

### **3.11. Autoclaving Procedures for Biological Waste**

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

#### **3.11.1. Container Selection**

##### **3.11.1.1. Polypropylene bags**

Commonly called biohazard or autoclave bags, these bags are able to withstand autoclaving and are tear resistant, but can be punctured or burst during autoclaving. Therefore, place bags in a rigid container such as a polypropylene or stainless steel pan during autoclaving. Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed. The biohazard bags should be clear or translucent. Red and orange autoclave bags are no longer permitted on Indiana University campuses.

Polypropylene bags are impermeable to steam, and for this reason must not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.

##### **3.11.1.2. Polypropylene Containers and Pans**

Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers do the following:

- Remove the lid (if applicable).
- Turn the container on its side when possible.
- Select a container with the lowest sides and widest diameter possible for the autoclave.

##### **3.11.1.3. Stainless Steel Containers and Pans**

Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

#### **3.11.2. Preparation and Loading of Materials**

- Fill liquid containers only half full.
- Loosen caps, or use vented closures.
- Always put bags of biological waste into autoclavable pans to catch spills.
- Position biohazard bags on their sides, with the bag neck taped loosely.
- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

- Add water to loads containing dry or absorbent material to facilitate proper steam generation and sterilization.

### **3.11.3. Cycle Selection**

- Use liquid cycle when autoclaving liquids, to prevent contents from boiling over.
- Select fast exhaust cycle for glassware.
- Use fast exhaust and dry cycle for wrapped items.

### **3.11.4. Time Selection**

- Bags of biological waste must be autoclaved in cycles that allow for a minimum of 20 minutes at 121°C and 15 psi to assure decontamination.
- Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- Non-select agent biological toxins may require extended autoclave times for inactivation. See **Appendix F** for additional instruction on biological toxin inactivation.

### **3.11.5. Removing the Load Safely**

**CAUTION - AUTOCLAVES MAY CAUSE SERIOUS BURNS. TO PREVENT INJURY:**

- Check that chamber pressure has returned to zero before opening door.
- Wear eye and face protection. Wear thermal protective gloves to handle materials.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware rush of steam as a burn hazard is present.
- Keep face away from door as it opens. Escaping steam may burn face.
- Wait 5 minutes after opening door before removing liquids.
- Liquids removed too soon may boil up and out of container, burning operator.

It is the responsibility of the autoclave user to transport autoclaved waste to the regular trash or dumpster. Follow the departmental specific procedures for your respective campus.