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# Autoclave Use

## 1. Training

Prior to operating an autoclave, the user must complete **Autoclave Safety Training** online. The training can be found in the Course Catalog via the Atlas Learning Center. Under Environment, Health, & Safety, select Biosafety, then [Autoclave Safety Training](#) (EHS00254w).

*Please note that not all autoclaves operate exactly the same. You should consult with someone in your lab that has used the autoclave, is familiar with the settings on your particular autoclave, and can show you the following:*

- *Standard autoclave operating procedure*
- *How to prepare items for sterilization*
- *How to select the appropriate cycle based on the contents of the load*
- *How to safely load and unload materials from an autoclave*

## 2. Personal Protective Equipment (PPE)

Since laboratory autoclaves use a jacketed construction, laboratory personnel should be aware that metal surfaces are hot even when the autoclave is not actively in use. Autoclaved materials can reach temperatures above 100°C and take a long time to return to room temperature. When operating the autoclave or handling autoclaved materials, make sure you are wearing the appropriate clothing and PPE as listed below.

- Long pants and closed-toed shoes
- Lab coat or vinyl apron



- Safety glasses or face shield



- Heat-resistant gloves that cover the hand and forearm



- **CAUTION:** Do not use cryo-gloves to handle autoclaved materials. They do not provide heat protection and can be damaged by high temperatures



Laboratories are responsible for providing this PPE and ensuring that it is in good shape. Please see your PI or supervisor if PPE needs to be ordered and/or replaced.

### 3. Preparing the Load

- Use a cart to transport items to the autoclave for loading and unloading.

- Place the items in a secondary container that can withstand autoclave temperatures, such as a **stainless-steel** tray or **polypropylene** container. This will collect any spills, leaks, or over-boiling of solutions. A secondary container is also recommended when doing dry runs for tubes, pipets, instruments, *etc.*
  - **Note:** *If using a plastic container, make sure it is autoclavable. Polystyrene will melt in the autoclave and should not be used.*
- Make sure all caps are loose. Never tighten caps on any bottles being sterilized as pressure will build up inside the vessel which can lead to a breakage or rupture.
- Cover each bottle with a small amount of aluminum foil and autoclave tape.
- If autoclaving stainless-steel tools, wrap them in aluminum foil and place a piece of autoclave tape on each item. (The tape is an indicator that the autoclave reached sterilization temperature.)
- Check the sediment collector located on the bottom surface of the autoclave. If it is dirty, clean and replace it.
- Check the door gasket to make sure it is not loose and that it is sealed correctly.
- Open the door and place the secondary container with the items in the autoclave.

Many autoclaves are equipped with metal wire rack shelves. When loading goods into an autoclave, place all items in such a way that they are easily accessible from the autoclave door without having to lean into the autoclave itself. The items also should not touch any piece of metal on the side of the machine.

#### 4. Running the Autoclave

- Close the door fully if you are using a hinged-door autoclave, engaging the locking pins and turning the locking mechanism until it stops moving. Do not overtighten.
- Make sure the jacket pressure gauge is between 15 – 20 psi. If not, press the steam button to allow the pressure to increase.
- Once the jacket pressure is at the appropriate level, turn on the autoclave using the “on” button. (This may be different for each autoclave.)
- Select the appropriate cycle type. The controls for each type are different for every autoclave, so make sure you select the proper cycle selection.
  - Typically, a “**liquid**” or “**slow exhaust**” cycle is used for sterilizing liquids or anything that will become liquid during autoclaving (broth, media, etc).
  - Typically, a “**dry**” or “**fast exhaust**” cycle is used for sterilizing solid materials like glassware, pipette tips, or Eppendorf tubes.
- Press the start button.

- Temperature should rise to 121°C or 250 F, and the chamber pressure should slowly rise to 15 psi.
- *Do NOT attempt to open the autoclave door once the cycle has started.*
- *Abort the cycle using the controller or emergency stop button if you suspect any issues with the autoclave.*
- *Do NOT attempt to open the door until the chamber pressure has returned to zero.*

## 5. Removing Items from the Autoclave

- Wait for the cycle to complete and the pressure within the autoclave chamber to return to zero.
- **Remember to wear the same protective clothing and PPE described above.** The steam from the autoclave and the heated metal jacket can cause severe burns.
- Stand to the side of the autoclave and ensure that no one else is near the unit while you open the door one inch. This allows the autoclave to vent residual steam while protecting yourself from the heat.
- Allow the steam to vent and the materials to cool for 10 minutes before you attempt to unload the autoclave.
- Liquids can become super-heated by the autoclave due to high pressure raising the boiling point. To reduce the risk of scalds or burns, do not agitate any liquid containers or remove caps while unloading.
- Transfer the secondary container from the autoclave to a cart for transport back to the lab and allow the items to continue cooling.
- Once the autoclave is unloaded, leave the jacket on and engaged in case other laboratory personnel need to use it.

**Note: If the autoclave is not functioning properly, or if you have any questions, please contact your EHS Coordinator or contact EHS directly at 617-452-3477 or [environment@mit.edu](mailto:environment@mit.edu).**