

Biological Waste Program

Purpose and Applicability

The purpose of this document is to establish the process through which biological waste, rDNA materials, and medical waste is handled; whether it is deactivated on-site, or stored, packaged and shipped for off-site deactivation and/or incineration.

Biological waste is generated within the laboratory/research facility and contains potentially biohazardous agents or recombinant DNA material. Laws and regulations are in place to ensure the proper handling of these materials and to offer guidance to those who must do so.

Generator requirements are listed in 105 Code of Massachusetts Regulations (CMR) 480.000:

<http://www.mass.gov/eohhs/docs/dph/regs/105cmr480.pdf>

Special waste facilities are addressed with: 310 CMR 19.000; New Source Performance Standards (NSPS) for hospital/medical/infectious waste generators (HMIWI): 40 CFR 60.50c to 60.58c. Additional regulatory agencies are the Massachusetts Department of Public Health (DPH) Community Sanitation Program and the Massachusetts Department of Environmental Protection.

Routes for Managing and Treating Biological/Infectious Waste

The University utilizes several means to manage biological waste for our campus.

- [Autoclaving](#)
- [Liquid Waste Treatment](#)
- [Contractor Removal](#) (Incineration or Medical Waste recycling)

Roles and Responsibilities

- **Biosafety Staff** – advises the Environmental Health & Safety Executive Director on all matters relating to the use of biological agents within UMass. The biosafety staff advises faculty, staff and students on waste disposal policies, disinfection policies, risk assessments, hazardous waste training, and autoclave management program.
- **Environmental Management Staff** – (Environmental Health & Safety) assists the University with the proper characterization, removal and disposal of hazardous waste. Environmental Management monitors the website where [waste removal requests](#) are processed by a commercial hauler.
- **Principal Investigator** – Lead research scientist in the laboratory and responsible person for training on proper laboratory practices including waste decontamination and disposal as well as safety.

- **Laboratory Staff** – Scientists, assistants, technicians, students, clerical staff and any other persons that are working in the laboratory.
- **Institutional Biosafety Committee:** University personnel plus two community members who are responsible for the annual review of the biological and medical waste management program.

Definitions

Animals and Animal Bedding: All animal carcasses (or animal tissues) that have been used for teaching and/or research purposes are to be disposed of by incineration. The carcasses must be packed in a leak-proof container, labeled appropriately, and frozen until disposal. If the animals or animal tissues have been placed in a formaldehyde solution, they must be separated from the solution before incineration.

Animal bedding must be bagged, placed in a dumpster and disposed of in a sanitary landfill. Bedding contaminated with biohazardous waste, (e.g., from animals shedding pathogens) must be decontaminated by autoclaving before disposal.

Autoclaves: An autoclave is a specialized piece of equipment designed to deliver heat under pressure to a chamber, with the goal of sterilizing the contents of the chamber. There are chemical and biological indicators that can be used to ensure an autoclave reaches the correct temperature for the correct amount of time.

Biohazardous Agent: Refers to infectious agents or other hazardous biologic materials that present a risk or potential risk to the health of humans, animals, or the environment. The risk can be direct through infection or indirect through damage to the environment. Biohazardous agents include, but are not limited to; bacteria, fungi, viruses, rickettsiae, Chlamydia, prion, parasites, recombinant products, allergens, cultured human and animal cells and the potentially biohazardous agents these cells may contain, infected clinical specimens, tissue from experimental animals, plant viruses, bacteria and fungi, toxins, and other biohazardous agents as defined by State and Federal regulations.

Biohazardous and Medical Waste: Is defined as human tissues, wastes from contact with patients; waste contaminated with infectious agents, live vaccines, all contaminated sharps (syringes, needles, scalpel blades, etc.), and animal carcasses/tissues. These wastes must be disposed of by incineration.

Experimental rDNA Material: In the context of the NIH Guidelines, recombinant DNA molecules are defined as either:

- a) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or
- b) Molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart.

All recombinant DNA waste must be deactivated prior to disposal.

Incineration: Is a waste treatment technology that involves the combustion of organic materials and/or substances. Incineration and other high temperature waste treatment systems are described as thermal treatment. Incineration has particularly strong benefits for the treatment of certain waste types in niche areas such as clinical wastes and certain hazardous wastes where pathogens and toxins can be destroyed by high temperatures.

Infectious Medical Waste: In Massachusetts this waste is defined as waste that, because of its characteristics, may cause or significantly contribute to an increase in mortality or an increase in serious irreversible or incapacitating reversible illness or pose as a substantial present potential hazard to human health or to the environment when improperly treated, stored, transported, disposed of, or otherwise managed. The following are included in infectious medical wastes: blood and blood products; pathological waste; cultures and stocks of infectious agents; contaminated animal carcasses, body parts, animal bedding; sharps (needles, scalpels, blades, etc.); and biotechnical by-product effluents.

Medical Waste Recycling: The waste contractor collects, sterilizes and recycles regulated medical waste. The treatment involves shredding the materials and then an EPA approved sanitization process takes place. Sterilized material is further shredded, melted, extruded, and converted into various grades, dimensions, and colors of plastic.

Sharps: Are items that are capable of puncturing, cutting or abrading the skin. Sharps include, but are not limited to; glass and plastic pipettes, broken glass, test tubes, razor blades, syringes, and needles. Sharps shall be segregated from other wastes and aggregated immediately after use in red, fluorescent orange or orange-red leak proof, rigid, puncture-resistant, shatterproof containers that resist breaking under normal conditions of use and handling, meet ASTM standard F2132-01, and that are marked prominently with the universal biohazard warning symbol and the word "Biohazard" in a contrasting color.

Autoclaving

Autoclaves are the primary means for laboratories to process waste on our campus. There are very few *exemptions from autoclaving* your laboratory waste. Your lab may be exempt if

- a) Your building does not have an autoclave on site (contractor removal is used)
- b) You are disposing of animal carcasses or large tissues (contractor removal is used)
- c) You are creating medical waste that must be incinerated by law (contractor removal is used)

An autoclave is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, 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:

1. Gravity: The most basic sterilization cycle. Steam displaces air in the chamber by gravity (i.e. without mechanical assistance) through a drain port.

2. 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.
3. Liquids: A gravity cycle with a slower exhaust rate to minimize boil-over.

Collection of Waste:

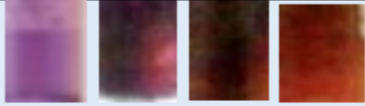



1. Waste is to be collected into a "Biohazard" labelled step can that is lined with a clear autoclave bag. EH&S can provide the "Biohazard" labels for the step can. Step cans are preferred as they may be opened without touching the container with your hands. Step cans allow you to have access to the container without having your face (and breathing zone) in close proximity to the waste.
2. Clear autoclave bags are available in the Fisher Stockroom. Clear autoclave bags are used as the final disposal will be to a landfill. Red, orange, or bags labelled with "Biohazard" are not allowed in landfills.
3. Do not overfill waste bags. Wearing appropriate PPE, remove when the bag is 2/3 full. Loosely close the bag by tying or taping it closed.
4. Place a chemical indicator on the bag (autoclave tape is an example of this).
5. Place the bag into secondary containment prior to transporting to the autoclave. Acceptable secondary containment: autoclavable bin (Nalgene) or a stainless steel tray with sides.
6. Transport the waste to an autoclave that is approved for waste disposal. There are signs posted on each autoclave to inform you if the autoclave is suitable for processing waste, or not.

Autoclaving Procedure:

1. There are signs that tell you to the appropriate waste cycles to use for solid vs. liquid waste.
2. 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.
3. Check the drain in the bottom of the autoclave chamber. If there is debris present, remove the debris prior to loading the autoclave.
4. Place the bag (within secondary containment) inside the autoclave.
5. Select the appropriate cycle and start the autoclave. If the autoclave is an older model and does not have preprogrammed cycles, be sure to add 60 minutes to the timer.
6. Allow the autoclave to complete the cycle. Allow some cool-time prior to opening the door.
7. Remove the autoclave bag and allow to cool at the end of the waste cycle.
8. Fill out an "Autoclaved Waste" sticker and adhere it to the clear bag.
9. Place the clear bag inside a black (dark or not see-through is also acceptable) plastic trash bag and dispose of in a dumpster.

Biological Indicators (spore testing):

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 a minimum of 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. (Do not break the spore vial prior to disposal)
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.
5. 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.

6. 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.
7. If none of the above gives a negative result (purple autoclaved ampoule), notify EH&S and call the autoclave repair technician for the autoclave.

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 “Biological Indicators” above.
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.

Safety Notes:

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.
- Keep face away from door as it opens. Escaping steam may burn face.
- Slowly open door to allow steam to leave the autoclave.
- Wait 5 minutes after opening door before removing liquids.

Of Special Note:

[MA 105 CMR 480.00](#) 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.”

Liquid Waste Treatment

Liquid wastes can be disposed of via the laboratory sink, under running water **after** it has been decontaminated by autoclave or chemical means. The sink should be rinsed well and disinfected if necessary, after the disposal procedure.

Collection of Waste:

1. Liquid biological waste should be collected in containers suitable for autoclaving or chemical disinfection.
2. These containers are to be labelled with the “Biohazard” symbol.
3. Transport these containers (close with caps) within secondary containment.

Treatment Procedure**Autoclaving Liquids:**

1. Loosen the caps on liquids once you have arrived at the autoclave.
2. Check the drain in the bottom of the autoclave chamber. If there is debris present, remove the debris prior to loading the autoclave.
3. Place the containers inside the autoclave. Do not allow bottles to touch each other or the sides/bottom of the autoclave.
4. Select the appropriate cycle (Liquid Cycle/60 minutes) and start the autoclave. If the autoclave is an older model and does not have preprogrammed cycles, be sure to add 60 minutes to the timer.
5. Allow the autoclave to complete the cycle. Allow 15 minutes of cool-down time prior to opening the door. **Gently** remove the bottles and allow them to cool for one hour.
6. The cooled waste can then be gently poured down the laboratory sink while flushing with copious amounts of water.
7. Do not pour melted agarose down the drain. Allow it to cool and solidify, then dispose of it as solid waste in biohazardous waste bags.

Chemical Disinfection of Biological Liquids:

1. Ensure that there are no chemicals present in the solution. Chemicals are not allowed down the drain and may react with the chlorine that is used to disinfect the liquid.
2. Select a container that is large enough to hold the fluid to be disinfected plus 10% volume.
3. Add concentrated germicidal bleach to the empty container. (1 Part concentrated bleach to 9 parts of the liquid to be disinfected).
4. Add the liquid to the bleach.
5. Gently mix the solution and allow it to stand for at least 30 minutes.
6. The disinfected waste can then be gently poured down the laboratory sink while flushing with copious amounts of water.

Contractor Removal

Solid biological waste may be removed from campus by a licensed waste hauler. Some waste must be incinerated according to law (sharps, BSL-3 materials, etc.). Some waste is removed from campus as the laboratory may not have access to an autoclave. Additionally, some waste is removed from campus as it would be too difficult to autoclave or may create terrible odors. This waste stream is managed very closely to maintain compliance with federal and state laws, as well as to keep costs down for the University.

Solid Biological Waste (Sharps disposal is addressed in a separate section below)

Obtaining Storage Containers:

1. The EH&S website has a form that you will use to order supplies:
<https://cems.unh.edu/umass/CEMS/FormSubmissionRecord?form=7&win=popup>
2. Place an order for 1 box and 2 red biohazard waste bags.

Collection of Waste:

1. Waste is to be collected into a “Biohazard” labelled step can (purchased by the lab) that is lined with a plastic bag. EH&S can provide the “Biohazard” labels for the step can. Step cans are preferred as they may be opened without touching the container with your hands. Step cans allow you to have access to the container without having your face (and breathing zone) in close proximity to the waste.
2. Do not overfill waste bag/containers. Wearing appropriate PPE, remove when the bag is 2/3 full. Close the bag by tying or taping it closed.
3. Transport the bag of waste to a bio-waste box provided by the waste contractor. This box should be lined with one of the red biohazard bags. Keep the lid closed on this box. (Some

labs that generate biological waste frequently will be supplied with red plastic containers that are recycled.)

Storage of Waste

1. Waste that may create an odor can be frozen prior to packaging and consequent removal.
2. Waste can also be held in a cold room or refrigerator for short periods of time.
3. Plan on removal of waste before odors are generated.

Removal of Waste

1. When the red bio-waste container is full, please seal the red bag and place a [Hazardous Waste Pickup Request](#) in CEMS to remove the full red containers.
2. Biological waste pickups are usually done on Thursdays.

Sharps Containers:

Obtaining Storage Containers

1. The EH&S website has a form that you will use to order sharps containers:
<https://cems.unh.edu/umass/CEMS/FormSubmissionRecord?form=7&win=popup>
2. Place an order for 1 box.
3. Sharps are provided at no cost to the laboratory.

Collection of Waste

1. Syringes, needles and other sharp implements are disposed of into sharps containers.
2. Seal the container when it is 2/3 full at the "FULL" line on the container.
3. Place the sealed container in the hazardous waste collection area.

Removal of Waste

1. Place a [Hazardous Waste Pickup Request](#) in CEMS to remove the full sharps container.
2. Biological waste pickups are usually done on Thursdays.

Resources

1. [Biosafety Manual](#)
2. [BMBL 5th Edition](#)
3. 105 CMR 480.000 Minimum Requirements for the Management of Medical or Biological Waste (Massachusetts State Sanitary Code Chapter VIII)
4. Bloodborne pathogen Standard 29 CFR 1910.1030, and OSHA Instruction CPL, 2-2, 44C, Occupational Health and Safety Administration, 1999
5. Code of Federal Regulations, Hazardous Waste Criteria, 40 CFR 261, Subpart C and Subpart D, 40 CFR 262.11, and 21 CFR 1200-1316.

6. National Institutes of Health for Research Involving Recombinant DNA Molecules, April 2002.