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 autoclavable 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.
- "Autoclave Waste" sticker on the autoclave bag.
- 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 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 ProSpore® 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: 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.

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

### Autoclave Waste Management Guidelines

<table>
<thead>
<tr>
<th>Choosing the right sterilization cycle to implement</th>
<th>Autoclave Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>There are three basic types of sterilization cycles. Choose the right one according to the type of goods to be sterilized:</td>
<td>All autoclaves used for waste decontamination must be tested monthly for effectiveness per state regulation</td>
</tr>
<tr>
<td><strong>Hard Goods (Dry Cycle with or without Vacuum)</strong></td>
<td>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&amp;S for permission for quarterly testing.</td>
</tr>
<tr>
<td>Suitable for items that are easy to sterilize, because air removal and steam penetration are highly effective on these items.</td>
<td>Use approved spare vials (Geobacillus stearothermophilus) from a reputable company. EH&amp;S recommends ProSpore® available from Thermo-Fisher #12-001-1.</td>
</tr>
<tr>
<td>e.g., open glassware and large diameter piping</td>
<td>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.</td>
</tr>
<tr>
<td>A typical hard goods cycle may draw one vacuum prior to introducing steam to reach the desired sterilization temperature.</td>
<td>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.</td>
</tr>
<tr>
<td><strong>Wrapped Goods (Dry Cycle with or without Vacuum)</strong></td>
<td>Each test requires one control ampule (un-autoclaved) and one or more autoclaved ampules.</td>
</tr>
<tr>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>e.g., empty bottles (glass or plastic) with lids or foil covers, gowns, long hoses/tubes, vent filters, biohazardous waste bags and cages.</td>
<td>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&amp;S if you need to incubate your ProSpore® in our incubator.</td>
</tr>
<tr>
<td>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.</td>
<td>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.</td>
</tr>
</tbody>
</table>

**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 implement sterilization vacuum draws and steam penetration are highly effective on these items. Suitable for items that are easy to sterilize, because air removal and steam penetration is not impaired.

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**Expected ProSpore® Results**

- **Non-Infectious Autoclaved Biological Waste from UMass Amherst Treated in Accordance with MA-DEP and MA-DPH Regulations**
- **Date Treated:** 6/11/2014
- **Building:** LCR 2 Room: 17
- **Principal Investigator:** Fisher #12-001-1.

**Date** | **Quantity** | **Type** | **Treatment Method** | **Process Parameters** | **Printed Name** | **QC Results** |
--- | --- | --- | --- | --- | --- | --- |
6/6/14 | 1 | Waste bag | Heat | 60 min | 15 psi | N/A | 121°C | Chemical or Spore + or - |
6/7/14 | 1 | Waste bag | Heat | 60 min | 15 psi | N/A | 121°C | Control: yellow Test: purple |

**Environment Health & Safety May 2014**