

Autoclaves

UMass Amherst has a comprehensive Waste Management Program, and the majority of biological waste is autoclaved on campus, prior to disposal into an approved landfill.

Biological Waste (with the exception of animal carcasses and medical waste from UHS) is placed into CLEAR autoclave bags for treatment. Clear autoclave bags are used, as landfills cannot accept red or orange bags, or materials with a biohazard symbol on them.

An autoclave is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents. In order to kill a cell through heat, its temperature must be raised to the point where the proteins in the cell wall break down and coagulate. Steam is a very efficient medium for transferring heat, therefore it is an excellent way to destroy microbes.

How they work: All autoclaves operate on similar scientific principles that they share with a common kitchen pressure cooker and use temperature, pressure and time. The door is locked to form a sealed chamber, and all air within the chamber is replaced by steam. The steam is then pressurized to reach the desired sterilization temperature and time, before exhausting the steam and allowing the goods to be removed. Here are the various phases of a sterilization cycle.

1. **Purge Phase:** Steam flows through the sterilizer beginning the process of displacing the air; temperature and pressure ramp slightly to a continuous flow purge.
2. **Exposure (Sterilization) Phase:** During this phase, the autoclaves' control system is programmed to close the exhaust valve causing the interior temperature and pressure to ramp up to the desired set point. The program then maintains the desired temperature (dwells) until the desired time is reached.
3. **Exhaust Phase:** The pressure is released from the chamber through an exhaust valve and the interior is restored to ambient pressure, although contents remain relatively hot.

Autoclave Cycles:

- **Gravity:** The most basic sterilization cycle. Steam displaces air in the chamber by gravity (i.e. without mechanical assistance) through a drain port.
- **Pre-vacuum or Post-vacuum:** Air is mechanically removed from the chamber and load through a series of vacuum and pressure pulses. This allows the steam to penetrate porous areas of the load that couldn't otherwise be reached with simple gravity displacement.
- **Liquids:** a gravity cycle with a slower exhaust rate to minimize boil-over.

Waste Processing in Autoclaves

1. Step cans (labelled with a biohazard symbol) are lined with clear autoclave bags.
2. Once the clear autoclave bag is full, loosely close the top of the bag, and remove it from the step can. Place the bag in a rigid, secondary container (an autoclavable bin) that is marked with the biohazard symbol. The bag is transported to an autoclave that is approved for waste processing.

3. Place the autoclavable bin on a rack inside the autoclave. Do not let the bag(s) touch the sides, rear wall, bottom or top of the autoclave chamber.
4. All autoclaves have signs affixed to them to determine whether they are approved for waste processing or not.
5. There are signs that direct you to the appropriate waste cycles to use for solid vs. liquid waste.
6. Record your load in the orange Biological Waste Record Keeping Log. Entries must be legible.
7. **All waste cycles are programmed for 60 minutes.** All entries are audited by the Biosafety team and reviewed by the Institutional Biosafety Committee on a monthly basis.
8. The autoclave bag is removed and allowed to cool at the end of the waste cycle.
9. An "Autoclaved Waste" sticker is filled out and affixed to the clear bag.
10. The clear bag is placed inside a black (dark or not see-through is also acceptable) plastic trash bag and disposed of in a dumpster.

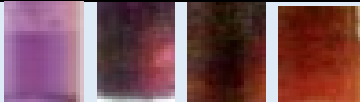



Record keeping

1. Records for biological waste processing are required by the state and federal governments.
2. Record all loads in the orange [Biological Waste Record Keeping Log](#). Entries must be legible.
3. These record books are specific to one autoclave. If there is more than one autoclave in a room, select the appropriate record book.
4. Log the load in the "Daily Log Sheets" section. Complete each column as necessary.
5. Chemical indicators are to be checked off for each load. Were they successful? Yes or No must be selected. The indicators are visual (change color) and demonstrate that the load reached a specific temperature. Examples of chemical indicators are [autoclave tape](#) and [indicator strips](#).
6. Once per month a spore test must be run for all autoclaves used to treat waste. See below.
7. Record spore test results in the section: "Spore Test Sheets". The results are to be recorded whether they pass or fail. Contact EH&S if failed tests occur.
8. Repairs, certifications and installation records are to be maintained for five years. These records may be submitted to EH&S or kept in the department. These records must be available for state or federal agents, and they are requested if individuals hold USDA or CDC permits.
9. Log books are kept on hand in EH&S for 3 years.

Spore Testing (Biological Indicators)

1. Spores tests can be [ordered](#) free of charge from EH&S.
2. Spore testing shall occur monthly for autoclaves that process waste.
3. Place a spore vial in the most difficult location to sterilize, usually in the center of the load or suspended in a volume of liquid. Dental floss is a useful way to retrieve vials from bags.
4. Run cycle for at least 60 minutes.
5. Place the spore vials in a vertical position in an incubator at 55-60°C. Mark a control vial as such and incubate for 48 hours along with processed vial to ensure spore viability. Contact EH&S if you need to incubate your spores in our incubator.
6. All vial results should be recorded and then disposed of immediately into a sharps container.
7. Control: The control vial 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.

8. 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-like color indicates an adequate sterilization cycle. Due to a caramelization of the sugars in the test vials, the colors can range from purple to a “bourbon” color. See the color chart below.
9. If the test failed, perform corrective action (see below), re-process the load and run spores again. Use a different autoclave for the waste load if necessary.

PASSING Results		FAILING Results	
Test		Test	
Control		Control	

Corrective Action:

1. Check temperature charts and verify that the correct cycle was run. Verify the spores are not expired and select a different lot number when retesting commences.
2. 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.
3. Density: Load the bag to 75% capacity or less, because steam cannot penetrate completely through densely packed bags.
4. 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.
5. Record all changed parameters on the log sheet. Revise your autoclave cycle and procedures so new parameters for autoclaving waste are included and inform EH&S.
6. If none of the above gives a negative result (purple autoclaved ampoule), notify EH&S and call the autoclave repair vendor.

Autoclave Safety:

Comprehensive safety training is available in [OWL](#) and required on an annual basis for autoclave users:

To prevent injury:

- Loosen screw caps on bottles and tubes of liquids before autoclaving.
- Check that chamber pressure has returned to zero before opening door.
- Wear eye and face protection.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware rush of steam.
- 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.

MA 105 CMR 480.00

The University of Massachusetts at Amherst is required to comply with the regulation: [105 CMR 480](#)

The University of Massachusetts at Amherst underwent an extensive review by the Massachusetts Department of Health that resulted in approval of our biological waste management program.

Of special note:

The Department of Health requires that an annual calibration of the autoclave be performed.

“An operations and mechanical systems assessment shall be conducted by a qualified individual who has received training from the manufacturer in the operations and maintenance of the equipment. Appropriate corrective actions shall be implemented, when warranted, including but not limited to mechanical adjustments and when applicable, recalibration of all parametric monitoring devices followed by re-treatment of the waste and additional challenge testing to confirm the effectiveness of any implemented corrective action. Retain results of annual calibration procedures for parametric monitoring equipment with the record-keeping log for three years.

Resources:

[Consolidated Sterilizers](#)

[Getinge](#)

[Primus Sterilizers](#)

[Tuttnauer](#)