

SOP BIO-006 USE OF AUTOCLAVE FOR STERILIZATION OF MATERIALS AND BIOLOGICAL WASTE

SCOPE

This policy describes the use of autoclave/steam sterilization for general sterilization of labware, liquid and solid materials, and biological waste. The policy is part of the Biohazardous Waste Disposal Program in compliance with Massachusetts Department of Public Health regulations State Sanitary Code Title VIII 105 CMR 480.00, Massachusetts Department of Environmental Protection regulations 310 CMR 19.00, and UMASS Lowell policies

This standard operating procedure (SOP) applies to all laboratories that generate regulated biological waste. This waste includes material such as labware contaminated with blood, blood products, non-fixed pathological waste, cultures and stocks of infectious agents and associated biological material, animal carcasses, animal bedding, and sharps.

This SOP also applies to the sterilization by autoclaving of different materials and items that need to be sterile for biological work in the laboratory.

DEFINITION

Autoclave is an airtight vessel utilized for sterilization of objects by using steam under pressure. During the autoclaving process, each item is exposed to direct steam at the required temperature and pressure for a specified time.

Biohazardous Agent is any agent that is biological in nature, capable of self-replication, and has the capacity to produce harmful effects upon biological organisms. Biohazardous agents include, but are not limited to: bacteria, fungi, viruses, rickettsia, chlamydia, parasites, recombinant products, allergens, cultured human and animal cells and the potentially biohazardous agents these cells may contain, clinical specimens, tissue from experimental animals, toxins of biological origin, other biohazardous agents like prions or as defined by State and Federal regulations.

Decontamination is a procedure that eliminates or reduces microbial contamination to a safe level with respect to transmission of infection.

Disinfection is a procedure that kills pathogenic microorganisms, but not necessarily their spores. Chemical germicides formulated as disinfectants are used on inanimate surfaces (i.e., medical devices) and not used on skin or any body parts.

Infectious Waste is waste containing, or potentially containing, pathogens of sufficient virulence and quantity so that exposure to the waste by a susceptible host could result in the development of a communicable disease.

Pathological Waste includes all animal and human non-fixed organs, tissues, body parts other than teeth; products of conception; fluids removed by trauma, during surgery, autopsy, or other medical procedure; and infected animal carcasses.

Regulated Biohazardous/Medical Waste includes any material such as sharps; blood and blood products; pathological waste; cultures and stocks of infectious agents and associated biologicals; and animal bedding that contains or has been contaminated by a biohazardous agent. Biohazardous waste can be separated into sharps (See Policies and Procedures for Disposal of Sharps), liquid waste, and solid waste.

Steam is the vapor created by heating water to 212°F (100°C).

Steam Sterilization is moist heat in the form of saturated steam under pressure. This is the most widely used and dependable methods available for sterilization. The exposure of any item to moist heat at 250°F or 121°C under pressure (at least 15 psi) for 15 to 30 minutes allows the destruction of all forms of microbial life on any item.

Sharps include objects that can cause a puncture or laceration. Sharp waste includes needles, scalpels, lancets, and shards of glass or plastic contaminated with biological agents.

Universal Precautions is an infection control method where all human blood and any other potentially infectious materials are treated as if known to be positive for bloodborne pathogens such as Hepatitis B Virus, Hepatitis C Virus, and Human Immunodeficiency Virus.

RESPONSIBILITIES

Employees

1. Familiarize themselves with this SOP and associated work instructions;
2. Abide by this SOP and associated SOP requirements;
3. Immediately report injuries, accidents, unsafe conditions, and unsafe acts to their supervisor and/or Environmental Health and Safety (EHS);
4. Attend applicable training classes.

Supervisors

1. Assure that permanent and temporary workers are trained in and follow the requirements of this SOP;

2. Assure that employees attend appropriate training sessions;
3. Investigate and report accidents and unsafe conditions to Environmental Health and Safety.

Environmental Health and Safety (EHS)

1. Provide professional guidance and resources related to management of biohazardous waste;
2. Conduct training for employees;
3. Monitor the implementation of this SOP in various labs;
4. Ensure that accidents and other hazardous situations, which may unnecessarily expose employees to biological hazards, are properly reported, evaluated, and corrected;
5. Oversee all aspects of contracts with outside biological waste vendors;
6. Act as site-wide liaison with other departments and institutions sharing common facilities and resources pertaining to biological waste management.

Principal Investigator/Laboratory Director/Laboratory Manager

1. Review and approve the implementation of this SOP;
2. Support managers and supervisors in their efforts to implement the SOP;
3. Investigate and report accidents and unsafe conditions to Environmental Health and Safety.

PERSONAL PROTECTION EQUIPMENT (PPE)

1. Before handling any type of biological waste to be put in or taken out of the autoclave, employees should wear proper PPE: lab coat, safety glasses, heat resistant gloves and closed-toe shoes;
2. When handling biological waste, all employees should follow universal precautions;
3. Employees should never reach into a biohazardous waste container to retrieve materials.

EQUIPMENT AND SUPPLIES

1. Supplies such as biohazard autoclave bags should be purchased by each department;
2. Occasionally and or in a case of emergency, you can get supplies from EHS by calling 978-934-2618;
3. Supplies such as biohazard signs or those for monitoring the autoclaves as chemical monitoring–strips and biological test indicators will be supplied by EHS Autoclave Program;
4. If the autoclave has been repaired and or any service has been done to it, a biological test needs to be performed. Contact the EHS at biosafety@uml.edu.

RECORDKEEPING

1. Record all data from any run in the Daily Autoclave log as date; time-in of treatment; the type of load (clean material or waste); quantity of waste treated; printed name and signature of the person responsible for treatment; and any relevant information when applicable;
2. The person in charge of the autoclave will be responsible to maintain all records and logs;
3. Biological testing results (growth/no growth) is coordinated by the BSO and all records from "Biological Test Logs" should be kept in the EHS office;
4. All records collected in the "Autoclave user log" and "Biological Test log" must be maintained for 3 years.
5. The autoclave operator (or person in charge) should notify their supervisor and record any incident or problems when working or monitoring the autoclave.

MONITORING THE AUTOCLAVE - GUIDELINES RECOMMENDED BY THE CDC

The CDC recommends monitoring the autoclave mechanical and chemically every time that a run is performed. A monitoring biological test should be performed monthly.

At UMass Lowell, EHS will monitor the autoclave using the *Geobacillus stearothermophilus* once a month. Autoclaves that run more than 10 runs per week (40 loads per month) are recommended to perform a biological test more often. The Biosafety Officer (BSO) will make recommendations for each particular case.

Daily Monitoring Mechanical Procedures

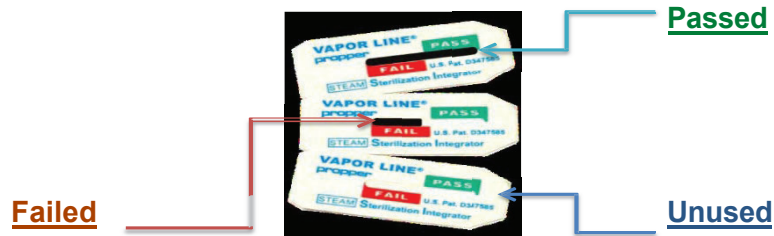
All users must follow the CDC recommendations for mechanical monitoring of steam sterilization. These include:

- The daily assessment of cycle time and temperature by examining the temperature record chart (or computer printout);
- An assessment of pressure checking the pressure gauge.

Chemical Testing Guidelines

- The Center for Disease Control (CDC) requires that a chemical indicator be placed on the inside of each waste package to verify steam penetration;
- Every run/load will carry a **Class 5 Vapor Line Steam Integrator**; in order to corroborate the correct time, steam, and temperature of each autoclave run;
- The Class 5 Vapor Line Steam Integrator strips clearly indicate Pass/Fail for the run by changing color. The sterilization cycle has achieved spore death when the indicator reaches the "Pass" area;
 - If the indicator only reaches the "Fail" zone, the batch needs to be autoclaved again using a fresh indicator;

IMPORTANT If Fail results continue, report the condition to the Autoclave Room Manager in charge;

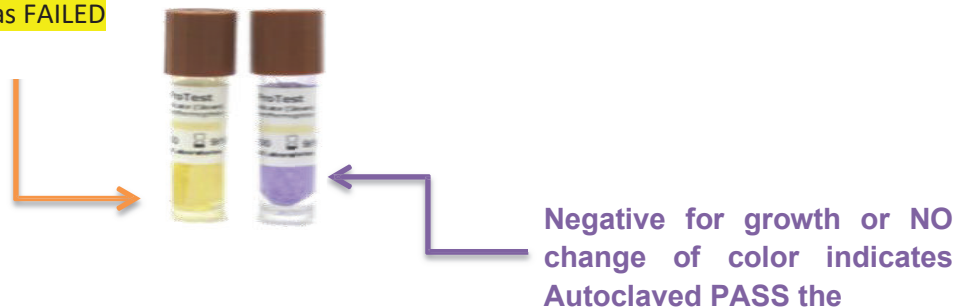


- All run results should be noted in the Daily Autoclave Use log;
- The Class 5 Vapor-line Steam Integrators will be provided by EHS Department and can be requested by calling the EHS extension 978-934-2618

Biological Testing Guidelines

- The CDC recommends that all autoclaves should be biologically monitored at least weekly with the appropriate commercial preparation of spores;
- At UMass Lowell sterilization is done **only** by steam in autoclaves monitored by the EHS;
- The biological testing is done once a month by the Biosafety Officer (biosafety@uml.edu);
- On an established monthly schedule, the Biosafety Officer will perform biological monitoring using a Bio-Test indicator;
- The Bio-Test indicator contains spores from the *Geobacillus thermophiles*, and will be used in each autoclave at standard conditions for the use of each autoclave.
- After the regular run, the Bio-Test indicators will be incubated for 24-48 hours at 65°C together with a control Bio-Test indicator that has being maintained at room temperature.

Positive for growth, color change indicates that Autoclave has FAILED



- Results of the biological indicator tests (indicating date and Pass or Fail) must be documented on the “**Biological Test Log**”

STEAM STERILIZATION PROCEDURES FOR CLEAN MATERIAL AND BIOHAZARDOUS WASTE

The following procedures apply for steam sterilization of clean material and for sterilization of biohazardous waste.

IMPORTANT: To Prevent Burns or Spills

Observe the following safety procedures during loading and unloading the autoclave:

1. Loosen screw caps on bottles and tubes of liquids before autoclaving;
2. Be sure to wear a face shield when you open the autoclave. Steam can burn your face;
3. Check that chamber pressure has returned to zero before opening door;
4. Stand behind door when opening it;
5. Slowly open autoclave door only a crack;
6. Beware rush of steam. Make sure that the door to the autoclave room is closed in order to prevent steam from escaping into corridor;
7. Wait 5 minutes after opening door before removing liquids. Keep face away from door as it opens. Escaping steam may burn face;
8. Do not put solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials in an autoclave;
9. Load the bags into the autoclave and operate the autoclave according to the manufacturer's operating instructions.

Packing and Loading the Autoclave

1. DO NOT sterilize clean material together with biohazardous waste in the same load and run;
2. All infectious waste must be placed in biohazard autoclave bags, obtained from the EHS (extension 4-2618), and be loosely sealed with autoclave tape to allow steam to penetrate;
3. Sealed autoclave bags must be brought directly to the autoclave room in a secondary container (cart with sides, tote, etc.);
4. Ensure sufficient water in load to allow steam penetration or add 250 mL water to bags containing solids to ensure steam penetration;
5. In order to corroborate the correct time, steam, and temperature of each run, every load will carry a **Class 5 Vapor Line Steam Integrator**. The Class 5 Vapor-line Steam Integrators will be provided by EHS Department and can be requested by contacting EHS at biosafety@uml.edu or Ext. 4-2618.
6. Biohazard waste bags to be autoclaved later should be located in specific area of the autoclave room and clearly marked with biohazard symbol;
7. Biohazard waste bags CANNOT be held in the autoclave room for more than 24 hours without being autoclaved;
8. The bags to be autoclaved must be placed in secondary containers or decontamination pans;
9. There should only be one bag per pan or enough space between bags to allow adequate steam circulation;

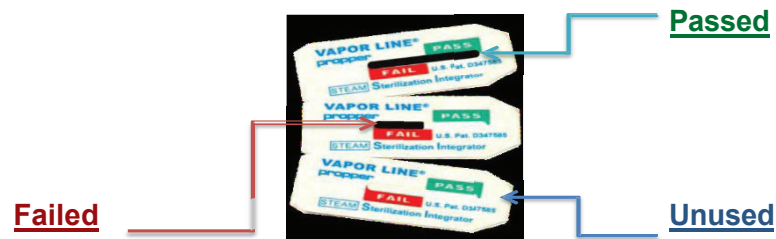
10. Do not overfill containers (prevent spill and boil over);
11. Do not allow bags to touch sides of autoclave;
12. Complete "Daily Autoclave Log".

Loading the Autoclave

2. Ensure the autoclave is operating properly before commencing;
3. Determine the appropriate exposure time, temperature and pressure for the load to autoclave based on spore testing;
4. Ensure the autoclave attains the desired temperature (normally 121⁰C) and pressure (minimum 15 psi) for the desired time (minimum 30 min.);
5. Record the information in "Autoclave Use Log";

Unloading the Autoclave

1. Wait until the chamber pressure gauge reads zero before opening;
2. Open slightly to allow steam to escape (protect yourself from the steam);
3. Wait 20-30 minutes, more if necessary, for the contents of the autoclave to cool;
4. Carefully remove the secondary container with the waste bag to reduce the risk of spillage;
5. Wait until the autoclaved plastic bag has cooled completely;
6. Verify temperature and duration of exposure has been met;
7. Verify that each chemical monitor-strip has changed color. Proper sterilizing conditions turn the indicator on the monitor strip black;

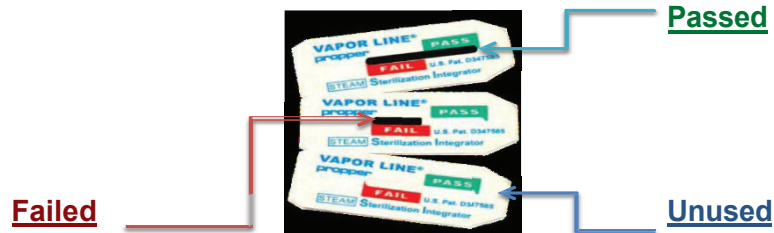


8. If any of the chemical monitor-strips did not turn black, then the entire load must be re-autoclaved.

Disposal of Autoclaved Biological Waste

Autoclaved bags of biological waste are noninfectious and can be disposed of in the same manner as non-infectious waste in the regular trash. Regular trash is not regulated by 105 CMR 480.000.

1. After verification that each chemical monitor-strip changed color by turning black, the operator should begin to unload the bags;



2. Transfer the cold autoclaved bag to a regular **black plastic trash bag**;
3. Close bag tightly and dispose of it in the regular trash;
4. **DO NOT** dispose the autoclaved red bag directly into the regular trash.

RELATED DOCUMENTS

The following SOPs are related to this document:

1. **SOP Bio-002** Sharp Usage and Disposal;
2. **SOP Bio-003** Disposal of Solid Biohazardous Waste;
3. **SOP Bio-004** Decontamination and Disposal of Liquid Biohazard Waste;
4. Exposure Control Plan (UMass Lowell Bloodborne Pathogens Program).

REFERENCES

1. 105 CMR, Department of Public Health 480.000
<http://www.mass.gov/eohhs/docs/dph/regs/105cmr480.pdf>
2. CDC Biological and Infectious Waste
3. <https://www.cdc.gov/infection-control/hcp/environmental-control/regulated-medical-waste.html>
4. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, Dec 2009
5. <https://www.cdc.gov/labs/bmbl/index.html>
6. Environmental Health and Safety web site
<http://www.uml.edu/eem/ehs>

For additional information on autoclaving, Biological testing, biological waste disposal and/or any biosafety issues; contact EHS at biosafety@uml.edu or 978-934-2618.