

What waste is autoclaved?

- Disposable labware contaminated with potentially biohazardous materials (i.e.: blood, body fluids, human cell culture media, bacteria culture media, viruses, recombinant DNA, animal cell lines, plant or animal cells, bacteria, and fungi, etc.)
- Culture plates and media
- Animal cages and non-infected animal bedding
- Contaminated Pasteur pipettes should be disposed of in a sharps container or autoclaved and placed in a glass disposal box
- Do NOT autoclave radioactive materials, hazardous chemicals, chemotherapeutics, or bleach

Background

Individuals that autoclave must be trained by a competent person and should refresh that training annually

Adequate waste processing (decontamination) time depends on:

- Load size
- Type of container
- Moisture content
- Type of cycle
- A **60 minute cycle** must be used to allow enough time for the center of the load to come up to temperature and have sufficient steam penetration.
- This will ensure that all parts of the load have reached 121°C for a minimum exposure time of 30 minutes.
- If you wish to use a shorter processing time you must verify effectiveness

In order to standardize the autoclaving of wastes and to assure that all loads, regardless of their size or content, are properly decontaminated, the following procedure must be followed.

Procedure

- Enter your waste information on the log sheets mandated by the MA-DEP ([see example below](#))
- Place materials in a clear autoclave bag
- No biohazard symbol may be visible.
- **No red or orange bags may be used as they cannot be disposed of as regular trash after autoclaving.**
- Bag should be loosely packed and not more than 3/4 full; add water (200 ml) to dry bags
- Do not seal bag shut—the opening should be an inch in diameter for steam penetration
- Use autoclave indicator tape on the exterior of the bag to show that the waste has been processed. This tape does NOT prove decontamination effectiveness (See Autoclave Testing)
- Place waste material on a large, metal, leak-proof tray; Metal containers transfer heat more effectively than plastic containers (Use only autoclaveable plastics)
- The container should be large enough and shallow enough to allow for ample steam circulation
- If autoclaving more than one bag at a time, be sure that there is ample room between the bags so that steam circulation is not impaired
- Autoclave at 121°C for **60 minutes**; Minimum Pressure: 15 psi;
- Once the pressure is back to zero, open the door slowly (stand behind the door) to allow steam to escape. Once the steam has abated you may open the door more fully.
- After the autoclaving is complete, allow the bag to cool. Tape the bag shut and place an "Autoclave Waste" sticker on the autoclave bag.
- Place the labeled bag inside a black or opaque trash bag and place in the regular waste receptacle (dumpster). No labels on the exterior of this bag.
- Complete your log sheet and print as well as signing your name; attach any autoclave printouts to the back of the log sheet
- To autoclave liquid waste, place the liquid in a beaker or flask, not in an autoclave bag
- Autoclaved liquid culture waste can be poured down a drain. If media has been melted, do NOT pour this down the drain, allow it to solidify and dispose of as solid waste.

Contact Biological Safety Services
with questions: 413-545-2682



Autoclave Waste Management Guidelines

Choosing the right sterilization cycle to implement

There are three basic types of sterilization cycles. Choose the right one according to the type of goods to be sterilized:

Hard Goods (Dry Cycle with or without Vacuum)

Suitable for items that are easy to sterilize, because air removal and steam penetration are highly effective on these items.

e.g., open glassware and large diameter piping

A typical hard goods cycle may draw one vacuum prior to introducing steam to reach the desired sterilization temperature.

Wrapped Goods (Dry Cycle with or without Vacuum)

Utilized for items that are difficult to sterilize/decontaminate, because air removal and steam penetration are harder to achieve on these items than on hard goods.

e.g., empty bottles (glass or plastic) with lids or foil covers, gowns, long hoses/tubes, vent filters, biohazardous waste bags and cages.

A typical wrapped goods cycle may draw three or more vacuums prior to reaching sterilization. A post-sterilization vacuum draws the steam from the load items.

Liquids (Non-vacuum)

Items that contain liquids generally cannot have a deep vacuum pulled or the liquid will be drawn out of them. Autoclave cycles for liquids generally heat up and cool down without a vacuum. Steam, introduced into the top of the chamber, displaces the air. The air is pushed to the bottom of the chamber and is removed.

Autoclaved Waste Label

NON-INFECTIOUS AUTOCLAVED BIOLOGICAL WASTE FROM UMASS AMHERST TREATED IN ACCORDANCE WITH MA-DEP AND MA-DPH REGULATIONS
DATE TREATED: _____
BUILDING: _____
PRINCIPAL INVESTIGATOR: _____

Expected ProSpore® Results



Autoclave Testing

- All autoclaves used for waste decontamination must be tested **monthly** for effectiveness per state regulation
- If the autoclave is only used for sterilizing of liquids or materials to be used in the lab, then the testing may be performed quarterly. Contact EH&S for permission for quarterly testing.
- Use approved spore vials (*Geobacillus stearothermophilus*) from a reputable company. EH&S recommends ProSpore® available from Thermo-Fisher #12-001-1.
- Each ProSpore® ampoule contains a spore suspension within a growth medium also containing Bromocresol Purple to function as a pH indicator. The acid production associated with growth causes a change in color from purple to or toward yellow.
- Ampoules should be purple and undamaged prior to use. Do not use after expiration date. Since ProSpore® contains live cultures, ampoules should be handled with care. ProSpore® is not intended for flash sterilization processes. This is a single use product. Sterilize all positive and expired units prior to disposal.
- Each test requires one control ampoule (un-autoclaved) and one or more autoclaved ampoules
- Place one or more ProSpore® Biological Indicators in the most difficult location to sterilize, usually in the center of the load or suspended in a volume of liquid. Run cycle. Caution: After sterilization, handle ampoules with care. Contents of the ampoule are hot and under pressure. Failure to allow sufficient cooling time (10-15 minutes) may result in bursting of the ampoule.
- Place the test ampoules in a vertical position in an incubator at 55-60°C. Mark a control ampoule as such and incubate for 48 hours along with processed ampoules to ensure spore viability. Contact EH&S if you need to incubate your Prospores® in our incubator.
- Examine the ProSpore® ampoules daily during incubation. Record observations. All positive ampoules should be recorded and then disposed of immediately into a sharps container.
- Control: The control ampoule should exhibit a color change to or toward yellow and/or turbidity, which is indicative of growth. If the control ampoule does not show signs of growth, consider the test invalid.
- Test: A failed sterilization/decontamination cycle is indicated by turbidity and/or a change in color to or toward yellow. A test ampoule that retains its purple color indicates an adequate sterilization cycle. Record your results on the log sheet.
- If the test failed, perform corrective action (see below), re-process the load and run ProSpores® again. Use a different autoclave for the waste load if necessary.
- Corrective action: Check temperature charts and verify that the correct cycle was run. Verify the Prospores® are not expired and select a different lot number when retesting commences.
- Time: increase the cycle by 15 minutes and re-test. If results are positive again, increase the cycle by 15 minutes until the results are negative. The cycle time will vary depending on the size of the load.
- Density: Load the bag to 75% capacity or less, because steam cannot penetrate completely through densely packed bags.
- Steam: Add approximately 200 ml of water to dry waste loads to facilitate steam generation.
- Loading: Allow steam to better move from the top of the chamber to the bottom. Do not cover the drain. Do not let materials touch the sides or top of the autoclave.
- Record all changed parameters on the log sheet. Revise your autoclave cycle and procedures so new parameters for autoclaving waste are included and inform others who use the autoclave.
- If none of the above gives a negative result (purple autoclaved ampoule), notify EH&S and call the autoclave repair vendor.

University at Massachusetts Amherst: Biological Waste Record for On-Site Treatment

Department: EH&S Building: LGRT Room: 17 Manufacturer: Steris Model #: N12345
Serial #: 67890 UMass Tag #: 91919 Date Placed in Service: May 2014 Autoclave was: New

Date	Quantity	Type	Treatment Method	Process Parameters			Printed Name	QC Results
				Time	Pressure	pH Temp		
5/6/14	1 24"X 30"	Waste bag	Heat	60 min	15 psi	N/A	121°C	Signature Sue Smith
								Chemical or Spore + or - Spore test: ok Control: yellow Test: purple
5/7/14	1 24"X 30"	Waste bag	Heat	60 min	15 psi	N/A	121°C	Signature Bob Jones
								Chemical or Spore + or - Tape = changed color to black; ok