

# Eastern Kentucky University



## Biohazardous Waste Handling Guide

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## Introduction

This document has been prepared to provide guidance to Eastern Kentucky University employees and students in the use and disposal of biohazardous materials in compliance with regulatory requirements. For this document “biohazardous waste” is defined as any discarded material which might include infectious laboratory materials or agents regulated by federal, state, and local authorities. At a minimum, the following categories should be considered as biohazardous waste materials:

1. Cultures and stocks of infectious biological agents, including laboratory waste, discarded live or attenuated viruses or related agents, culture dishes and other laboratory supplies used in the production or use of these agents, and any other related devices.

2. Human blood is a biohazardous waste. Human blood should be treated in accordance with the “[Eastern Kentucky University Bloodborne Pathogens Exposure Control Plan](#)”. This plan can be found at the Risk Management Insurance/ Environmental Health and Safety website <https://ehsrmi.eku.edu/occupational-safety>.

3. Sharps: These are defined as needles, syringes, scalpels, etc., as well as any object sharp enough to puncture the skin (i.e. microscope slides, cover slips) that is used in the laboratories that could possibly come in contact with material that may be considered biohazardous waste.

4. Contaminated waste, blood and bodily fluids from animals that have been exposed to biological agents that are infectious to humans which used in research and/or teaching labs, as well as bedding from the cages of these animals. ECU has a special committee for research or work with animals exposed to hazardous agents, known as the Institutional Animal Care and Use Committee or IACUC. For more information, go to the Institutional Animal Care and Use Committee webpage: <http://sponsoredprograms.eku.edu/institutional-animal-care-and-use-committee>.

Anyone in the university that intends to do animal research which involves a hazardous agent is required to submit an Animal Care and Use Application through the office of Sponsored Programs (the application can be found online through the link above or on the Sponsored Programs home page: [www.sponsoredprograms.eku.edu](http://www.sponsoredprograms.eku.edu)). As part of the Animal Care and Use application, the faculty member is required to fill out (and submit with the application) Form H, “Use of Hazardous Agents” (Appendix 1). All the procedures and forms are found on the IACUC webpage (see above). The Animal Care and Use Application (with all attachments) is reviewed by ECU’s IACUC (Institutional Animal Care and Use Committee) and must be approved by the IACUC before any research activities can be conducted. For more information or assistance with this application, contact sponsored programs.

Note: Bio hazardous waste does NOT include animal carcasses that have not been exposed to chemical or biological reagents. This is to be treated by double bagging and then placing the specimen in the normal trash disposal system

## Definitions

**Employee:** An individual employed in a laboratory workplace who may be exposed to hazardous chemicals in the course of his or her assignments.

### **Hazardous**

**Chemical:** The Occupational Safety and Health Administration (OSHA) defines a hazardous chemical as any chemical that is either a:

- i) Physical Hazard: For a physical hazard, a chemical has scientifically valid evidence that it is a combustible liquid, a compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable (reactive), or water-reactive.

Or

- ii) Health Hazard: For a health hazard, a chemical which is classified as posing one of the following hazardous effects: acute toxicity (any route of exposure); skin corrosion or irritation; serious eye damage or eye irritation; respiratory or skin sensitization; germ cell mutagenicity; carcinogenicity; reproductive toxicity; specific target organ toxicity (single or repeated exposure); or aspiration hazard.

**Laboratory:** A facility where the “laboratory use of hazardous chemicals” or biological material occurs. It is a workplace where relatively small quantities of hazardous chemicals are used on a non-production basis.

**Laboratory Scale:** Working with substances in which the containers used for reactions, transfers, and other handlings of substances are designed to be easily and safely manipulated by one person. “Laboratory scale” excludes those workplaces whose function is to produce commercial quantities of materials.

### **Autoclave Point of Contact**

**Person:** The person that has been designated as the individual to

contact for the safe maintenance and handling of the autoclave operations. This person should be notified of all problems and should coordinate the repair of the autoclave(s) for which they are responsible.

**Designated Person  
in Charge:**

Includes individuals with the titles of Laboratory Supervisor, Principle Investigator, Lab Coordinator, etc. Faculty, staff, teaching assistants, and/or research assistants that have been placed in charge of supervising specified laboratories as well as the training and safety of laboratory workers. **A Designated Person in Charge must be identified and assigned to each individual laboratory.**

**Laboratory**

**Workers:**

Any person, employee or student, working in a laboratory or involved in laboratory activities.

**Biohazardous**

**Waste:**

Discarded laboratory material which may be considered infectious to humans or materials and agents regulated by federal, state, and local authorities as biohazardous or infectious; includes lab supplies contaminated with potential (but not defined) biohazardous waste.

## **Roles and Responsibilities**

The proper management, treatment and disposal of biohazardous waste can only be accomplished through the cooperation of all Eastern Kentucky University personnel involved in the handling of the biohazardous material at every level.

The primary division of these levels are as follows:

Public Safety/EHS, Dean of the College, Chair of Department, CSO of College, Point of Contact Person for Autoclave Operations, Designated Person in Charge of Laboratory, Supervised Employee/Student, and Housekeeping.

### **Public Safety/EHS shall:**

1. Provide technical assistance as needed to be compliant with regulatory agencies and achieve safe protocols as established.
2. Remain current on rules and regulations concerning biohazardous materials on EKU campuses.
3. Maintain and update a current copy of the Eastern Kentucky University Biohazardous Waste Handling document.
4. Perform audits to verify safe handling of biohazardous waste and biohazardous material procedures, including verification of document training.

### **Dean of the College shall:**

1. Identify those departments within their college to which this biohazardous safety standard applies.
2. Work with departmental chairs and university administration to secure resources for compliant operations as well as safety improvements.
3. Have the authority to halt operations if any laboratory within the college is operating in a non-compliant or safe manner (typically this is done after consultation with chairs and responsible person in charge of the laboratory).
4. Ensure that the college has procedures in place to properly handle storage and handling of biohazardous materials and biohazardous waste.

### **Chair of the Department shall:**

1. Support the biohazardous material/waste safety protocols as they apply to their department/faculty.
2. Work with the faculty and staff to adapt lab specific procedures to comply with biohazard material/waste protocols.

3. Ensure that each laboratory where biohazard material is used has a designated person in charge (i.e. supervisor, principle investigator, lab coordinator, etc.) to oversee compliance to the protocol and safety practices. In the absence of a designated person in charge of a laboratory, the chair is by default the designated person in charge.
4. Make budget requests and/or secure resources to comply with biohazardous material/waste protocols and safety procedures.
5. Ensure that documented training is kept within the department for a time frame in accordance with university record retention policy.

**Primary point of contact person for autoclave(s) shall:**

1. Be the person to which all problems and concerns about a designated autoclave or autoclaves is reported.
2. Coordinate via the chair of the department the repair and maintenance of designated autoclave(s).
3. Assist in the training or guidance of autoclave users.

**Designated Person in Charge of Laboratory (lab supervisor, principle investigator, lab coordinator, etc.) shall:**

1. Comply with this document and follow accepted safety practices.
2. Ensure that all laboratory workers receive appropriate and documented training with respect to handling biohazardous material/waste.
3. Ensure that all assigned laboratory workers comply with the biohazardous waste handling document and accepted safety practices.
4. Identify biohazardous material handling techniques or problems unique to their laboratories, and develop and maintain written procedures to address these issues.
5. Control access to their laboratories.
6. Ensure that all equipment and supplies are present and functioning properly as well as oversee that laboratory workers and/or users receive proper training of such equipment and supplies.
7. Request funding via upper administrative channels for supplies or other items required to comply with this document or safety procedures.
8. Report in writing all laboratory accidents or incidents (especially where injuries occurred) to their departmental chair as well as the Public Safety/ EH&S Compliance Office.
9. Report in writing repairs or maintenance of equipment needed for biohazardous material operations to their departmental chair. For autoclaves, report all problems or repairs to the designated point of contact person for that autoclave.

**Laboratory worker shall:**

1. Comply with all the requirements of this documents and follow provided procedures and /or training by designated person in charge of the laboratory in which they work.
2. Report any and all hazardous conditions to the designated person in charge of the laboratory.
3. Wear or use all Personal Protective Equipment (PPE).
4. Report any suspected job-related injuries or illnesses to the designated person in charge of the laboratory and seek immediate treatment.
5. Refrain from the operation of any equipment or instrumentation without proper instruction and authorization.
6. Request additional information and/or training when unsure of procedures used to handle biohazardous materials.

## BIOHAZARDOUS WASTE MATERIALS HANDLING AT EKU

### Definition of biohazardous waste:

- Human blood, blood components, fluids, tissues and cell lines
- Animal cell lines, animal blood, fluids, tissues
- Animal carcasses (infected and uninfected)
- Lab ware and personal protective equipment (PPE) that are contaminated with any of these biohazards
- Sharps items (needles, scalpels, lancets, or materials capable of puncturing the skin)
- Biotoxins requiring BSL-1 or BSL-2 containment

### Biosafety level 1 (from Wikipedia):

This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.

It includes several kinds of bacteria and viruses including non-pathogenic *Escherichia coli*, as well as some cell cultures and non-infectious bacteria. At this level, precautions against the biohazardous materials in question are minimal and most likely involve gloves and some sort of facial protection. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Usually, contaminated materials are left in open (but separately indicated) waste receptacles. Decontamination procedures for this level are similar in most respects to modern precautions against everyday microorganisms (i.e., washing one's hands with anti-bacterial soap, washing all exposed surfaces of the lab with disinfectants, etc.). In a lab environment all materials used for cell and/or bacteria cultures are decontaminated using an autoclave sterilizer. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

### Biosafety level 2 (from Wikipedia):

This level is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It includes bacteria and viruses that cause only mild disease to humans, or are difficult to contract via aerosols in a lab setting, such as *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus*, and other pathogens. BSL-2 differs from BSL-1 in that:

1. Laboratory personnel have specific training in handling pathogenic agents and are directed by scientists with advanced training;
2. Access to the laboratory is limited when work is being conducted;
3. Extreme precautions are taken with contaminated sharp items; and
4. Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Biohazardous material at EKU must be disposed of in biohazardous waste bags, as discussed in the following sections. Biohazardous waste bags must be placed in approved labelled biohazardous waste containers, as shown in the image below. Biohazardous waste bags are never to be placed (even temporarily) on the floor, lab benches, or other surfaces. The containers are usually bright red in color with the biohazard symbol and the word “Biohazard” clearly displayed on them. The red color is the industry standard. It is best to keep this consistent to make such hazardous materials easily identified. If your container is a different color than red, please make sure the biohazardous markings are large enough that the container is easily identifiable; the symbol should also appear on several sides. Biohazardous materials containers must have a closable lid. This lid is to be shut whenever the container is not in use.

Below are examples of approved biohazard containers, as well as examples of containers that are not safe to use for biohazardous materials. The main difference is that approved biohazard containers have lids and are capable of containing the biohazardous material if the integrity of the bag is compromised due to a tear or puncture.



When removing the full biohazardous waste bags from the approved containers proper PPE should be worn. The bags should then be taken directly to the autoclave location or autoclave holding areas and placed in proper secondary containers for processing. If there is no on-site autoclave available for use, the full biohazardous containers should be taken to the designated holding area previously identified as a location for a licensed biohazardous waste hauler to pick up. Biohazardous waste bags should NEVER be left on the laboratory floor, bench top, or anywhere that is not a designated biohazardous waste container. The containers that are used to

hold biohazardous waste bags prior to autoclaving should be disinfected using a certified disinfecting agent. Disinfectant should be given sufficient contact time before being wiped down using appropriate personal protective equipment (PPE) and gloves used during disinfection should be discarded into the biohazard waste receptacles. Supervisors for each laboratory or teaching area should establish best practice for such disinfection for the containers located in their areas.

There are two approved methods for disposing of biohazardous waste at ECU; these methods are outlined in the following sections. These choices are either to autoclave in-house or to have an outside contractor pick up the biohazardous waste. The majority of biohazardous waste generated in the College of Science is disposed of by autoclaving. Some biohazardous waste is autoclaved in the College of Health Sciences. Most of other areas and Colleges that do not have access to autoclaves must collect the biohazardous waste material and coordinate with Public Safety/ EHS compliance office to arrange either routine or special pickups.

### **Biohazardous waste that is autoclaved at ECU:**

After proper autoclaving of wastes, the material is safe enough to be disposed using housekeeping or custodial services. Currently, housekeeping and/or custodial duties are performed via an outside contracted company (Aramark). Therefore all procedures must be coordinated with that company.

Autoclaving is universally accepted as a dependable method to kill or inactivate all forms of microbial life. If done correctly at appropriate temperatures, pressures, and exposure time, this method is sufficient to treat biohazardous waste before the materials are then placed into the facility trash streams. Saturated steam must be established to ensure correct temperature (at least 121°C) using pressure (at least 15 pounds per square inch) for a minimum of 15 minutes. Basic cycles for autoclaving are shown in the table below.

| Basic Cycles                  | Description                                                                                                                                                                                                                            | Typical Application or Load Type                                              |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Gravity                       | The most basic sterilization cycle. Steam displaces air in the chamber by gravity (i.e. without mechanical assistance) through a drain port.                                                                                           | Glassware, unwrapped goods, waste, utensils, red bags.                        |
| Pre-Vacuum and/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. | Wrapped goods, packs, animal cage bedding, cages, porous materials, red bags. |
| Liquids                       | A gravity cycle with a slower exhaust rate to minimize boil-over.                                                                                                                                                                      | Media, LB broth, water, etc.                                                  |

Flash  
(Healthcare  
sterilizers only)

High temperature cycle (over 270F) for a shorter period  
of time.

Unwrapped goods

The hazards of autoclaving include extreme heat, pressure, and scalding steam. In addition, if the autoclave is used incorrectly, all materials may not be sterilized, leaving the material hazardous if handled by custodians or housekeeping. Therefore, only properly trained personnel are allowed to operate the autoclaves.

### **Autoclave Point of Contact Persons:**

In the Science Building East Wing at ECU, which is supervised by the Department of Biological Sciences, the faculty member in charge of overseeing the autoclave operations is Dr. Marcia Pierce ([marcia.pierce@ecu.edu](mailto:marcia.pierce@ecu.edu)). Dr. Pierce must approve all users of the autoclaves in room 5215. All faculty and undergraduate or graduate students wishing to use the research autoclaves in 5215 should contact Dr. Pierce. The autoclaves in 5203 are reserved for class use, unless both autoclaves in 5215 are simultaneously out of order.

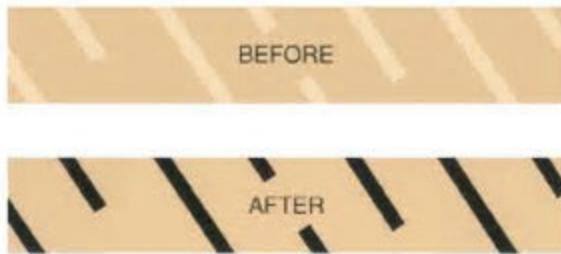
In the Science Building East Wing at ECU, the faculty member in charge of overseeing autoclave operations in the vivarium is Dr. Robert Pace ([robert.pace@ecu.edu](mailto:robert.pace@ecu.edu)). Any questions regarding use/operation, maintenance/repairs, and protocols should be directed to Dr. Robert Pace .

For the autoclave in the Science Building North Wing at ECU (room 5108), which is supervised by the Department of Chemistry. Any questions regarding the use and operation of this autoclave, as well as the maintenance, repairs, or protocols for use, please contact Tracy Gastineau-Stevens ([tracy.gastineau-stevens@ecu.edu](mailto:tracy.gastineau-stevens@ecu.edu)) or Laura Rowe ([laura.rowe@ecu.edu](mailto:laura.rowe@ecu.edu)).

For the autoclave in the Dizney building (room 218), the faculty member that oversees operations is Dr. Travis Altheide ([travis.altheide@ecu.edu](mailto:travis.altheide@ecu.edu)). Any questions regarding the use and operation of this autoclave, as well as the maintenance, repairs, or protocols for use, please contact Dr. Altheide.

### **Biohazardous waste to be autoclaved must be collected in red biohazardous waste bags either with:**

1. A large "X" made with autoclave tape that discolours after correct exposure to the autoclave conditions. This should be taped so as to mark out the biohazardous symbol, indicating that the material has been autoclaved sufficiently.



2. Alternatively, red biohazard bags with autoclave indicator strips incorporated into the bag by the manufacturer may be used.

The primary goal is that any biohazardous waste must have an easily recognizable indicator that proper autoclaving was achieved and the material is safe for disposal through the regular trash stream. Once properly autoclaved, the bags should be disposed of by placing them in large autoclave waste containers lined with black plastic bags that can then be picked up by the custodians or housekeepers. See below for examples of disposal containers for autoclaved materials.



Housekeeping can then remove the black bags and dispose of them by adding them to the regular waste stream.

#### **Biohazardous waste removal by outside biohazardous waste handling services:**

An alternative to autoclaving in-house is disposal by a biohazardous waste hauler at regular intervals. These companies are contracted by ECU. The removal of wastes using this method

usually only occurs every 4- 6 months, although more frequent pickups can be scheduled. This is arranged through EKU's Public Safety/EHS compliance office. ***In the event autoclave is not operational, best practice during these events should always be to minimize waste. Laboratory configurations may need altering to meet best practice.***

This method is for biohazardous waste that is not autoclaved here at EKU. It **should not** be used for labs or classes that generate large quantities of biohazardous waste if autoclaving is a reasonable alternative. The pickup of this type of waste is coordinated through the Public Safety/EHS compliance office. Identify the building and room where you like to have a contractor biohazard container delivered. A red biohazardous waste bag liner will be provided with the container. The frequency of when the containers are removed by the disposal company will be determined by the biohazardous waste producer, and a routine pickup will be scheduled. A contact person and phone number within the department requesting this type of biohazardous waste removal must be given to handle operations when the pickup occurs. If additional containers are needed, the producer should contact the Public Safety/EHS compliance office.

#### **Use of disposable biohazardous waste containers at EKU:**

Some laboratories prefer to use disposable biohazardous waste containers. This is a good method for collecting gloves, disposable pipets, etc., that have trace amounts of biohazardous materials. Typically, these containers fill very slowly, as the items placed in these containers are usually sharps (needles, scalpels, etc.), PPE, or lab supplies that have very minor amounts of biohazardous contamination. Choosing a suitable disposable biohazardous container is essential when considering use.



Users of these types of containers **must buy** their own disposable containers. These containers usually work well for areas that have items that do not autoclave easily, such as broken glass, needles, etc. Once the user fills these disposable containers, they should contact the Public Safety/ EHS Compliance office, after which the waste container should be removed within a

timely manner. New containers are the responsibility of the end user. They are **not supplied** by the Public Safety/EHS compliance office. The contents of the biohazardous waste in your individual bags must be labeled. Containers should be documented within the lab with their contents, as well as in the email requesting pickup.

### **Animal Carcasses**

Animal or other biological carcasses should **never be autoclaved**. Such an action would create a very unpleasant odor and could cause evacuation of work areas due to health and safety reasons.

If there are animal or other biological specimens that need to be disposed of, there are two ways to proceed. First, if the carcass has not been exposed to chemicals, then it can be handled in the same manner as roadkill. Simply double bag it and place it in the regular trash stream. It is suggested that this type of trash be taken directly to outside dumpsters or other outside receptacles, to prevent obnoxious smells from permeating the building.

In the case where a biological specimen carcass has been exposed to or preserved in chemicals, it is required that such specimens be taken by a **licensed hauler**. If there are large amounts of chemical preserving the carcass, the chemical must be decanted into a separate container. The carcass must be placed in a sealed **plastic** container. The chemical can be decanted into any suitably sealed container. Both containers should be labelled appropriately to convey the chemical present. For example if you decanted ethanol from a preserved fish specimen, the fish should then be bagged and labeled as “fish specimen with trace ethanol” before placing it in a plastic sealable container. The decanted ethanol is then labeled “ethanol from preserved specimens”.

If the animal tissues and animal bedding must be disposed of as pathological waste due to the source animal being infected with a BSL-2 agent or higher. All animal wastes that are collected as biohazardous **must be sent for incineration** via a licensed vendor who retrieves the waste from each building at pre-determined intervals.”

If there are more questions about animal carcass disposal, please contact the Public Safety/EHS compliance office.

This is a working document to outline the basics for handling biohazardous waste at ECU. It is meant to provide guidance and good protocols to all personnel at ECU. As with all safety documents, it depends on the individual to practice and achieve safe and appropriate laboratory methods.

## APPENDIX 1

### USE OF AUTOCLAVE Dizney 218

**I. INDIVIDUALS USING THE AUTOCLAVE MUST READ THESE DIRECTIONS AND SIGN AT THE BOTTOM THAT THEY COMPLY WITH THE AUTOCLAVE RULES! A COPY WILL BE MAINTAINED IN EACH AUTOCLAVE ROOM AT ALL TIMES!**

#### **II. TRANSPORTING PACKAGED MATERIAL TO THE AUTOCLAVE**

- When transporting material to be autoclaved, use a cart with guard rails.
- Ensure the use of a secondary, leak-proof closed container to collect any spillage should any accident happened to the cart during transport.
- Surface decontaminate the container prior to and after transport, unless there is no risk of contamination.

#### **III. AUTOCLAVE USER LOG**

- **MUST BE FILLED OUT COMPLETELY BY EACH USER.**
- This log identifies the users, nature of the load (media, waste, etc.), cycles used and exposure times.

#### **IV. FUNDAMENTALS OF LOADING TO ENSURE SUCCESS**

- Prior to autoclaving, twist tie bags with temperature sensitive autoclave tape and place two strips of tape to form an “X” across the biohazard symbol of the biohazard bags, if autoclaving waste.
- **SPECIAL NOTE ON SHARPS:**
  - When autoclaving Sharps containers, place tape on the top where it is easily visible. Sharps containers can be autoclave directly without a bag.
- Ensure containers do not touch each other as this will ensure all surfaces are sterilized.
- No items should touch the top or sides of the autoclave container as the container is pushed inside.
- A load of liquid filled containers should be of similar size, shape, content and volume, as exposure time is based on these characteristics.
- Run material to be sterilized separate from those to be decontaminated.

#### **V. OPERATING INSTRUCTIONS**

- Open silver-colored valve (water supply) at the lower left front of the autoclave.
- Close the chamber drain valve located directly beneath the door to the autoclave. Note: do not open drain valve during operation of the autoclave.
- Fill bottom of chamber with **deionized water** to just below ledge at the bottom of the door opening – about 6 quarts of water.
- Close and latch autoclave door.
- Set exhaust selector switch: Slow for liquids and decontaminating biohazard waste, fast for all other load types.
- Set timer for desired length of sterilization, never less than 15 minutes. For biohazard waste, the cycle should be at least 30 minutes.

- Heaters will switch on first
- White pilot light will illuminate when set temperature/pressure has been reached and timer is running
- Autoclave will exhaust automatically at end of sterilizing period

## V. UNLOADING AN AUTOCLAVE

- ***THE GREATEST RISK OF PERSONAL INJURY OCCURS DURING THE PROCESS OF UNLOADING THE AUTOCLAVE!***
- Wear all necessary personal protective equipment, including lab coat, glasses, and heat-resistant gloves to remove hot containers.
- The chamber pressure gauge of the autoclave should be zero before opening the autoclave door.
- To minimize the risk of accidents caused by steam escape, the person who opens the autoclave door should lean away from the door as it opens.
- Slowly open autoclave door. Opening the autoclave door too quickly may result in glassware breakage and/or steam burns to the skin.
- Remove items from the autoclave using the heat-resistant gloves and personal protective equipment.
- If immediately removing items from the autoclave room, load onto the lab cart using heat resistant gloves.
- Verify that the temperature sensitive tape has changed, as black, diagonal lines should have appeared on the autoclaved objects. If no change appears on the tape, the load is required to be re-autoclaved after placing new tape on material.

## VI. DISPOSING OF AUTOCLAVED BIOHAZARDOUS WASTE

- Once the waste has been successfully autoclaved, the waste is no longer considered biohazardous.
- Once cooled to room temperature, the autoclaved waste bags should be placed inside the waste container next to the autoclave. This container should be lined with black plastic trash bags designed for domestic applications.
- It is highly recommended to place only two biohazardous bags inside a single black trash bag to prevent overloading the bags, leading to the risk of breakage. Ensure the black bag is securely tied.
- Waste may now be disposed of by custodial services.

---

Signature of individual

---

Date

---

Signature of professor (if student above)

---

Date

## APPENDIX 2

### Departmental- or College-Specific Procedures or Protocols

## USE OF RESEARCH/TEACHING AUTOCLAVES IN NEW SCIENCE BUILDING

**I. INDIVIDUALS USING THE AUTOCLAVES MUST READ THESE DIRECTIONS AND SIGN AT THE BOTTOM, INDICATING THAT THEY WILL COMPLY WITH THE AUTOCLAVE RULES! A COPY WILL BE MAINTAINED IN EACH AUTOCLAVE ROOM AT ALL TIMES!**

### II. TRANSPORTING PACKAGED MATERIAL TO THE AUTOCLAVE

- When transporting material to be autoclaved, use a cart with guard rails.
- Ensure the use of a secondary, leak-proof closed container to collect any spillage should any accident happened to the cart during transport.
- Use the most direct but not heavily populated route possible to transport the cart.
- Surface decontaminate the container prior to and after transport, unless there is no risk of contamination. Transporting autoclaved waste to outside waste bin should be done in a similar manner.

### III. AUTOCLAVE USER LOG

- **MUST BE FILLED OUT COMPLETELY BY EACH USER.**
- This log identifies the users, nature of the load (media, waste, pipet tips, etc.), cycles used and exposure times as well as any issues that occurred during autoclave cycles.

### IV. FUNDAMENTALS OF LOADING TO ENSURE SUCCESS

- On biohazardous waste-containing autoclave bags, place two pieces of autoclave tape to form an “X” across the biohazard symbol. In addition, after closing the bag with a twist tie, wrap a piece of autoclave tape around the twist tie so that the autoclave tape is visible for post-autoclaving confirmation that the material has been successfully sterilized.
- **SPECIAL NOTE ON SHARPS:**
  - When autoclaving Sharps containers, tape the opening shut with sufficient autoclave tape to ensure it is sealed.
  - Using a black sharpie please write the contact number, date, and lab room number across the tape.
- **USE THE FOOT PEDAL TO OPEN AND CLOSE THE AUTOCLAVE DOOR ON THE AUTOCLAVES THAT HAVE A FOOT PEDAL OPTION!**
- Load the items in the autoclave in the best arrangement to result in the least resistant passage of air exchange through the load, from the top of the chamber to the bottom.

- When autoclaving flat items, place packages on their edges to enhance steam penetration, place a rack or other item against these items to prevent them from slipping.
- Make sure containers do not touch each other so that all surfaces are sterilized.
- No items should touch the top or sides of the autoclave container as the container is pushed inside.
- A load of liquid filled containers should be of similar size, shape, content and volume, as exposure time is based on these characteristics.

## V. UNLOADING AN AUTOCLAVE

- ***THE GREATEST RISK OF PERSONAL INJURY OCCURS DURING THE PROCESS OF UNLOADING THE AUTOCLAVE!***
- Wear all necessary personal protective equipment, including lab coat, glasses, and heat-resistant gloves to remove hot containers.
- The chamber pressure gauge of the autoclave should be zero before opening the autoclave door.
- USE THE FOOT PEDAL to crack door slightly and stand back to allow steam to escape.
- To minimize the risk of accidents caused by steam escape, the person who opens the autoclave door should lean away from the door as it opens.
- Slowly open autoclave door. Opening the autoclave door too quickly may result in glassware breakage and/or steam burns to the skin.
- Remove items from the autoclave using the heat-resistant gloves and personal protective equipment.
- If immediately removing items from the autoclave room, load onto the lab cart. Bring the lab cart to the chamber, using heat resistant gloves; otherwise, set items on the counter to cool before removal.
- For autoclaved biohazardous waste, allow to cool to room temperature before labeling and disposing in the waste containers provided inside the autoclave room.
- After every use, it is advised to close the autoclave door but do not seal the door as this will shorten the life span of the rubber gaskets on the door. USE THE FOOT PEDAL to operate door at all times on autoclaves that have one.
- Verify that the temperature-sensitive tape has changed, as black, diagonal lines should have appeared. If no change appears on the tape, the load is required to be re-autoclaved after placing new tape on the material.
- Also check the autoclave log recorder to verify the temperature attained and the time spent at the required temperature. If minimum time and temperature is not attained on the second cycle, users should contact the person in charge of the autoclave for your department. For Biological Sciences, the autoclaves in SB 5215 and 5203 are maintained by Dr. Pierce and the autoclave in SB 1119 is maintained by Dr. Kraemer. For Chemistry, the autoclave in SB 5108 is maintained by Dr. Brock and Dr. Paumi. This way there will be a designated contact person or persons in each department college that coordinates repairs to minimize confusion. Dr. Pierce or Dr. Paumi are in charge of contacting Getinge customer service (800-660-1687) to request that a technician service the autoclave.

## VI. DISPOSING OF AUTOCLAVED BIOHAZARDOUS WASTE

- Once the waste has been successfully autoclaved, the waste is no longer considered biohazardous. It is now important to alter the hazard awareness signage and symbols to reflect this.
- When waste bags have cooled to room temperature, use a large felt marker to further deface the biohazard symbols.
- Inspect the autoclave tape to insure it still securely is attached to the biohazardous waste container and appropriately marks out the biohazard symbol with an obvious “X”. Also verify that the autoclave tape has changed color to confirm proper autoclave conditions were achieved to neutralize the biohazardous nature of the material. If this is not the case then the bag will have to be autoclaved again.
- Once this has been accomplished, autoclaved waste bags should be placed inside the trash cans provided for this purpose. These should be lined with black plastic trash bags designed for domestic applications.
- It is **highly recommended to place only one or two biohazardous bags inside a single plastic trash bag (domestic applications) to prevent overloading the bags**, leading to the risk of breakage. Ensure the black bag is securely tied when full.
- Waste may now be disposed of to the appropriate municipal waste stream.

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Signature of individual

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Date

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Signature of professor (if grad student above)

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Date

## APPENDIX 3

IACUC Protocol Number: \_\_\_\_\_

### **IACUC Form H: Use of Hazardous Agents**

**Principal Investigator:**

**Project Title:**

1. Check which of the following the project will involve.

☐ Radioactive materials

☐ Human, plant, animal pathogens

☐ Recombinant DNA/RNA

☐ Chemical carcinogens

☐ Acute toxins

☐ Other: \_\_\_\_\_

2. Identify agents.

| Species | Agent | Dose | Route | Frequency |
|---------|-------|------|-------|-----------|
|         |       |      |       |           |
|         |       |      |       |           |
|         |       |      |       |           |
|         |       |      |       |           |

3. Describe the potential health risks to humans and/or animals.

4. Describe in detail experimental procedures to be used.

5. Describe special animal care related to the use of hazardous materials.

6. Describe special containment facility requirements.

7. Describe special precautions for animal handlers.

8. Describe waste and animal disposal requirements

# Autoclave: Sterilization Performance

Autoclaves are used to sterilize biological waste products prior to disposal in regular trash. These devices must be tested to ensure that sterilization performance meets the requirements.

There are three indicators that may be used to detect the efficacy of the autoclave process:

- (1) Physical: pressure and temperature recording devices,
- (2) Chemical: indicators that change color after being exposed to specific temperatures, such as temperature sensitive tape. The color change upon exposure to the given temperature, and
- (3) Biological: *Bacillus stearothermophilus* spores are used, due to its resistance to heat, for the testing that measures the biological performance of the autoclave process.

## Introduction:

- ☐ Biological spore testing is performed monthly on all research autoclaves used for biological waste, and results will be recorded on a monthly autoclave log form. Log records should be kept for a minimum of three years.
- ☐ Obtain biological indicator ampules, or self-contained vial and strip sampling systems. Supply companies such as Hardy Diagnostics and Fisher Scientific sell these systems. Regardless of which system is used, follow all instructions-of-use and/or recommendations from the manufacturer.

## Autoclave Testing Process:

- ☐ It is recommended that at least two spore (*Bacillus Stearothermophilus*) are used per cycle.
- ☐ Label the spore indicator with the proper information. Such as date, autoclave number, etc.
- ☐ Place the spore indicator in a horizontal position inside a Biohazardous bag with representative materials to be sterilized.

**(Biohazardous bag should be closed with autoclave tape)**

- ☐ Select appropriate cycle to process the load.
- ☐ Once the cycle is completed, allow autoclave to decompress and cool down (min.10 minutes).
- ☐ Remove the load from autoclave and allow it to further cool down (10 - 15min).
- ☐ Retrieve the spore test indicator from the load.
- ☐ Note: the chemical indicator tape changes from white to black when exposed to steam.

## Incubation process:

- ☐ Use an adjusted microbiological incubator to a temperature for 131F – 140F (55C to 60C).
  - Take both indicators from the loads that were autoclaved, and follow manufactures recommendation for type of indicator chosen and place those in the incubator.
  - Take a third spore indicator (labeled as “control”) that was not run in the load. prepare sample spore indicator according to manufacture to also incubate in the same incubator, allow incubation and verify all three spore indicators at time frame specified by manufacturer.

**Interpretation:**

- ☐ Examine the indicators for any color change according to manufacturer specifications. The presence of yellow color change indicates a fail test and positive bacterial growth. No color change indicates a passing test and proper sterilization of sample. **The control indicator should produce a yellow color change**
- ☐ If a fail test is confirmed run the test again and discontinue normal use of the autoclave. If the second test fails again, post a sign “**Autoclave Not In Use**” and inform all appropriate personnel including primary point of contact. Discontinue full use of the autoclave device until approved for use.
- ☐ If a passing test is confirmed from the first round or second round, you may proceed with regular use of the autoclave.
- ☐ Record all results in your monthly autoclave log form.
- ☐ Ensure disposal of all used Bacillus indicators by autoclaving first before discarding.

# Autoclave Log For \_\_\_\_\_

A COMPLETE entry must be made in this log for each use of the autoclave

| Date/<br>Time | Generator<br>Name | Location | Time at<br>121°C | Medical<br>Waste | Biohazard<br>Waste | Other:<br>Specify | Sterilization<br>Check Pass/Fail | Comments | Operator<br>Name |
|---------------|-------------------|----------|------------------|------------------|--------------------|-------------------|----------------------------------|----------|------------------|
|               |                   |          |                  |                  |                    |                   |                                  |          |                  |
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## Autoclave Monthly Log for Spore Tests

**Location:**

Department

Autoclave: Serial #

[illegible]

## AUTOCLAVE LOG

- 1. INDIVIDUALS USING THE AUTOCLAVES MUST READ THE AUTOCLAVE RULES AND SUBMIT THEIR SIGNED COPY TO THE AUTOCLAVE SUPERVISOR!**
- 2. EACH TIME YOU USE THE AUTOCLAVE, YOU MUST LOG IT IN THE TABLE BELOW!**
- 3. IF THE AUTOCLAVE MALFUNCTIONS, PLEASE CALL LET THE AUTOCLAVE SUPERVISOR KNOWN ASAP, AND LOG ERROR IN THE TABLE BELOW!**

| Individual | Date      | Items       | Cycle used | If autoclave(s) malfunctions, write down problem here! |
|------------|-----------|-------------|------------|--------------------------------------------------------|
| STA        | 6/26/2023 | Biowaste    | Slow       | N/A                                                    |
| JM         | 6/30/2023 | Instruments | Fast       | N/A                                                    |
| IEA        | 7/01/2023 | Media       | Slow       | N/A                                                    |
|            |           |             |            |                                                        |
|            |           |             |            |                                                        |

| <b>Individual</b> | <b>Date</b> | <b>Items</b> | <b>Cycle used</b> | <b>If autoclave(s)<br/>malfunctions, write down<br/>problem here!</b> |
|-------------------|-------------|--------------|-------------------|-----------------------------------------------------------------------|
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| <b>Individual</b> | <b>Date</b> | <b>Items</b> | <b>Cycle used</b> | <b>If autoclave(s)<br/>malfunctions, write down<br/>problem here!</b> |
|-------------------|-------------|--------------|-------------------|-----------------------------------------------------------------------|
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| <b>Individual</b> | <b>Date</b> | <b>Items</b> | <b>Cycle used</b> | <b>If autoclave(s)<br/>malfunctions, write down<br/>problem here!</b> |
|-------------------|-------------|--------------|-------------------|-----------------------------------------------------------------------|
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