

AUTOCLAVE PROCEDURE

LOADING AND UNLOADING THE AUTOCLAVE SAFELY:

- Bags that are closed and ready for autoclaving must be placed in secondary containment. Minimize contact with biohazard waste as much as possible.
- Never crush or push down biohazard waste.
- Biohazard waste containers should be removed for autoclaving when they are 2/3 full. Indicator tape should be applied in an "X" pattern over the biohazard symbol.
- The heat sensitive tape is to be of the type that changes color, such as the type that the word "autoclaved" appears after treatment. This tape is available from Fisher Scientific.
- Once the autoclave disinfection is complete, the tops of the bags may be sealed tightly with lab tape.
- After the proper autoclave waste decontamination steps are followed as listed below, the decontaminated waste is then placed in a container lined with black plastic garbage bags. These containers are to be labeled "AUTOCLAVED/ DECONTAMINATED WASTE ONLY".
- When the black plastic garbage bags are full, they are offered as garbage waste to housekeeping.

AUTOCLAVING PRECAUTIONS:

Autoclaving, or steam sterilization, is the most dependable procedure for the destruction of all forms of microbial life. Proper temperature and exposure time are critical factors in ensuring the reliability of this method. These critical factors are dependent upon steam penetration to every part of the waste load. Therefore, the autoclave user must be mindful to prevent the entrapment of air. If all the air is not allowed to escape from the waste during the cycle, it cannot be replaced by steam. Saturated steam is employed under pressure (at least 15 pounds per square inch) to achieve a chamber temperature of at least 121 C (250 F) for a minimum of 15 minutes. This time is measured after the temperature of the steam saturated material being sterilized reaches 121 C.

When operating an autoclave the following safety procedures must be followed:

- Become familiar with the autoclave's owner's manual. Though the principle is the same for each, manufacturer recommendations for use can vary widely.
- Firmly lock autoclave doors and gaskets in place before you run the autoclave to prevent a sudden release of high-pressure steam.
- Do not stack or store combustible materials (cardboard, plastic, volatile or flammable liquids, compressed gas cylinders) next to an autoclave.
- Do not autoclave toxic, volatile or radioactive material. If you have biohazard waste that contains any of these materials, please contact EH&S for guidance.
- When a cycle is complete, wait approximately 1-2 minutes after the pressure gauge reads zero before opening the door of the autoclave.
- Wait at least 30 seconds after opening the door before reaching or looking into the autoclave.
- Open the door slowly, keeping head, face, and hands away from the opening.
- Allow contents to cool before removing them from the autoclave.
- Remove solutions from the autoclave slowly and gently; some solutions can boil over when moved or when exposed to room temperature. Heat-resistant gloves and safety glasses or face shield should be

worn when removing hot liquids from the autoclave. Liquids should stand for over 1 hour before being handled without heat-resistant gloves.

- Clean up any spills immediately.

AUTOCLAVE WASTE DECONTAMINATION PROCEDURES:

The autoclave is to be operated at 121°C (250°F) or higher for a minimum of 60 minutes for most biohazard waste (see chart below). The time and temperature used for each type of waste in the laboratory must be validated using biological indicators to ensure effective sterilization (see procedure below).

Material	Temperature	Time
Laundry	121°C (250°F)	30 minutes
Trash (biohazard bags containing infectious waste)	121°C (250°F)	1 hour
Glassware	121°C (250°F)	1 hour
Liquids	121°C (250°F), each gallon	1 hour
Animals	121°C (250°F)	8 hours

Criteria for Autoclaving Typical Materials

Use the appropriate autoclave settings.

Solid waste: Do not overfill waste bags or the autoclave. This will interfere with steam penetration. Add about 50-100 ml (~¼ to ½ cup) of water to each bag of solid waste to facilitate steam penetration in the bag. If there is naturally occurring water in the load, adding additional water is not necessary. Keep the waste bags slightly open to allow for steam penetration. Bags are placed into stainless steel or polypropylene trays prior to autoclaving.

Liquid waste: Liquids should be placed in borosilicate (Kimax or Pyrex) or polypropylene containers for autoclaving. The containers should not be filled to more than 75% capacity. The caps or stoppers on the containers should be loosened. **Never autoclave sealed containers of liquid. This could result in an explosion of superheated liquid.** Liquid containers should be placed in a stainless steel or polypropylene

tray with ¼ to ½ inch of water in the bottom of the tray. The tray should be placed on a shelf in the autoclave and not on the bottom of the chamber.

AUTOCLAVE WASTE DECONTAMINATION CYCLE TESTING AND VERIFICATION:

The N.C. Medical Waste Rules require that autoclaves be monitored under conditions of full loading for effectiveness monthly through the use of **biological indicators**. *Geobacillus stearothermophilus* indicators must be used with average spore populations of 10^4 to 10^6 organisms. There are many commercially available biological indicators with a choice of spore ampoules or spore strips with growth media.

- Follow the instructions provided by the manufacturer of the biological indicators. Most require refrigeration when kept in storage.
- Place the indicator in the middle of the waste bag or material to be autoclaved. It is best to put the indicator in the waste bag before it is filled completely. To aid recovery of the indicator after sterilization, tape it to a brightly colored sheet of paper or to a long string allowed to protrude from the bag. Indicators can also be placed in test waste bags filled with materials that simulate full loading for the test.
- Autoclave the waste, following normal procedures. Once the cycle is complete and contents have cooled, remove the indicator from the waste bags wearing appropriate protective equipment. Prepare and incubate the indicator and a control indicator that was not autoclaved as recommended by the manufacturer.
- **Check for signs of growth** at regular intervals during the incubation period (8, 12, 24 and 48 hours). There should be signs of growth on the control indicator that was not autoclaved or the test is invalid. If there are signs of growth on the indicator placed in the waste, the waste was not sterilized properly. The time, temperature and autoclave procedures should be re-evaluated.
- A log of each test should be maintained, which includes the type of indicator used, date, time, and result of the test.
- The waste does not have to be held until the results of the testing confirm effectiveness. If test results indicate that the autoclave is not sterilizing properly, the autoclave should not be used for waste until it has been repaired. The first load run in the autoclave should be tested with a biological indicator to insure proper functioning of the autoclave.

AUTOCLAVE PREVENTATIVE MAINTENANCE:

Autoclave operators should perform the following preventative maintenance on their autoclave to maintain the autoclaves effectiveness:

- Remove the plug screen or drain strainer to make sure it is free of dirt, dust, or sediment that may collect in it and it should be cleaned as necessary.
- Clean the interior surfaces of residues collected from the steam or materials being sterilized as needed.
- Visually inspect the gaskets, doors, shelves and walls for residue buildup or wear regularly.