Materials NOT appropriate for autoclaving:



- a. **Never put materials containing solvents, corrosives or radioactive materials in the autoclave** (e.g., phenol, chloroform, pyridine, or bleach²).
- b. **Pathological laboratory** materials including pathological specimens (i.e., whole cadavers, recognizable human body parts, animal carcasses or tissues) must not be autoclaved. Contact EHS Biosafety (982-4911) for disposal instruction.

8 Monitor and perform validation tests



Note: 3M Attest Biological Indicator Test kits available through the Hospital Storeroom.

REQUIRED VALIDATION TEST (MONTHLY):

- a. **Biological Indicators (BI)** are the most accepted means of monitoring the sterilization process because they directly determine whether the most resistant microorganisms (e.g., *Geobacillus* or *Bacillus* species) are present rather than merely determine whether the physical and chemical conditions necessary for sterilization are met.³
- b. **For the BI test, ampoules of *B. stearotherophilus* are autoclaved along with a load of waste.** Upon completion of the cycle and following manufacturer's instructions, ampoules are incubated for 24-48 hours and then observed for any sign of growth that would indicate that autoclave is not sterilizing properly. Record results and keep in the autoclave log book.
- c. BI tests **performed on a monthly basis and:**
 - Whenever a new type of packaging material or tray is used.
 - After training new sterilization personnel.
 - After a sterilizer has been repaired.
 - After any change in the sterilizer loading procedures.
- d. **Other types of indicators** such as Mechanical Indicators (gauges and digital displays) or Chemical Indicators (autoclave tape or strips) do not ensure sterilization, but can be used to show that autoclaving has been performed. Additionally, mechanical indicators can identify when a problem has occurred with the sterilization cycle.

9 Perform preventative maintenance



- a. **Autoclaves should be maintained on a regular service contract.** Contact the unit manufacturer or the Biosafety Office for more information.
- b. **Calibration should be performed annually⁴** (requirement of the ASME Boiler and Pressure Vessel Code and Public Law PL 104-113) and can be performed by the manufacturer or other qualified third party autoclave service technician as part of an annual maintenance contract.
- c. **If you suspect there is a problem** with your autoclave's performance, contact your autoclave repair representative for assistance.

References

¹Le, R.N., et al (2005) Autoclave Testing in a University Setting. *Applied Biosafety*, 10(4), 248-252.

²Trace amounts of bleach used to decontaminate laboratory materials during procedures (e.g., decontaminating pipette tips or other labware) may be autoclaved.

³Centers for Disease Control and Prevention, Oral Health Resources "Sterilization – Monitoring FAQs," April 2005. www.cdc.gov/oralhealth/infectioncontrol/faq/sterilization_monitoring.htm

⁴ASME Boiler and Pressure Vessel Code and Public Law PL 104-113.

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