



## Enter PI's Name's Laboratory Biosafety Manual

Building and Lab Room Number(s): Bldg Name and Rm #(s)  
Biosafety Containment Level: Choose an item.

IBC Protocol/BUA Number(s): Enter Number(s)  
IACUC Protocol Number(s) (if applicable): Enter Number(s) or NA

Date: Click or tap to enter a date.

This manual must be reviewed annually or when laboratory scope of work has changed.

Manual Template Reviewed by IBC: 11/9/2022

## Introduction

This manual is provided to assist Principal Investigators (PIs) in the development of a *laboratory-specific* biosafety manual with instructions to safely handle and manipulate biological agents in the laboratory.

The PI is responsible for:

- Completing this Manual
  - Title Page
  - Page 4 contact information table
  - Page 5 emergency equipment locations
  - Page 6 additional PPE requirements
  - Page 13 description of decontamination procedures
  - Page 16 list all biological agents present in the laboratory
  - Page 17 complete exposure risk section
- Including background information for each agent (see [MSU Pathogen Safety Data Sheets](#) (PSDS)).
- Making protocols (IBC, IACUC, etc.) and experimental SOPs available to lab staff.
- Writing an exposure risk assessment.
- Detailing surface decontamination and disinfection procedures.
- Writing standard operating procedures (SOPs) for experiments and specific laboratory procedures.

Laboratory staff must be trained and understand the risks associated with the laboratory. Training dates for the following must be captured on a signature sheet in Appendix A. Signatures may be physical or digital (e.g., DocuSign).

Training items:

- This document.
- All relevant MSU IBC Protocols or Biological Use Agreements (BUAs) including:
  - How to access protocols.
  - Biological materials and their hazards (see PSDS).
- Laboratory specific procedures and equipment.
- Emergency procedures.

If any laboratory determines the need to deviate from standard work practices outlined herein, then such deviations must be submitted to the BSO in written form with an explanation for approval.

In addition to this manual, PIs and research staff must follow requirements outlined in the [MSU IBC Manual](#), [MSU Biosafety Manual](#), [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\) 6th Edition](#), and the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#).

**Prior to working in the lab, personnel must read this entire manual and all relevant IBC Protocols or BUAs. Staff must sign the signature sheet indicating they have read and understand all items and have had the opportunity to ask questions. The signature page is found at the end of this manual in Appendix A.**

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## Responsibilities

### Principal Investigator (PI)

The PI has the primary responsibility for ensuring that the laboratory is safe and is responsible for the safe use of biological materials used in the lab.

In addition, the PI is responsible for the following:

- Limit personnel, student, and visitor exposure to hazards to the lowest practical level.
- Be familiar with the required medical surveillance for each type of biological agent used in the laboratory.
- Develop written lab-specific safety procedures and train personnel on them.
- Maintain documentation of training in this Biosafety Notebook.
- Provide the appropriate Personal Protective Equipment (PPE) and instruction on proper use.
- Ensure all forms of waste are properly disposed of.
- Report spills, exposures, or incidents to the Biosafety Officer.

### Laboratory Staff/Students

- Complete all required training before conducting any lab activity.
- Knowledge of the biological agents and procedures used in the laboratory.
- Follow approved lab procedures and safety guidelines.
- Know emergency procedures.
- Report any unsafe conditions to the PI and/or the Biosafety Officer.
- Utilize appropriate lab equipment and containment facilities.

## Emergency Information

### Emergency Contacts

<b>Principal Investigator:</b>	<b>First, Last</b>
Lab Location:	Lab Location
Office Phone:	Office Phone
Cell Phone:	Enter number
<b>Secondary Contact</b>	<b>First, Last</b>
Office Phone:	Office Phone
Cell Phone:	Enter number
<b>Biosafety Officer</b>	<b>Amy Robison</b>
Office Phone:	406-994-6733
Cell Phone:	406-451-3511
<b>Chemical Safety Officer</b>	<b>Ryan Brickman</b>
Office Phone:	406-994-7760
<b>MSU Work Control</b>	<b>Building Emergencies</b>
	406-994-2107

In the event of a medical emergency, call 911.

If there is a fire or explosion immediately pull the fire alarm and evacuate the building. Then call 911 from a safe location.

For building emergencies (e.g., water leak) call MSU Work Control.

If any emergency or spill/exposure occurs in the laboratory, immediately notify the Biosafety Officer and your lab supervisor/PI. Any spills involving Recombinant/Synthetic Nucleic Acid Molecules (r/sNA) must be reported to the Biosafety Officer.

## Fire Alarms/Extinguishers

Locations of fire pull stations: Enter locations

Locations of fire extinguishers: Enter locations

Know the location of each of these and identify the location of the extinguisher closest to your lab bench. If the fire alarm sounds, leave the building immediately and move away to a safe distance and call 911.

## Eyewash and Emergency Showers

Locations of eyewash stations: Enter locations

Person responsible for weekly flush: Enter Name

Locations of emergency shower: Enter locations

Person responsible for monthly flush: Enter Name

**Flush and document that eyewash stations are functioning, and water temperature is tepid on a weekly basis.**

In case of biological exposure, proceed to nearest eyewash station. Hold eyelids open with thumb and forefinger and rinse for at least 15 minutes. Wash from outside edges towards the inside to prevent washing back into the eye.

Rinse should be aimed at the inner corner of the eye (near the nose) not directly at the eyeball. "Roll" eyes around and up and down to ensure full rinsing.

Contact lenses (if worn) should be removed as soon as possible. Have another member of the lab call for emergency response immediately. The area around the eye wash station must always remain clear.

## Standard Operating Procedures (SOPs)

### Standard Microbiological Practices and Guidelines

These refer to the safe laboratory work practices when experimenting with biological agents. For additional information refer to:

- Montana State University Biosafety Manual
- BMBL, 6<sup>th</sup> ed.
- NIH Guidelines

## Hygiene and Housekeeping

Keep work areas clean and uncluttered to reduce the chance of cross-contamination and inadvertent exposure to biohazards. To avoid ingestion of contaminated material, use a mechanical pipetting device. Food and drink are not permitted in the laboratory. Refrigerators, freezers, microwaves, ice machines, and other equipment must be labeled with biohazard symbols and a notice that no food or drink is permitted. Eat, drink, or apply cosmetics only in designated areas outside the laboratory.

The following must be adhered to by lab staff:

- Wash hands with soap and water after removing gloves, and before leaving the lab.
- Clean work surfaces and decontaminate with the appropriate disinfectant(s) after work and at the end of each day.
- Remove gloves and wash hands before leaving the lab, touching the face, keyboards, cell phones, or control panels.
- Remove lab coats prior to leaving the laboratory. PPE is not to be worn in common areas (including hallways).

## Laboratory Specific SOPs

The purpose of this section is to develop lab specific SOPs. Detailed, step-by step protocols describing entire experiments with materials and methods are not necessary. Staff must be trained on lab specific SOPs prior to

performing the work. A sample training document can be found at the end of this manual for tracking of SOP training. Examples of SOPs where safety is emphasized are bulleted below:

- Propagation of viruses/bacteria
- Experiments that require PPE in addition to a lab coat and gloves
- Experiments that require manipulation of a BSL2 agent outside a biosafety cabinet
- How to properly vortex or sonicate a viable agent
- Safety concerning the handling of human or non-human primate cell lines or tissues
- Safety when injecting a research animal with a biological agent
- How to safely centrifuge a sample

**Place copies of SOPs and signature sheets at the end of this manual.**

## Personal Protective Equipment (PPE)

The following PPE must be worn when working with biological agents in the laboratory:

- Lab coat
- Disposable gloves (nitrile or latex)
- Safety glasses

NOTE: Personal prescription glasses are not considered safety glasses. Staff must wear approved safety glasses over top of personal glasses or obtain safety prescription glasses.

Please check appropriate boxes for additional PPE that is required:

- |   |  |   |                                      |
|---|--|---|--------------------------------------|
| <input type="checkbox"/> Safety glasses | <input type="checkbox"/> N95 Respirator    | <input type="checkbox"/> PAPR           | <input type="checkbox"/> Shoe covers |
| <input type="checkbox"/> Face shield    | <input type="checkbox"/> Surgical mask     | <input type="checkbox"/> Medical scrubs |                                      |
| <input type="checkbox"/> Hair net       | <input type="checkbox"/> No Additional PPE |   |                                      |

### *PPE Considerations*

- Remove rings or other jewelry that could puncture gloves.
- Wear the appropriate glove for the hazard. Latex or nitrile glove is recommended for working with biological material.
- Do not reuse gloves.

Wear protective eyewear appropriate for the hazard:

- Standard lab safety glasses, goggles, or face shield when performing operations that pose a splash hazard.
- Always wear a face shield (and protective gloves) when opening a hot autoclave, to prevent steam burns.

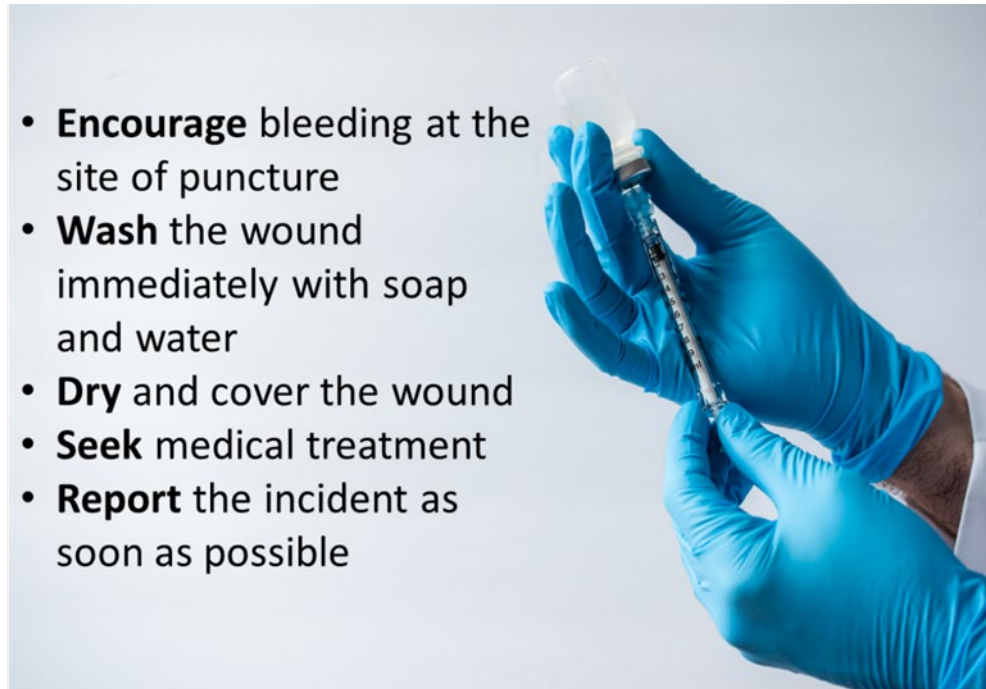
## Injuries

Injury to an individual in the laboratory (i.e., needle stick, cut, biological agent exposure incident, etc.):

1. Immediately stop work.
2. If the injury is a Medical Emergency call 911.
3. Flush affected area with soap and water for 15 minutes.
4. Secure all infectious materials.
5. Notify the PI and Biosafety Officer at x6733 or University police by dialing 911 after working hours.
6. Use the nearest First Aid Kit located in the laboratory.
7. Seek medical treatment. Montana State University offers occupational health via the preferred locations below. Or you may seek initial treatment at a medical professional of your choice.
  - a. **During business hours (8AM-6PM Monday through Friday; 9AM-5PM Saturday and Sunday):**
    - i. Bridger Orthopedic West - Occupational Health & Urgent Care 3400 Laramie Dr., Bozeman, MT; 406-587-0340
  - b. **After business hours (between 6 PM and 8 AM, and on weekends):**
    - i. Bozeman Deaconess Hospital Emergency Room 915 Highland Blvd, Bozeman, MT

8. Complete the [First Report of Injury Form](#) documenting the route of exposure and the circumstances under which the incident occurred.

### Needle Stick/Animal Bite/Cut Procedures



**To report any injury:** <https://firstreportinjury.mus.edu/>

Montana State University Occupational Health: Safety and Risk Management: 406-994-2711

Bridger Orthopedic West, 3400 Laramie Dr, Bozeman, MT 59718; M-F 8AM-6PM and S-S 9AM-5PM; 406-587-0340  
Bozeman Health Deaconess Hospital: 915 Highland Blvd, Bozeman, MT 59718; After Hours

### Biological Spills

Call the Biosafety Officer when a significant spill occurs. A lab incident report form must be filled out for significant spills.

A significant spill is defined as:

- Spills containing recombinant/synthetic nucleic acids or genetically modified materials
- Spills greater than 5 ml outside primary containment.
- Spills that result in an exposure.
- Spills that present an inhalation hazard.
- Spills that cannot be easily cleaned.
- Spills that endanger people or the environment.

### Biological Spill Kit

Ensure the lab has a stocked biological spill kit. All staff must know the location of the spill kit. Label the spill kit storage location.

Note that 1:10 bleach must be made up at time of spill clean-up; other more stable disinfectant solutions (e.g., 5% Microchem) may be considered to extend the shelf life of your spill kit. Spill clean-up procedures are outlined on the next page. Print and hang this sign throughout your laboratory.

### *Spill Kit Contents*

Every biosafety laboratory that works with biological agents must have a biohazard spill kit on hand, that is readily accessible and easy to find in the laboratory. It must have appropriate equipment and supplies for managing spills and accidents involving biohazardous materials.

The supplies available in a biohazard spill kit should include, but are not limited to:

- Container to keep all contents in.
- A copy of the Spill Cleanup Procedures.
- PPE - Nitrile disposable gloves, eye protection, lab coat (nearby if not in kit).
- Disposable shoe covers (booties).
- Absorbent material, such as paper towels.
- All-purpose disinfectant, such as normal household bleach (freshly diluted 1:10).
- Tongs/forceps, and/or dustpan and hand broom for cleaning up broken glass or contaminated sharps.
- Sharps waste container (in lab, nearby).
- Autoclavable biohazard waste bags.
- Biohazardous spill warning signs.

All non-disposable items should be autoclavable or compatible with the disinfectant to be used. Most of the listed items, as well as other biohazard spill control items, are available at Central Stores, and often are contained within various commercially available biohazardous spill control kits.





### Biological Spill Clean-Up Procedures

1. Alert others in the laboratory and leave the immediate spill area.
  2. Mark the area to prevent others from walking into the spill area.
  3. Remove contaminated PPE and dispose of in biohazard bag—place any reusable PPE into a separate biohazard bag (e.g., lab coat). Autoclave when available.
  4. Wash your hands.
  5. Don new lab coat, gloves, shoe covers, and safety glasses.
  6. Cover spill with absorbent material (e.g., Pig Pad, paper towels).
  7. Prepare fresh disinfectant (e.g., 1:10 bleach:water)
  8. Carefully pour disinfectant on the spill, working from the outside to the middle.
  9. Allow disinfectant to contact spill for no less than 20 minutes.
  10. If broken glass, or other sharps (e.g., needle, razor) are part of the spill, use tongs or plastic scoops to pick up absorbent material and sharp material. Dispose of in a sharps container, or broken glass container.
  11. If no sharps are present, dispose of absorbent material in biohazard waste.
  12. Remove PPE and wash your hands.
  13. Report all spills to your supervisor. All spills involving recombinant/synthetic nucleic acid molecules must be reported to the Biosafety Officer.
- If spill is inside BSC- Keep BSC running for at least 15 minutes after the cleanup. Clean spill tray below work area and trough below air intake grill while BSC is running.

### Wound/Cut Procedures

CALL 911 FOR ALL LIFE EMERGENCIES

1. Expose the wound—remove gloves.
2. Express blood from the wound and wash affected area for 5 minutes.
3. Cover wound with clean gauze or bandage.
4. Complete First Report of Injury form and seek medical treatment.

### Compliance/Ethics Anonymous Reporting

Call: 855-753-0486

Visit: [www.mus.ethicspoint.com](http://www.mus.ethicspoint.com)

## Biological Waste Disposal

### Liquid Biohazardous Waste:

All liquid biological waste from the lab must be treated prior to disposal. Examples of biological waste include cell lines, recombinant DNA, recombinant proteins, biological agents, and any associated media or buffer.

The procedures below are an example of the steps to treat liquid biohazardous waste generated in the lab:

1. Always wear appropriate PPE such as disposable gloves, lab coat and eye protection (safety glasses or goggles) when working with biohazardous waste.
2. When liquid biohazardous waste is anticipated to be generated, add 100 ml of undiluted bleach into a 1-liter beaker. Label the beaker appropriately.
3. As experiments are performed and completed pour the biological waste into the beaker with the bleach.
4. When experiments are completed (and if the beaker contents are less than 1-liter) add water to bring the volume to 1-liter. This provides for a 1:10 bleach:waste (0.5% sodium hypochlorite) solution.
5. Let bleach and biological waste solution stand for at least 30 minutes.
6. Dispose of the solution down the lab sink, flushing with excess water.
7. Rinse the beaker thoroughly with water.

### Solid Biohazardous Waste:

The procedures below outline the steps to take to treat solid biohazardous waste generated in the lab:

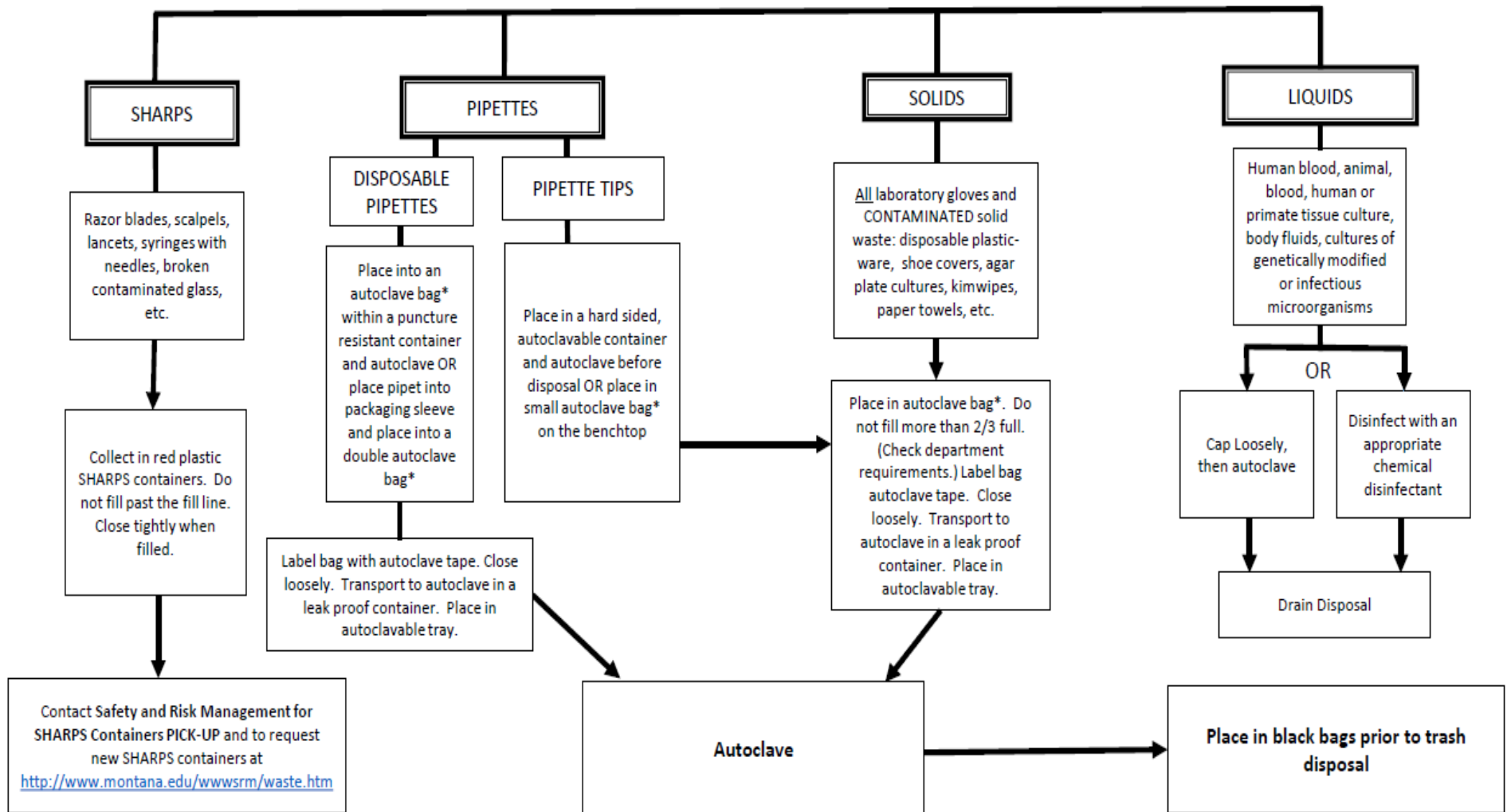
1. All solid lab waste that has come in contact with biological waste must be treated prior to disposal. Examples include used PPE, paper towels, pipette tips, Petri dishes, pipettes, culture flasks.
2. Always wear appropriate PPE such as disposable gloves, lab coat, and eye protection (safety glasses or goggles) when working with biohazardous waste.
3. Place all potentially contaminated items in biohazardous waste bag.
4. Once the bag is  $\frac{3}{4}$  full close bag and place autoclave tape on the bag.
5. Take the biohazard bag to the autoclave room per your buildings transportation guidelines as follows and place in the autoclave.
6. Complete autoclave logbook entry and autoclave biohazardous waste for at least 60 minutes at 121°C.
7. Once the autoclave cycle is complete the load within has been sterilized. Ensure cycle has completed properly by checking the autoclave print out/chart recorder. Confirm waste has been exposed to steam by observing if the autoclave tape has turned color. Allow sterilized bag to cool in a bin.
8. Place the biohazardous bag into a black garbage bag and dispose of in the dumpster.
9. If the autoclave tape did not turn color and/or the autoclave display indicates errors occurred during operation or an incomplete cycle the load has not been sterilized. Notify the Biosafety Officer to have the autoclave serviced.

### Sharps Biohazardous Waste:

The procedures below outline the steps to dispose of sharps:

1. All used sharps must be immediately discarded into a sharps container.
  - a. Sharps containers must be kept upright and never filled past the "fill-line" marked on the container.
  - b. Put sharps containers in immediate work area for easy use.
2. When sharps waste has reached the pre-marked "fill-line" of the container close and lock the lid.
3. Contact Safety and Risk Management to have sharps containers picked-up and replaced.

**BIOHAZARDOUS WASTE DISPOSAL CHART**  
(MSU Research Laboratories)



\* Use only clear or orange autoclave bags. No RED bags.

# Good Laboratory Practices

## Sharps go in dedicated waste containers



**Do not Re-cap Needles!!!**



Fill Line

Seal, mark with tape,  
Autoclave, submit to SRM

## Proper Biohazard Waste Disposal

### Biohazard waste

Gloves  
Pipet tips  
**NO SHARPS**



Seal bag loosely.  
Mark with tape.  
Autoclave  
(150-180 c/ up to 2h)

Take out, let cool.  
Put into regular  
dark garbage bag>>>  
Normal waste stream.



## Hazardous Chemical Waste Management

Please contact Safety and Risk Management (SRM) to dispose of hazardous chemical waste materials.

Disposal Guidelines: <https://www.montana.edu/srm/programs/waste.html>

- Hazardous waste must never go down any MSU drain at any time or in any amount.
- Hazardous waste is collected by Safety and Risk Management using the waste pickup request: <https://www.montana.edu/srm/forms/waste/>

Hazardous Materials Contacts:

[Safety & Risk Management](#)

Ryan Brickman, Chemical Safety Officer, x7760

Patrick Ryan, Hazardous Materials Manager, x7803

## Decontamination

Select the reagents (disinfectants) and/or processes used to inactivate biological agent(s) and the method to decontaminate surfaces.

- 1:10 Bleach:water
- 70% Ethanol
- Accelerated hydrogen peroxide (e.g., Rescue)
- 5% MicroChem
- 1% SDS
- Iodophor
- Virkon
- CaviCide
- Other

Describe Other

# Decontamination (decon)

Decontamination- Any procedure that reduces pathogenic microbes to a level where items are safe for handling and disposal



**Cleaning** – A process that removes foreign material (dirt, organic matter)  
Usually done with soap and water or detergent

- **Disinfection** – Destruction of most but not necessarily all pathogenic microbes or their spores.  
Generally done with Chemicals (bleach, ethanol)



- **Sterilization** – Killing of all living forms of microbes including spores  
Autoclave



## Training

All laboratory research personnel must take institutional provided training. Training must be documented. Personnel should not initiate research until training is completed. Training can be confirmed via CITI and/or TOPAZ – certificates do not need to be printed and stored with this manual.

### Agent-Specific Training

Laboratory personnel are not allowed to work with biological agents until they have been trained by the PI who supervises their work, or a designated technical expert. The worker should demonstrate good microbiological skills and an understanding of SOPs prior to being permitted to work with agents.

### Training Requirements:

#### *Biosafety Training:*

**Biosafety for BSL1 and BSL2 Laboratories** – Required for all researchers; *3-year certification*.

**NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids** – Required for all researchers working on r/sNA; *3-year certification*.

**OSHA Bloodborne Pathogens** – Required for any work with human-sourced material; *1-year certification*.

#### *Chemical Safety Training:*

**Hazard Communication** - Required for all employees that work with/around Hazardous Chemicals (cleaning and industrial chemicals, laboratory chemicals, solvents or acids, mechanical fluids, etc.)

## Training Options

### *In House Training*

Biosafety & Hazard Communication Training. This is a 2-hour course that covers all training requirements listed above.

- Details: <https://www.montana.edu/orc/training/biosafety/biosafety-hazard-com-training.html>

### *Online Options*

Biosafety: [Collaborative Institutional Training Initiative \(CITI Program\)](#)

Chemical Safety: [SRM Online Training Options](#)

## Biohazard Warning Signs and Postings

Each laboratory must clearly display a sign at the entrance door that provides safety information to visitors and service personnel. SRM will provide the signs.

- All areas and laboratories which contain biohazardous agents must be posted with a biohazard sign.
- The sign must have information regarding biosafety level, materials used, entry requirements, exit requirements, emergency contact name and phone number.

Please confirm that your laboratory entry signs are accurate and up to date. If they need correction, please contact Amy Robison (BSO x6757) or Ryan Brickman (CSO x7760).

## Biological Safety Cabinet (BSC)

BSC's should be positioned in the laboratory away from normal traffic patterns to minimized airflow disruption.

Some work may be done on the open bench by persons wearing appropriate protective clothing or gear. Any work that may produce aerosols of infectious materials must be done inside a biological safety cabinet (BSC).

Clean materials should be kept to one side of the work surface, dirty items on the other. Management of workflow within the BSC is crucial to preventing spills and cross-contamination.

Rapid air movement outside the cabinet (caused by co-workers walking past, air supply vents directed across the face of the BSC, fans, doors opening, etc.) will interrupt the air curtain.

The chair should be adjusted so that the lower portion of the sash is even with the worker's armpits. Any paper or plastic materials introduced into the BSC should not be allowed to interfere with air flow through the front or rear grilles.

The downward airflow from the supply filter "splits" about one third of the way into the cabinet; in the front third, air moves to the front grille, with the remainder of the air flowing to the rear. This means that aerosol-generating activities should be performed towards the rear of the cabinet to provide further worker protection.

## Biological Agents and Exposure Risks

### List of Biological Agents

List all biological agents used in your laboratory:

- Enter biological agents



## Exposure Risks

Describe how laboratory personnel could be exposed to the agent(s). Include practices that pose potential for exposure, such as those that could create aerosols.

Enter Details.

## Agent(s)-Specific Pathogen Safety Data Sheets

Pathogen Safety Data Sheets can be found at the [MSU Biosafety website](#). If needed, [Public Health Agency of Canada \(PHAC\)](#) has additional resources. Attach copies of PSDS to your IBC Protocol/BUA.



## Appendix B: Laboratory Training Documentation – Individual Qualification

Staff Name:	Principal Investigator/Supervisor:
MSU Email:	

### **BIOSAFETY PROCEDURES AND DOCUMENTATION OF TRAINING/EXPERIENCE**

MSU Policy requires that the Principal Investigator assures that individuals performing procedures with biohazardous material are adequately trained/experienced to perform those procedures. Activities requiring training include, but are not limited to:

- Understanding the characteristics of biological agents and the risks associated with their work (See MSU Pathogen Safety Data Sheets)
- Aerosol control
- Laboratory practices (e.g., pipetting, vortexing, centrifuging, etc.)
- Personal Protective Equipment (PPE)
- Proper use of biological safety cabinets
- Decontamination procedures
- Waste procedures
- Lab specific Standard Operating Procedures

Appropriate training can be obtained by a number of means, including:

- Principle Investigator
- Qualified senior researchers in your lab or other labs
- External training provided by vendors for specialized equipment

List all procedures/topics you have been trained on, the training dates, the trainer, and when proficiency was achieved. Create additional lines/pages as necessary.

Procedures/Topic	Training Date	Trainer Initials	Training Date	Trainer Initials	Training Date	Trainer Initials	Date Proficiency Achieved	Trainer Initials