

Autoclave Standard Operating Procedures

Department of Biological Sciences

RISK MANAGEMENT

1. Associated Risks

- a. Autoclaves, also known as steam sterilizers, operate at high temperatures and pressures and as a consequence pose a serious risk to operator safety.
- b. Specific risks include:
 - i. Heat, or burns from hot materials and autoclave chamber walls and door.
 - ii. Steam burns from residual steam venting from autoclave and materials on completion of the cycle.
 - iii. Hot fluid scalds from boiling liquids and spillage in autoclave

2. Risk Control

- a. Autoclaves to be regularly inspected and an inspection service and repair record maintained.
- b. An authorized training session must be successfully completed prior to use of autoclaves.
- c. Procedure and instruction documentation must be followed
- d. Personal protection must be worn
 - i. Heat-insulating gloves that provide complete coverage of hands and forearms must be worn when loading and unloading the autoclave.
 - ii. Eye protection, a lab coat and closed-toed footwear are required to protect against steam and scald burns.

OPERATION INSTRUCTIONS

1. Training

- a. All operators must have successfully completed an authorized training session.

2. Material Preparation

- a. Ensure that the material is autoclavable. Oils, waxes, some plastics, flammable materials, and samples containing solvents or substances that may emit toxic fumes should not be autoclaved.
 - i. Never sterilize items containing chlorides or producing chloride gases in the automatic autoclaves
- b. Package material suitably; loosen all lids.
- c. Bottles with liquids must be a maximum of one-half full and lids loosened.
- d. Place items in secondary containers, e.g. Nalgene trays provide in the autoclave room.
 - i. If your lab has trays confirm with the Departmental Laboratory Supervisor that they are the correct material.
- e. Contaminated materials must be secured in a containment vessel or an autoclavable bag and processed as soon as possible according to licensing requirements for the handling of infectious materials.

3. Log Book

- a. Entries must be placed in the log book each time the autoclave is used. These records are used for maintenance/service schedules and reporting incidents, accidents, and/or faults.
- b. Entries should include:
 - i. Full name
 - ii. Lab number
 - iii. Date + time

iv. Cycle information
(gravity/liquid + durations)

v. Other relevant not

4. Loading the Autoclave

- a. Wear heat insulating gloves, eye protection, lab coat, and closed-toed shoes
- b. Place items in secondary container into the autoclave chamber
- c. Ensure there is enough room in the autoclave for steam to circulate. The bottom rack must always be used to allow steam to circulate underneath the secondary container.

5. Operating Manual Barrel Autoclaves

- a. Close the door firmly.
- b. Close the waste valve (see diagram on white board for valve locations)
- c. Open the steam valve
- d. Set a timer on your phone or watch
 - i. Allow for 20 minutes for pre-sterilization to ensure proper pressure and temperatures are reached
 - ii. Add additional sterilization time based on your protocol
 - iii. The University Biosafety Officer recommends a total of 60 minutes for autoclaving biohazardous waste (infectious and/or genetically modified materials)
- e. Unload the autoclave
 - i. Gravity or Fast Exhaust
 1. This method is for solid materials (pipette tips, empty glassware, etc)
 2. Close the steam valve
 3. Open waste valve and allow the chamber pressure to come back to zero
 4. Wearing personal protection equipment, carefully crack door open to release residual steam
 5. Allow sterilized materials to stand for 1-2 minutes. This will allow steam to clear and trapped air to escape.
 6. Take out items.
 - ii. Liquid or Slow Exhaust
 1. This method is for liquid materials or anything that will become liquid while autoclaving (media, broth, etc.)
 2. Close the steam valve
 3. Allow the chamber pressure to come back to zero *while leaving the waste valve closed*. This will take approximately 40 minutes.
 4. Wearing personal protection equipment, open the waste valve and carefully crack door open to release residual steam.
 5. Allow sterilized materials to stand for 1-2 minutes. This will allow steam to clear and trapped air to escape.
 6. Take out items.

6. Operating Automatic Autoclave

- a. Check to ensure the unit is powered on and the jacket is on by looking on the touch screen. The units automatically power on at 8 AM and turn off at 11 PM each day.
 - i. If the units are powered off, turn the power switch to the ON position.
 - ii. If the jacket is off, then tap the "Jacket is Off" button on the touch screen to turn it on. It will take about 15 minutes for the jacket pressure to reach the proper 15 psi.
- b. Gently slide the door completely closed
- c. From the main menu, touch CYCLE SELECT

- d. Select cycle type: Gravity or Liquid
 - i. Gravity cycles are used for solid materials (pipette tips, empty glassware, etc.)
 - ii. Liquid cycles are used for liquid materials or anything that will become a liquid while autoclaving (medial, broch, etc.)
- e. Select the specific cycle you want to run by pressing the SELECT button.
 - i. Temperature should never exceed 121 Celsius (250 Fahrenheit). Please contact the Departmental Laboratory Supervisor if you require a different temperature.
- f. Review the parameters
 - i. If correct, then select YES
 - ii. If incorrect, then select NO and you will be brought to the previous screen
- g. Once the cycle is complete, and audible noise will sound and message will appear on the screen instructing you to open the door to take out items.

7. Waste Disposal and Cleanup

- a. If container says "Biohazard" or is a "Sharps Box" it must be placed in a Biohazrd collection box after cooling.
- b. All other wastes must be double bagged in a regular garbage pages
 - i. Haul trash to a dumpster outside loading dock
 - ii. Do not dump liquid contain melted agar down the drawn. Instead, collect it in a disposable container.
 - iii. Clean up and spills in the autoclave or on the floor.
 - iv. Rinse out secondary containers and invert to dry.
- c. Spills inside the autoclave, must be cleaned immediately with Consolidated C3 Chamber Cleaner. Report any spills to the Departmental Laboratory Supervisor.