Version 2 – 2026

Environmental Health & Radiation Safety

3160 Chestnut Street, Suite 400

Philadelphia, PA 19104-6287

215-898-4453

Contents

EMERGANCY CONTACT INFORMATION

PENN MAIN CAMPUS

IF YOU ARE INJURED AND REQUIRE EMERGANCY ASSISTANCE

From a CAMPUS LAND LINE: 511

From a CELL PHONE or non-Campus Line: 215-573-3333

Smilow and **South Tower/PCAM Laboratories** should also call HUP Emergency to report building emergencies: **215-615-5656**

For medical assistance FACULTY and STAFF report to:

HUP OCCUPATIONAL MEDICINE

Ravdin Building, 2nd Floor
3400 Spruce Street
215-662-2354
8AM – 4:30PM

STUDENTS (graduate and undergraduate non-paid staff) report to:

STUDENT HEALTH SERVICES

3535 Market Street, Suite 100
215-746-3535
see website for varying hours

VISITING SCHOLARS, NON-AFFILIATES, and ALL EMERGANCIES AFTER WORK HOURS and HOLIDAYS report to:

HUP EMERGENCY DEPARTMENT

HUP PAVILION
1 Convention Avenue
215-662-2354

EMERGANCY CONTACT INFORMATION

NEW BOLTON CENTER

IF YOU ARE INJURED AND REQUIRE EMERGANCY ASSISTANCE

From a CAMPUS LAND LINE: 911

Campus Emergency Line: 610-444-5800

For medical assistance FACULTY and STAFF report to:

OCCUPATIONAL HEALTH CENTER OF KENNETT SQUARE

830 West Cypress Street
Kennett Square, 19348
Hours: Mon-Fri, 7:30am - 4:00
pm 800-789-7366

STUDENTS (graduate and undergraduate non-paid staff) report to:

STUDENT HEALTH SERVICES

3535 Market Street, Suite 100
215-746-3535
see website for varying hours (https://wellness.upenn.edu/)

VISITING SCHOLARS, NON-AFFILIATES, and ALL EMERGANCIES AFTER WORK HOURS and HOLIDAYS report to:

CHESTER COUNTY HOSPITAL

701 East Marshall Street West Chester, 19380
610-431-5000

Section 1 - INTRODUCTION

PURPOSE

The purpose of the Biosafety Program is to protect all employees, students, the public, and the environment from exposure to biological agents or materials being used at the University that may cause disease or be harmful to humans. This manual provides a comprehensive overview of proper work practices, regulations, and requirements for proper containment and disposal of biological hazards. The guidance in this manual is based on the **Biosafety in Microbiological and Biomedical Laboratories**, published by the Centers for Disease Control and Prevention and the National Institutes of Health (https://www.cdc.gov/labs/BMBL.html)

POLICY

The University of Pennsylvania is committed to providing a healthy and safe learning, teaching, research, and work environment. Accordingly, the goals of the University's Biological Safety Program are to:

- Ensure a **HEALTHY** and **SAFE** research environment.
- **PROTECT** staff, students, and community from exposure to infectious agents.
- **PREVENT** environmental contamination.
- **SECURE** experimental materials.
- **COMPLY** with Federal, State and Local Regulations.

The Office of Environmental Health & Radiation Safety (EHRS), under the direction of the University's Institutional Biosafety Committee (Penn IBC) and The Office of the Vice Provost for Research, developed this Biological Safety Manual. This manual provides university-wide safety guidelines for working with biological hazards (biohazards). It also outlines general policies and procedures for using and disposing of infectious or other potentially infectious materials (OPIM). Penn biosafety policies ensure compliance with federal, state, and local laws, regulations, and guidelines. This manual is a guidance document that is revised annually to include new and amended Federal, State, and City regulations that apply to the University. It may not address all hazards encountered by faculty, students, staff, and the Penn community.

Biological safety practices and procedures in all University laboratories must comply with those outlined in this manual. Principal investigators (PIs), laboratory supervisors, or laboratory managers must contact the Office of Environmental Health & Radiation Safety by phone (215-898-4453) or email (ehrs@ehrs.upenn.edu), if they are uncertain how to categorize, handle, store, treat, or discard any biologically derived material.

ROLES AND RESPONSIBILITIES

Office of the Vice Provost for Research

The Vice Provost for Research has responsibility for the development and implementation of research policies and procedures across the University.

The Vice Provost chairs the Provost's Council on Research which has representatives from the 12 component schools of the University and advises the Vice Provost for Research on formation and implementation of research policies.

The Vice Provost is also responsible for administering, overseeing, and coordinating a wide variety of activities. To provide comprehensive services to researchers, the Office of the Vice Provost for Research unites and coordinates the following offices:

- Office of Research Services (ORS) is responsible for supporting University investigators and administrators by managing externally sponsored research projects through the pre-award and post-award processes.

- Human Research Protections Program (IRB) promotes and protects the welfare of human research participants by reviewing and approving all human subject research conducted on Penn's campus.
- Office of Animal Welfare (OAW) provides comprehensive support and guidance to the animal research community on matters related to Institutional Animal Care and Use Committee (IACUC) protocol development and ensuring regulatory compliance with the animal program.
- University Laboratory Animal Resources (ULAR) is a campus wide research service organization that provides daily animal husbandry, veterinary care and diagnostic services.
- Research Integrity Office manages the University's Research-related Financial Conflicts of Interest Program and oversight of the Office's commitment to Responsible Conduct of Research and Research Integrity.
- Office of Environmental Health and Radiation Safety (EHRS) promotes health, safety, and environmental protection in teaching, research, and healthcare programs and facilities by providing services, advice, and compliance assistance.
- Penn Center for Innovation (PCI) helps to translate Penn discoveries and ideas into new products and businesses for the benefit of society.

In addition, the Office of the Vice Provost for Research works closely with the Office of Human Research and serves as the University of Pennsylvania's Research Integrity Officer.

Compliance with University, federal, state, and local regulations is a condition of acceptance of research funding from the NIH and various other granting agencies.

The Institutional Biosafety Committee (IBC)

- The Institutional Biosafety Committee (IBC) has University-wide oversight of synthetic and recombinant nucleic acid research as mandated by the National Institutes of Health (NIH) Office of Science Policy (OSP) and is additionally charged by the Vice Provost for Research with formulating policy and procedures related to the use of biohazardous agents including human pathogens, oncogenic viruses, other infectious agents.
- The IBC is responsible for the review and approval of projects involving recombinant and synthetic nucleic acid research, gene-editing technology, and human gene transfer protocols. Additionally, the IBC reviews work with Select Agents, biohazardous agents that are animal or human pathogens requiring BSL-3 or ABSL-3 containment, oncogenic viruses, and other potentially infectious agents on an as needed basis.
- The committee sets containment levels in accordance with the National Institutes of Health (NIH) and Centers for Disease Control and Prevention (CDC) guidelines and adopts emergency plans covering accidental spills and personnel contamination.
- The Vice Provost for Research appoints members of the IBC.

The Office of Environmental Health & Radiation Safety (EHRS)

- The Office of Environmental Health and Radiation Safety (EHRS) houses the Biosafety Team, which is the operational arm of the Institutional Biosafety Committee (IBC). The Biosafety Team provides instruction and training on safe work practices, conducts routine inspections of work areas, investigates accidents and recommends preventive/corrective actions, reviews animal research protocols involving hazardous materials, reviews renovations and new construction design for safety features, and responds to emergencies.
- The Institutional Biosafety Officer (IBSO) is an EHS Associate Director and is responsible for oversight and daily

implementation of the Biosafety Program.

- The IBSO is responsible for compliance with all federal, state, and local regulations that apply to biosafety and keep University regulations up to date.
- Biosafety Officers (BSO) are EHRS staff members and make up the Biosafety Team. They report to the Institutional Biosafety Officer (IBSO). BSOs perform risk assessments on labs as needed, help PIs develop SOPs, offer appropriate training to all staff and students, and perform annual laboratory inspections of research spaces where biological materials are used.
- BSOs pre-review work requiring BSL-3 or ABSL-3 containment as well as all IBC registration submissions. They maintain the BioRAFT Biological Registration, and administer a Select Agent Program, as needed, at the University of Pennsylvania. BSOs also provide guidance and regulatory support regarding biological containment and registration of biological materials to the Office of Animal Welfare in pre-reviews of IACUC protocols
- BSOs will respond to and follow up on any major biological incident, as needed. This includes situations such as spills and accidental releases of recombinant and/or hazardous agents. BSOs must be called to assist with large spills containing infectious material and accidental releases of recombinant organisms outside of containment. They will assist the PIs to ensure that all corrective actions and emergency procedures are followed in accordance with applicable University regulations and guidelines.

Deans/Department Chairs

- Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their schools or departments.

Facilities Departments

- The Facilities Department in each school is responsible for the removal, packaging, and shipment of all infectious waste in accordance with local, state, and federal regulations.
- Building Administrators are responsible for keeping common laboratory spaces clean and in safe working conditions. They are responsible for getting broken infrastructure in the laboratory fixed.

Principal Investigators (PIs)

- The Principal Investigator (PI) is responsible for full compliance with approved research protocols, trainings required by the University, the University Biological Safety Manual, the NIH Guidelines (NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules), the Occupational Safety and Health Administration(OSHA) Bloodborne Pathogen Standard (human-derived materials) and other local, state, and federal regulations that apply to research.
- In the laboratory, PIs must conduct and document risk assessments to identify potentially hazardous procedures involving infectious agents, develop and document Standard Operating Procedures (SOPs), instruct all faculty, staff, and students working in the lab on safe work practices, including keeping the lab space clean and up-to-date and follow regulations for disposal of infectious waste. The PI must provide all required PPE to their staff.
- PIs must register research projects that require review by the Penn IBC and/or EHRS, such as the generation and/or use of rsNA, work requiring BSL-3 or ABSL-3 containment, Select Agents, and other work with infectious agents as needed.
- PIs must complete the Biological Registration and update it annually or as needed.
- PIs are responsible for hands-on training for all laboratory procedures. They must ensure that all laboratory staff has fulfilled University training requirements and are current in all required training.
- The PI and/or lab personnel are responsible for initiating cleanup and disinfection in the event of a biohazard spill in a laboratory. If assistance is required, in the case of a larger spill, contact EHRS. Once the

material has been contained, absorbed, and removed, housekeeping/facilities management may be contacted to finalize the cleanup and disinfection of the area. The PI is responsible for ensuring that all corrective actions and emergency procedures are followed in accordance with applicable University procedures and regulations.

Employees and Students

- Laboratory staff and students must follow the PI's instructions for working in the laboratory.

- All personnel working in Penn laboratories with potentially infectious materials must be familiar with University training requirements and the University Biological Safety Manual. Additionally, laboratory staff and students must be familiar with the approved research protocols, the NIH Guidelines, and the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen Standard.

- Laboratory staff and students must understand how to safely work with potentially infectious agents, be provided with and trained on how to wear appropriate personal protective equipment (PPE), keep their laboratory space clean and up-to-date, and follow regulations regarding the disposal of infectious waste.

- All employees must receive proper training for their specific tasks, including hands-on training for laboratory procedures. They must also be current in University training requirements. This requirement relies upon mandatory, annual completion of Penn Profiler which will result in assignment of appropriate training in University's Learning Management System.

Section 2 - FREQUENTLY ASKED QUESTIONS

- Why does my door sign say Biosafety Level 2?

  Biosafety Levels (Section 3.2)

- How does a Biosafety Cabinet work?

  Biosafety Cabinets (section 4.1)

- How do I safely work with my lab equipment?

  Common Lab Equipment (section 4.2)

- What is recombinant and synthetic nucleic acid?

  rsNA (section 5.2)

- What should I know when working with human material (blood, tissue, cells, etc.)?

  Human Source Material (section 5.3)

- How do I work safely with non-human primate materials?

  Non-human Primate Material (section 5.4)

- I want to work with a Select Agent.

  Select Agents (section 5.5)

- What does a BSO look for during a lab audit?

  Biological Research Laboratory – Annual Lab Audits (section 6)

- Help! My room sign is out of date.

  Biological Research Laboratory – Room signs (section 6)

- I purchased a new centrifuge to spin down my virus supernatant. Does it need a special label?

  Biological Research Laboratory – Hazard Labels (section 6)

- I purchased a new centrifuge to spin down my virus supernatant. Does it need a special label?

  Biological Research Laboratory – Hazard Labels (section 6)

- I spilled my 50 ml tube of viral supernatant in the BSC. What do I do now? Decontamination (section 7)

SECTION 2 – FREQUENTLY ASKED QUESTIONS

Biological Research Laboratory – Hand Protection (section 6)

- My biohazard bag is full. What do I do with it?
  Infectious Waste Management (section 8.1)

- How do I dispose of my used syringe?
  Sharps Waste Management (section 8.2)

- Can I throw my used serological pipette in the biohazard bag?
  Sharps Waste Management (section 8.2)

- What do I do with infectious waste that has been contaminated with a chemical?
  Mixed Waste Management (section 8.3)

- Do I need to wear PPE while using the autoclave?
  Autoclaving Infectious Waste (section 8.5)

- I've dissected a research mouse in my lab. Where do I dispose of it?
  Research Animals Infectious Waste (section 8.4)

- Do I need training to ship a package with dry ice?
  Transport & Shipping of Biohazards (section 9)

- Do I need a permit to ship my virus sample to my research friend in New Jersey (or anywhere else)?
  Transport & Shipping of Biohazards (section 9)

- What are animal biosafety levels (ABSL)?
  Animal Biosafety Levels (section 3.3)

- How do I properly connect a vacuum line and vacuum flask?
  Vacuum System Protection (section 4.1)

- Do I need a natural gas connected to a biosafety cabinet?
  Alternatives to Continuous Flame Burners (section 4.1)

Section 3.1 - RISK ASSESSMENT

Based on the information ascertained during the risk assessment, a Biosafety Level (BSL) can be assigned to the planned work, appropriate personal protective equipment selected, and standard operating procedures (SOPs) incorporating other safety interventions developed to ensure the safest possible conduct of the work.

Conduct a risk assessment to determine the proper work practices and containment requirements for work with biohazardous material. Risk assessments should identify microorganisms, their NIH established Risk Groups (RG) (see Table 3.1), and host/environmental factors that influence potential exposure risks for workers. The following points can be used as a guide, but a Biosafety Officer should be consulted to ensure full compliance with established guidelines and current regulations.

1. Identifying the infectious agent or material
2. Identifying laboratory procedures.
3. Identifying Laboratory Acquired Infections (LAIs)
4. Identifying laboratory hazards
5. Identifying potential routes of exposure resulting from laboratory manipulations of material and equipment, such as sonication, aerosolization, centrifugation, etc.
6. Understanding the stability of the agent in and out of its environment
7. Identifying the concentration of the agent and understanding its infectious dose
8. Being aware of potentially zoonotic diseases associate with agents being worked with
9. Identifying the host of an agent or cell culture
10. Understanding the outcome of genetic manipulation of an organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens, making it more susceptible to spread and infect
11. Assessing the experience and skill level of all personnel, including those potentially at higher risk, and providing safety training and hands-on experience to execute projects safely

IMPORTANT CONSIDERATIONS WHEN PERFORMING A RISK ASSESSMENT?

Identifying the agent or infectious material

Directors and principal investigators of microbiological and biomedical laboratories have the important responsibility to perform a risk assessment of their laboratories to ensure their staff have knowledge of the hazards being worked with in their laboratory. Such a risk assessment will help to develop best practices and procedures to handle the biological hazard and use containment equipment to prevent Laboratory Acquired Infections (LAIs).

Each microbiological agent is measured by its hazardous characteristics and put into a classification or Risk Group described by the World Health Organization (WHO) and NIH in the *NIH Guidelines*. The criterion in which each agent is placed depends on the following:

- Capability to infect and cause disease in a susceptible human or animal host.
- Its virulence as measured by the severity of disease.
- Availability of preventative measures and effective treatment of the disease.
- Probable routes of transmission of laboratory infection.

- Capability of transmitting disease through respiratory exposure.
- Infectious dose
- Stability of agent in the environment.
- Host range.
- Endemic nature of the agent.
- The use of laboratory animals.
- The origin of the agent. Non-indigenous agents are of special concern because of the potential to introduce risk of transmission or spread of human and animal or introduce infectious diseases from foreign countries to the United States.
- Genetically modified agents involve the same consideration for risk assessment as for wild- type organisms.

Laboratory procedures and Laboratory Acquired Infections (LAI)

Working with biological agents may result in exposure and infection, considered a laboratory acquired infection (LAI). The principal routes of laboratory transmission must be identified before work may commence.

The most common routes of transmission in the laboratory are:

1. Direct skin, eye or mucosal membrane exposure such as spills or splashes of contaminated materials directly onto the skin and/or mucous membranes.
2. Parenteral inoculation by a contaminated sharp object such as: syringe needles, broken glass, scalpels, razor blades, etc.
3. Bites and scratches from infected animals and arthropod vectors.
4. Inhalation of infectious aerosols.

Annual data on LAIs supports these common routes of transmission and shows that prevention of LAIs depends on the conscientious and proficient use of standard microbiological practices and procedure (see Section 4.2: Lab Equipment and Materials) and the correct use of laboratory equipment.

LABORATORY HAZARDS

Biohazardous Agents

- Human Pathogens
- Plant & Animal Pathogens
- Biological Toxins
- Select Agents
- Prions
- Bloodborne Pathogens
- rsNA
- Animals
- Cell lines

Equipment Hazards (see Table 3-2 for description and examples)

- Aerosol generating
- Cryogenic temperatures
- High temperatures

- High pressure
- Oxygen deficiencies
- Rotational energies
- Sharps
- Ultraviolet (UV) C radiation

Table 3-1: Classification of Infectious Microorganisms by Risk Group

Risk Group	**NIH Guidelines for Research involving Recombinant DNA Molecules**	**Examples**
Risk Group 1	Agents not associated with disease in healthy adult humans.	Adeno-associated virus (AAV), asporagenic Bacillus subtilis, Escherichia coli K-12 Host Vector Systems
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.	Staphylococcus aureus, Cryptococcus neoformans, Toxoplasma, Hepatitis B virus, SARS-CoV-2
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	Mycobacterium tuberculosis, West Nile virus, Yersinia pestis
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	Lassa virus, Herpesviruses simiae, Ebola virus

Source: adapted from the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules – updated April 2024

Table 3-2: Equipment Hazards

Equipment Type	Hazards	Examples
aerosol generating	The diameter of aerosols generated from certain types of equipment will vary from 0.1to 100 microns. • Bacterial cells and spores are 0.3 to 10 microns in diameter. • Viruses are 0.02 to 0.3 micron in diameter. • Biological particles generated from liquid orpowder form particles that are 0.5 micron diameter.	• blender: 2 micron diameter particles • sonicator: 4.8 micron diameter particles • dropping bacterial flask: 3.5 microndiameter particles • dropping lyophilized culture: 10 microndiameter particles • pipette blow out: 4.9 micron diameterparticles • vortex culture: 4.8 micron diameterparticles • centrifuge: 4 micron diameter particles
cryogenic temperature	Cryogenic temperatures of -80°C are used to remove moisture frommaterials and contain low-temperature refrigerants. If protective equipment is not used, exposure to low temperature may cause cryogenic burns and frostbite.	• -80°C freezers • lyophilizers (freeze dryers) • use of dry ice in shipping and receiving
high temperature	The use of heat to decontaminate or sterilize materials is widely used in the biological research laboratory. Physical injury from burns may occur from sudden accidentalreleases of heat sources or from thehandling of hot items.	• dry heat sterilization temperatures rangefrom 80°C to 200°C • autoclaves utilized wet heat to sterilize materials and can range between 80°C to 500°C • saturated steam operates at 121°C
high pressure	Compressed gas cylinders and pressurized equipment are commonly used in the laboratory. Injury may occur from rupturehigh-pressure lines.	• autoclaves operate at high pressures of 1,000 kilo Pascal (145 psig)
oxygen deficiencies	Oxygen deficiency environment may result from the displacement of oxygen by expanding gases (i.e., 700 parts of air to 1 part liquid nitrogen) or the linear displacement of oxygen from carbon dioxide(gas)	• liquid nitrogen or liquid carbon dioxidecompressed gas cylinders or tanks • dry ice
rotational energies	Sudden release of such rotational energies can cause serious physical injury from unbalanced equipment or flying shrapnel.	Tabletop and floor-mounted low, high, and ultracentrifuges rotate at speeds ranging from less than 5,000 to more than 100,000 rpm with rotor masses up to several kilograms.
sharp objects	Any material having the potential to puncture. Such material used in the manipulation of infectious material carries a higher risk.	• needles with or without syringes, withvacutainers, or attached tubing • blades (razors, scalpels, X-ACTO knives) • broken glass • serological pipettes and pipette tips
ultraviolet radiation (UV)	UV radiation is used for inactivating microorganisms. Its usefulness, however, islimited by a variety of factors (wavelength, distance, exposure). The eyes and skin can be damaged by exposure to UV radiation.	UV lights in Biosafety cabinets and transilluminators.

Section 3.2 – BIOSAFETY LEVELS

WHAT ARE BIOLOGICAL SAFETY (BIOSAFETY) LEVELS (BSL)?

Biosafety Levels are the degrees of physical containment, safe practices, and personal protective equipment, through which microbiological agents can be manipulated allowing the most protection to the worker, occupants in the building, public health, and the environment. Each level of containment describes the microbiological practices, safety equipment, and facility design for the corresponding level of risk associated with handling a particular agent. There are four laboratory biosafety levels (Table 3-3).

Facility Design

The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory.

Primary Barriers: To protect those working in the laboratory, safety equipment such as biosafety cabinets and sealed centrifuge rotors are controls designated to remove or minimize exposures to hazardous biological.

Secondary Barriers: A secondary barrier will depend on the risk of transmission of the specific agents being used. For a laboratory that may be working with risk group 2 agents and require a biosafety level 2 containment, the barrier would be the separation of the laboratory from public areas. For other risks such as aerosols, the secondary barriers would need to prevent infectious materials from escaping into the environment. Designs that include specialized ventilation systems to ensure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances or separate buildings or modules to isolate the laboratory are all examples of secondary barriers or containment.

Practices and Procedures

Persons working with infectious agents or potentially infectious materials must be aware of the potential hazards. They must be trained and be proficient in the practices and techniques required for handling hazards safely. An important element of containment is strict adherence to standard microbiological practices and techniques.

Personal Protective Equipment (PPE)

PPE is the last line of containment for individuals working in a containment laboratory and may be the primary barrier (as when wearing gloves in a BSC) between a worker and an infectious agent. The type of PPE appropriate for a specific agent is to be determined by a risk assessment based on the agent and type of work being performed

Biosafety Level I (BSL-1)

Practices and Procedures

Persons working with infectious agents or potentially infectious materials must be aware of the potential hazards. They must be trained and be proficient in the practices and techniques required

for handling hazards safely. An important element of containment is strict adherence to standard microbiological practices and techniques.

Personal Protective Equipment (PPE)

PPE is the last line of containment for individuals working in a containment laboratory and may be the primary barrier (as when wearing gloves in a BSC) between a worker and an infectious agent. The type of PPE appropriate for a specific agent is to be determined by a risk assessment based on the agent and type of work being performed.

Laboratory Design

- A sink is dedicated to handwashing.
- Doors can be locked for access control.
- Laboratory must be designed so that it can easily be cleaned (no carpets or rugs).
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned.
- Laboratory windows that open to the exterior must be fitted with screens

Signage

- If necessary, proper signage must be posted at the entrance to the laboratory to warn of any non-biological hazards present in the laboratory. The name and phone number of the laboratory supervisor and other responsible personnel must be listed.
- Room Signage is requested either through EHRS or, for the Perelman School of Medicine, through Space Planning Operations (SPO)

Training

Principal Investigator must ensure that ALL laboratory personnel, including students, receive:

- Required university training
- Appropriate procedure specific safety training
- Updates and additional training when procedures have changed.

Principal Investigator must enforce the University of Pennsylvania's policies to only allow authorized personnel access to the laboratory.

Laboratory Procedures

- Don appropriate personal protective equipment (PPE) prior to working in the lab; lab coat, gloves and eye protection.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Do not wear gloves out of the laboratory space to prevent possible contamination to high touch surfaces

- Eating, drinking, smoking, handling contact lenses, applying facial cosmetics, and storing food for human consumption is not permitted in laboratory areas.
- Food must be stored outside the laboratory area in cabinets or refrigerators designated and used only for this purpose. Research materials that may be considered food (i.e. apple juice, sunflower seeds) must be labeled "Not for human consumption".
- Always perform procedures in a controlled manner to minimize the creation of splashes.
- Mouth pipetting is prohibited; mechanical devices must be used.

Personal Protective Equipment (PPE)

- Cotton (non-flammable) laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Protective eyewear should be worn routinely and must be worn when conducting procedures that have the potential for creating splashes of microorganisms or other hazardous materials.
- Gloves must be worn to protect hands from exposure to hazardous materials.
- Gloves should be selected to provide the best protective characteristics based upon an appropriate risk assessment of the biological, chemical, and physical hazards that can be encountered.
- Change gloves when contaminated, when glove integrity is suspected or known compromised, or when necessary for other reasons.
- Remove gloves and wash hands after work and before leaving the laboratory.
- Do not wash or reuse disposable gloves.
- Do not take lab coats home to launder. Lab coats must be laundered using a service provided by an EHRS approved vendor.

Equipment

- Special containment devices or equipment, such as biosafety cabinets, are not generally required.

Waste

Disposal of sharps such as needles, scalpels, pipettes, and broken glassware must be done in accordance with the University of Pennsylvania's waste disposal regulations

Biosafety Level II (BSL-2)

BSL-2 builds upon BSL-1 containment described above. BSL-2 is appropriate for work using a broad- spectrum of biological agents and toxins that are associated with causing disease in humans of varying severity and for which preventive or therapeutic interventions are *often* available. With good practices and procedures, these agents and toxins can generally be handled safely on an open bench, provided the potential for producing splashes and aerosols is low.

Laboratory Design

- Restricted access to authorized personnel only.
- Separate laboratory work area from public spaces and eating areas

- Inward, single pass airflow is required forBSL-2 laboratories at Penn

Signage

- The international biohazard warning symbol must be displayed on the room signs of laboratories where microorganisms of Risk Group 2 or higher risk groups are handled.
- All equipment (centrifuges, water baths, cryogenic freezers, incubators, etc.) that comes in contact with biohazardous materials must be labeled with the universal biohazard symbol.
- All areas and laboratories that contain biohazardous or toxic agents must be posted with signs stating, "EATING, DRINKING, SMOKING, AND APPLYING COSMETICS ARE PROHIBITED IN THIS AREA."
- Room Signage is requested either through EHRS or, for the Perelman School of Medicine, through Space Planning Operations (SPO)
- All personnel must complete specific training in handling pathogenic agents and must be supervised by scientists competent in handling infectious agents and associated procedures.

Laboratory Procedures

- All procedures in which infectious aerosols or splashes may be created are conducted in a biosafety cabinet (BSC) or other physical containment equipment.

Work surfaces are decontaminated after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectants

Personal Protective Equipment (PPE)

- May include, but are not limited to, job appropriate gloves, cotton lab coats, face shields, safety glasses or goggles. Additional PPE, such as gowns, shoe covers, boots, may be required depending on the specific task.
- PPE is used in combination with BSCs, Down Draft Tables, and other devices that contain biohazardous agents, animals, allergens, and/or other materials, to provide protection for workers reaching into the device and in case there is a splash/splatter that exits the device. If it is impractical to work in BSCs or with other engineering controls such as Down Draft Tables, a risk assessment should be conducted to identify the appropriate PPE to protect personnel from infectious and potentially infectious materials. Examples may include certain animal studies, animal necropsies, agent production activities, and activities relating to maintenance, service or support of the laboratory facility.

Training

- certain animal studies, animal necropsy, agent production activities and activities relating to maintenance, service or support of the laboratory facility.

Equipment

- Biosafety Cabinets are required for all work involving the manipulation of Risk Group 2 or higher agents, which may create aerosols or splashes.
- Eyewash must be readily available to lab personnel within a 10 sec walk from their location of work.
- Eyewashes must be activated weekly to ensure they are functioning properly.

Safety devices must be used to contain potential aerosols created during processing of known or potentially infectious material. Safety Centrifuge Cups are enclosed containers designed to prevent aerosols from being released during centrifugation. Safety Blenders will contain aerosols during homogenization of know or potentially infectious material. These containers should only be opened and manipulated in a Biosafety Cabinet

Waste

- Disposal of sharps such as needles, scalpels, pipettes, and broken glassware must be done in accordance with state waste disposal policies and state regulations.
- Decontaminate all cultures, stocks, and other potentially infectious materials prior to disposal using an appropriate decontamination or disinfection method.
- Solid waste that is potentially infectious must be disposed of appropriately through the infectious waste stream. See the Biohazardous Waste Disposal Guides on the EHRS website for additional guidance.

For assistance in the selection of a Biosafety Cabinet or other lab safety equipment, contact a Biosafety Officer by phone (215-898- 4453) or email: BIOSAFETY@LISTS.UPENN.EDU

Biosafety Level III (BSL-3)

BSL-3 is suitable for work with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Primary hazards to personnel working with these agents (e.g., *Mycobacterium tuberculosis, Yersinia pestis*, and Yellow Fever virus) include percutaneous inoculation, ingestion, and exposure to infectious aerosols. Incubation times, infectious periods, and environmental hardiness can vary from agent to agent and there is a possibility of unknowingly spreading these high consequence agents to others. Greater emphasis is placed on primary and secondary barriers as well as strict adherence to protocols to protect the investigator, other researchers and facility occupants, the community, and the environment from exposure to infectious aerosols and reducing the risk of disease transmission to others.

See the BSL-3 Manual for more information & consult with a Biosafety Officer if you are planning to pursue work with agents that require this level of containment.

Laboratory Design

- In addition to design elements at BSL-2, the laboratory has enhanced entry requirements.
- Walls, windows, and ceiling are sealed and easily decontaminated.
- All penetrations are sealed or are sealable to allow for space decontamination.
- Secondary barriers include controlled access to the laboratory and a specialized ventilation system that ensures containment of infectious aerosols from the laboratory.
- Directional air-flow:

  All lab spaces are under negative pressure to the common hallway with decreasing negative pressures separated by each doorway leading into the laboratory to ensure inward airflow.

  No air passing through the lab is recirculated. Penn policy dictates that all air is passed through a HEPA filter before being released into the environment.

- Doors remain locked and are self-closing.

- Hands-free handwashing sinks are provided throughout the labs.
- Floors are slip resistant and impervious to liquids.
- Walls, windows, and ceiling are sealed and easily decontaminated.
- Only one person may enter the facility at a time via their Penn ID and iris scan.

Signage

- The outside door of the entrance anteroom will have a sign posted with the following information:
  - Universal Biohazard Symbol
  - BSL-3
  - Names of responsible personnel and numbers
- Additional signage is posted inside the laboratory. See the BSL-3 Manual for additional information.

Training

- Laboratory personnel receive specific training in handling pathogenic and potentially lethal agents and are supervised by scientists competent in handling infectious agents and associated procedures.
- Work with agents or materials at BSL-3 requires registration, approvals, and training beyond that required for other basic research at Penn.
- The Institutional Biosafety Committee is responsible for approving all BSL-3 research proposals and for granting access to users based upon prior proficiency and training.
- See the BSL-3 Manual for more information.

Laboratory Procedures

- Only authorized personnel are allowed to enter these laboratories.
- Only one person may enter the facility at a time under their own Penn ID and iris scan.
- PPE is removed in a manner in which potentially exposed surfaces do not come in contact with unexposed surfaces.
- All non-authorized personnel that need access to the facility (e.g., maintenance) must be escorted by designated personnel (e.g., biosafety officer, senior lab personnel) and supervised at all times.
- See the BSL-3 Manual for more information.

Personal Protective Equipment (PPE)

- Disposable gloves, gowns, and booties are worn.
- Additional PPE may be required depending on agent or procedure, including, but is not limited to, 2 pairs of gloves, gown, shoe covers, safety glasses or goggles. Additional PPE such as face shields and respirators may be required depending on the specific task.

Equipment

- Containment devices or equipment, such as sealed centrifuge rotors or biosafety cabinets, are used for work that has the potential to form aerosols or splashes.

Waste

- All waste is autoclaved out of the facility.

Biosafety Level IV (BSL-4)

BSL-4 is applicable for work with dangerous and exotic agents that pose a high individual risk of life- threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy.

There are **NO** Biosafety Level 4 laboratories permitted to operate at Penn.
Agents requiring this level of containment may **NOT** to be brought to Penn's campus

Table 3-3: SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FORINFECTIOUS AGENTS

BSL	AGENTS	PRACTICES	PRIMARY BARRIERSAND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	*Laboratories; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities*
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	*Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available*
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory
4	Dangerous and exotic agents that pose high risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal. **(NOT ALLOWED AT PENN)**	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; gloves; full-body, air-supplied, positive-pressure suit	Entry sequence; entry through airlock with airtight doors; walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door, pass-through autoclave required

adapted from *Biosafety in Microbiological and Biomedical Laboratories*; BMBL 6th Edition, 2020

Section 3.3 – ANIMAL BIOSAFETY LEVELS

WHAT ARE ANIMAL BIOLOGICAL SAFETY (BIOSAFETY) LEVELS(ABSL)?

Animal Biosafety Levels are comparable to containment recommendations for Biosafety Levels, except with enhancements for animal control. Laboratory animal facilities, operational practices and quality of animal care must meet applicable standards and regulations (e.g. Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations). There are four animal biosafety levels (Table 3-4).

Facility Design

The design of a facility is important in providing a barrier to protect people working inside and outside the animal facility, and to protect people or animals in the community from infectious agents or zoonotic disease which may be accidentally released from the facility.

Practices and Procedures

Persons working with animals that may be inoculated with infectious or non-infectious agents must be aware of the potential hazards. They must be trained and be proficient in the practices and techniques required for handling live animals.

Animal protocols must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to beginning work.

Animal Biosafety Levels (ABSL) are ONLY to protect the worker. Quarantine and isolation labs should be used to protect the research animal from animal-to-animal spread of diseases.

All animal work must follow University Laboratory Animal Resources (ULAR) and IACUC policies for working with animals.

Animal Biosafety Level I (ABSL-1)

ABSL-1 is appropriate for work done with uninfected animals, with well characterized strains of viable microorganisms not known to cause disease in healthy adult humans, or microorganisms not known to spread as zoonotic diseases. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

Laboratory Design

- Restricted access to authorized personnel only.

Signage

- Proper signage must be posted at the entrance to the facility to warn of hazards present.
- Signage is posted by ULAR.

Training

ULAR requires species specific animal handling training.

Laboratory Procedures

- Don appropriate personal protective equipment (PPE) prior to entering animal facility.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the facility.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in the animal facility.

Personal Protective Equipment (PPE)

- Disposable gowns are recommended to prevent contamination of personal clothing
- Face mask may be recommended to minimize exposure to animal allergens.
- Protective eyewear should be worn routinely and must be worn when conducting procedures that have the potential for creating splashes of microorganisms or other hazardous materials while handling animals.
- Persons that wear contact lenses should consider wearing safety glasses as protection.
- Gloves must be worn to protect hands from exposure to hazardous materials.
- Gloves should be selected based upon an appropriate risk assessment.
- Change gloves when contaminated, when glove integrity is suspected or known compromised or when necessary for other reasons.
- Remove gloves and wash hands after work and before leaving the laboratory.
- Do not wash or reuse disposable gloves.

Equipment

- Special containment devices or equipment, such as biosafety cabinets, are not generally required.

Waste

- Disposal of sharps such as needles, scalpels, pipettes, and broken glassware must be done in accordance with the University of Pennsylvania's waste disposal policies and state regulations.
- All animal carcasses must be returned to ULAR for disposal.

Animal Biosafety Level II (ABSL-2)

ABSL-2 builds upon ABSL-1 containment described above. Animal Biosafety Level 2 (ABSL-2) refers to the practices and procedures required to work with animals infected with agents associated with human disease. These infectious agents are typically moderately hazardous and can be contracted by direct exposure through ingestion, percutaneous injury, and mucous membrane exposure.

See the "Penn Policy for working in Animal Biosafety Level 2 Containment" in Appendix D.

Laboratory Design

- Restricted access to authorized personnel only.
  Directional, single pass airflow is required.

Signage

- A room sign that incorporates the universal biohazard symbol must be posted on the door of every ABSL-2 room that includes the following information:
- The universal biohazard symbol.
- Animal Biosafety Level 2 (ABSL-2).
- Personal Protective Equipment (PPE) required to enter the space
- Principal Investigator(s) (PIs) responsible for the project.
- Laboratory contact person(s) and emergency contact number(s).
- Infectious agent(s) used in the room (listed on a second sign and posted on the same door).
- To request ABSL-2 signs, please contact your ULAR manager.

Figure 3.1 ABSL-2 Room Signage

Training

- Access to ABSL-2 rooms is limited to researchers and support staff who have been adequately trained.
- **ULAR Training**: All ULAR training requirements must be completed before access to ABSL-2 labs is granted. Please visit ULAR Training Services on the ULAR website for more information about required training.
- **EHRS Training**: All research personnel must be current with their laboratory safety training. Additional EHRS training, including Bloodborne Pathogens and rsNA training may be required depending on the material listed in the IACUC protocol.

HAZARD BRIEFINGS

It is the **PI's responsibility** to inform ULAR staff of the hazards and any special procedures required for work with animals housed at ABSL-2.

PIs are also responsible for the hands-on training of their research staff regarding the hazards of working with specific infectious.

Contact ULAR Management to schedule a **HAZARD BRIEFING** prior to working with Risk Group 2 organisms or human material

Personal Protective Equipment (PPE)

Before entering any ABSL-2 room, the following PPE must be worn:

- 2 pairs of foot covers
- 2 pairs of gloves
- 1 surgical gown securely tied in the back
- 1 face mask covering both the mouth & nose
- Eye protection if there are procedures generating a splash risk

After all work is complete the following steps must be taken before exiting the individual AGSL-2 suite:

- remove the outer shoe covers and the outer pair of gloves
- Step through the door to the hallway
- Re-gown and put on a new pair of gloves before entering another animal room.
- When preparing to exit the facility, remove all PPE except for the initial pair of shoe covers, which should be worn to exit the facility and then removed and thrown out.
- Wash hands with soap and waste OR with an alcohol-based hand sanitizer prior to leaving the facility

Equipment

Animal Cages: All rodents housed in ABSL-2 rooms must be housed in filtertop cages. These cages may only be opened inside a functioning biosafety cabinet. These rodents must remain in ABSL-2 designated containment at all times, including transport. Larger animals may be housed in regular cages, however additional PPE may be required to work in the room.

Biosafety Cabinets: Biological Safety Cabinets must be used for all manipulations of small animals housed in ABSL-2 rooms, including, but not limited to:

- Opening rodent cages Changing rodent cages
- Transferring rodents to new cages
- Injecting small animals with infectious agents

Any other procedure that may generate infectious aerosols

Rodent Cage Changes Procedures

- Rodent cages must be changed inside a certified BSC using the following procedure:
- Perform cage changes and other animal procedures in a biosafety cabinet, following proper sanitary procedures.
- Disposal of all waste from ABSL-2 rooms through the infectious waste stream.
- All rodents housed at ABSL-2 must be housed in filtertop rodent cages at all times. Cages must only be opened within a biosafety cabinet. Empty cages must be autoclaved prior to dumping the content.
- See the "Penn Policy for working in Animal Biosafety Level 2 Containment" in Appendix D.

Transport

- All rodents transported outside of the ABSL-2 room or the animal facility must be transported in filter-top rodent cages. If cages are changed in a lab outside of the animal facility, the same cage change procedures outlined above must be followed. Empty cages must be returned to the facility of origin.

Waste

- All waste generated in the manipulation of animals housed in ABSL-2 rooms is considered biohazardous.
- All animal carcasses removed from the ABSL-2 rooms must be returned to ULAR for disposal.
- Autoclaves within the ULAR facility must be monitored by ULAR for proper function by using biological indicators
- All infectious waste removed from the ABSL-2 rooms must be either disposed of directly into a hard-sided infectious waste tote or autoclaved and subsequently disposed of through the infectious waste stream.

Emergency Procedures

Know what to do and where to go after a potential exposure or injury with infectious material:

a. Irrigate exposed mucous membrane with running water for 15 minutes.
b. Wash out wounds with soap and water for 15 minutes.
c. Report exposure or injury to your supervisor and immediately seek medical attention: Penn Occupational Medicine or Penn Student Health during normal business hours, or HUP-Pavilion ER or Penn Presbyterian Hospital ER after hours.

Animal Biosafety Level III (ABSL-3)

ABSL-3 is applicable to work done in animals infected with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection.

See the **ABSL-3 Manual** for more information & consult with a Biosafety Officer if you are planning to pursue work with agents that require this level of containment.

Laboratory Design

- Design is similar to BSL-3 containment.

Signage

- Restricted access
- PPE requirements

Training

- Training is similar to BSL-3 training requirements.

Laboratory Procedures

- See the ***ABSL-3 Manual*** for more information.

Personal Protective Equipment (PPE)

- Disposable gloves, gowns, and booties are worn.
- Additional PPE may be required depending on agent or procedure, including, but is not limited to, 2 pairs of gloves, gown, shoe covers, safety glasses or goggles. Additional PPE such as face shields and respirators may be required depending on the specific task.

Equipment

- All work is performed in containment devices or equipment, such as sealed centrifuge rotors or biosafety cabinets.

Waste

Autoclaves must be used to decontaminate all waste created in the ABSL-3 facility.

Table 3-4: SUMMARY OF RECOMMENDED ANIMAL BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

ABSL	AGENTS	PRACTICES	PRIMARY BARRIERSAND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices.	Only what is required for normal care of each species.	Standard animal facility: • no recirculation of exhaust air • directional air flow • designated handwashing sink
2	Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 practice plus: • Limited access • Biohazard warningsigns • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies • Decontamination of animal cages prior to washing	Primary barriers: • Class I or II BSCs or other physical containmentdevices • containment appropriate for animal species PPE: Laboratory coats; gloves; face, and respiratory protection as needed	ABSL-1 plus: • Autoclave available • Mechanical cage washer recommended
3	Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequence	ABSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containmentdevices • containment for housing animals and cage dumping activities PPE: appropriate respiratory protection	ABSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetration and windows • Autoclave
4	Dangerous/exotic agents which pose high risk of life-threatening disease (**NOT ALLOWED TOBE USED AT PENN)**	ABSL-3 practices plus: • Clothing: change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	ABSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems

Source: adapted from the BMBL 6th Ed., 2020

Animal Biosafety Level IV (ABSL-4)

ABSL-4 is applicable for work with animals infected with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy.

There are NO Animal Biosafety Level 4 laboratories permitted to operate at Penn.

Agents requiring this level of containment may **NOT** be brought to Penn's campus.

Section 3.4 – PLANT BIOSAFETY LEVELS

What are Plant Biological Safety Levels (BL1-P – BL4-P)?

Plant biosafety levels provide a description of a combination of administrative controls, work practices and procedures, equipment, and facility features required to achieve a designated level of containment. The purpose of containment is to prevent the transfer of propagules and other organisms from inside the laboratory, greenhouse, or growth chamber to receptive environments outside these facilities. Section III of the *NIH Guidelines* describes four physical containment levels for experiments involving recombinant DNA molecules. An appendix of the *NIH Guidelines* was created to categorize experiments for recombinant DNA research involving plants according to specific risk criteria. Experiments may be assigned to one of four plant biosafety levels, BL1-P through BL4-P, using the criteria in the *NIH Guidelines.* Plant biosafety levels differ from laboratory biosafety levels (BSL-1 through BSL-4) in that environmental protection is the primary concern when setting plant biosafety levels whereas human safety is the primary concern when setting laboratory biosafety levels. Only BL1-P and BL2-P may occur at Penn. Any generation and/or use of transgenic plants (even those readily purchased from a vendor) must be reviewed by the Institutional Biosafety Committee (IBC) through the submission of an IBC registration. Experiments involving the generation and/or use of transgenic plants must meet the guidance outlined in the *NIH Guidelines.*

Biosafety Level 1 for Plants (BL1-P)

The BL1-P designation is used to provide a low level of containment for experiments involving transgenic plants in which there is no evidence that the modified organism would be able to survive and spread in the environment and, if accidentally released, would not pose an environmental risk. Examples of research that may be done at BL1-P include the use of sterile or non-propagative plants. It also includes transgenic microorganisms that cannot spread rapidly and are not known to have any negative effects on natural or managed ecosystems.

Biosafety Level 2 for Plants (BL2-P)

The BL2-P designation is used to contain experiments with transgenic plants and associated organisms, which, if released outside the greenhouse or laboratory, could be viable in the surrounding environment but would have a negligible environmental impact or could be readily managed. Examples of research requiring BL2-P containment include the use or creation of transgenic plants that may exhibit new weedy characteristics that may be capable of interbreeding with plants growing in the vicinity. BL2-P containment is also required when the research involves the entire genome of an indigenous infectious agent, pathogen, plant associate insect or other animal where release into the surrounding environment could be potentially harmful but manageable.

Biosafety Level 3 for Plants (BL3-P)

The BL3-P containment is designed to prevent the accidental release of transgenic or wild-type plants, plant pathogens, or other plant associated organisms that have a recognized potential for significant detrimental impact on the surrounding environment. In some cases, the transgenic plant may pose no threat, but the plant associated organisms would require containment. Experiments using transgenic plants or plant associated organisms that contain genes coding for vertebrate toxins must also be conducted at BL3-P.

Biosafety Level 4 for Plants (BL4-P)

The BL4-P designation is used to contain experiments involving certain exotic, readily transmissible infectious agents that are potentially serious pathogens of major US crops. These could include experiments involving USDA Plant Protection and Quarantine (PPQ) Select Agents and their insect vectors. Again, this work is not permitted at Penn.

Practices and Procedures

Facility Design

Facility design should incorporate best practices to ensure that pests are limited access to the laboratory, greenhouse, or

growth chambers. Pests can damage the plants located inside and can be difficult to remove once established. To limit the use of harmful pesticides, disrupting other experiments, and causing harm to the environment due diligence in ensuring that pests are not allowed into or out of growing areas is paramount as the pests can be mechanical vectors that can carry pathogens, pollen, and seeds from one area to another.

Facility design should also incorporate measures to capture viable reproductive plant material and plant pathogens to prevent any unauthorized releases to environment, especially if the plants, plant derived materials, and plant pathogens are transgenic and/or have been genetically modified.

Laboratory Design

Signage

Signage must be posted with the name of any transgenic plants and plant pathogens that are contained within the laboratory, greenhouse, and/or growth chamber.

Signage must be posted when plants are being transduced by recombinant plant pathogens and when transgenic plants are being brought to flower and seed. This is to bring awareness to personnel that this is a sensitive part of the plants life cycle where reproductive materials can be inadvertently carried out of the greenhouse or growth chamber containment which constitutes as an unauthorized release by US agricultural regulatory bodies.

Training

Lab specific training is required to handle transgenic plants. Lab staff must also be listed on the corresponding IBC registration and have completed *Recombinant or Synthetic Nucleic Acid Guidelines* training.

Laboratory Procedures

- Access to the laboratory, greenhouse, and growth chamber is restricted to authorized personnel.
- Records must be kept of all experimental plants and microorganisms brought into or out of the laboratory, greenhouse, and/or growth chamber.

Transport

Transport of transgenic plants and their associated materials must be carried in a sealed, lockable, unbreakable container. Shipping transgenic plants must meet Department of Transportation (DOT) packaging requirements.
The interstate, intrastate, and international shipping of plants should always be reviewed with the Biosafety Office and referenced with the USDA APHIS Regulated Materials List, Pending Review List, and USDA BRS for proper containment during transport and permitting requirements into other states or the USA. See the Transport & Shipping of Biohazards Section for more details.

Waste

All transgenic plant material including soil used to grow transgenic plants must be inactivated before disposal as per the *NIH Guidelines*. The use of steam devitalization and verifying/validating inactivation satisfies these requirements. All plant material and soil must be autoclaved prior to disposal in the municipal waste stream.

All transgenic plant material that has been exposed to recombinant plant pathogens including soil used to grow plants that harbored the recombinant plant pathogens must be autoclaved per the *NIH Guidelines*.

Integrated Pest Management

Care should be taken on how to manage pests and infestations in the laboratory, greenhouse, and growth chambers. Pesticides can be harmful to human and animal health when not applied properly. A risk assessment is made prior to the use of biological pesticides (organisms released into an environment to control pest populations). These organisms such as nematodes, wasps, and mites are motile and can act as mechanical vectors that can compromise containment via unauthorized release of pathogens or genetic material.

Section 4.1 – BIOSAFETY CABINETS

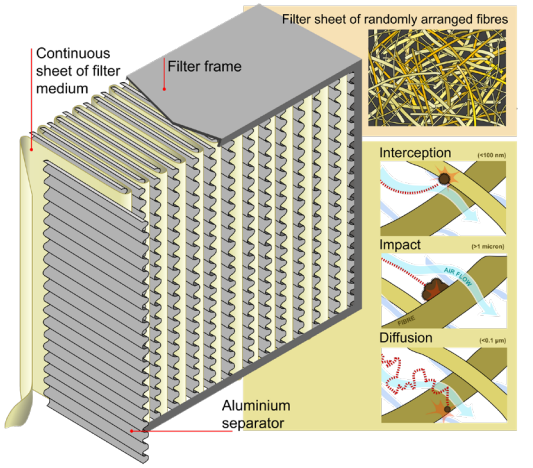
Biosafety Cabinets (BSCs) are the primary means of aerosol containment for working safely with infectious materials and microorganisms. They are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed. Other engineering controls offer different levels of protection. See Table 4-1 for the different protection of various engineering controls.

Please see Appendix A for detailed diagrams on air flow inside biosafety cabinets

Biosafety Cabinet Design

HEPA Filter

BSCs are equipped with High Efficiency Particulate Air (HEPA) filters in their exhaust and/or supply systems (Figure 4-1). These filters have a minimum efficiency of 99.99% removal of particles at 0.3µm, BUT particles both larger and smaller are removed with even greater efficiency.



Types of BSCs

There are several different types of BSCs that can be used for different purposes. See Appendix A for details on all the different types of cabinets and their air flow. See Table 4-2 for different protection levels of biosafety cabinets. The main categories of cabinets are as follows:

- Class I cabinets exhaust HEPA filtered air back into the room or exhaust it from the room. No HEPA filtered air flows down onto the work surface of the cabinet.
- Class II Type A1 & A2 cabinets exhaust 30% of HEPA filtered air back into the room, while the remaining 70% of HEPA filtered air flows down onto the cabinet work surface. Significant quantities of volatile toxic chemicals must not be used in these types of cabinets. Because 70% of the air is recirculated, there is potential for volatile chemicals to accumulate in the BSC creating a toxic or explosive environment.

Figure 4.1 Hight Efficiency Particulate Air (HEPA) filter

- Class II Type B1 & B2 cabinets are hard ducted to the building exhaust system.
  - In type B1 cabinets, 70% of HEPA filtered air is exhausted through the building exhaust system, while the remaining 30% of HEPA filtered air flows down onto the cabinet work surface.
  - Type B2 cabinets exhaust 100% of HEPA filtered clean air through the building exhaust. A separate supply HEPA filter allows clean air to flow onto the work surface.

TABLE 4-1: Protection Provided by Different Types of Engineering Control Cabinets.

Biosafety Cabinet:	**Clean Bench:**	**Fume Hood:**
• Use for infectious agents. • Provides product, user, and environmental protection. • Use for volatile chemicals must be done under EHRS guidance. 	NOT A BIOSAFETY CABINET • Provides protection for the product only. • **DO NOT** use to handle infectious agents or chemicals. • Air blows directly at the user. 	• Use to handle chemicals. • Provides chemical inhalation protection for the user. • **DO NOT** use to handle infectious agents. 

TABLE 4-2: Protection Provided by Different Types of Biosafety Cabinets.

Shaded rows highlight BSCs that are widely used on Penn's campus. Contact a biosafety officer prior to purchase of a BSC to help select the proper type of cabinet for your specific needs.

Re-Circulating Biosafety Cabinets

	Personnel Protection	Product Protection	Environmental Protection	Chemical Protection
Class I	Yes	No	Yes	No
Class II, Type A1	Yes	Yes	Yes	No
Class II, Type A2	Yes	Yes	Yes	No (without canopy)

Ducted Biosafety Cabinets

	Personnel Protection	Product Protection	Environmental Protection	Chemical Protection
Class II, Type B1	Yes	Yes	Yes	Yes (small amounts in back1/2 of cabinet)
Class II, Type B2	Yes	Yes	Yes	Yes (small amounts)
Class III	Yes	Yes	Yes	Yes (small amounts)

Operation of a Biosafety Cabinet

Before Use:

1) Raise the front sash to 8 or 10 inches, as indicated on cabinet frame.
2) Turn on the BSC let it run for approximately 5 minutes before use with biologicals.
3) Wipe down the cabinet surfaces with an appropriate disinfectant. (see Section 7 for appropriate disinfectants)
4) Check the magnehelic gauge for variations of +/- scale divisions. Document divisions and report major variations to supervisor. Possible maintenance may be required.
5) Transfer necessary materials (pipettes, pipette tips, waste bags, etc.) into the cabinet.

During Use:

1) Work clean to dirty across the BSC
2) See figure 4-2 for setup where clean cultures (left) can be inoculated (center) and contaminated pipettes can be discarded in a collection container (right).

Collect other contaminated materials in a biohazard bag

After Use:

1) Leave the cabinet running for at least 5 minutes after use. The surface under the main work surface must be cleaned and decontaminated regularly.
2) Empty the cabinet of all research materials after use. The cabinet should never be used for storage. Material left in the cabinet does not remain sterile after the blower has been turned off.

Wipe down cabinet surfaces with an appropriate disinfectant. See Section 7 for information about disinfectants. Clean and decontaminate the plenum under the work surface regularly. Use a Swiffer style cleaning device, a dust pan, or a vacuum to clean the cabinet and various surfaces, never bare hands to avoid puncture wounds.

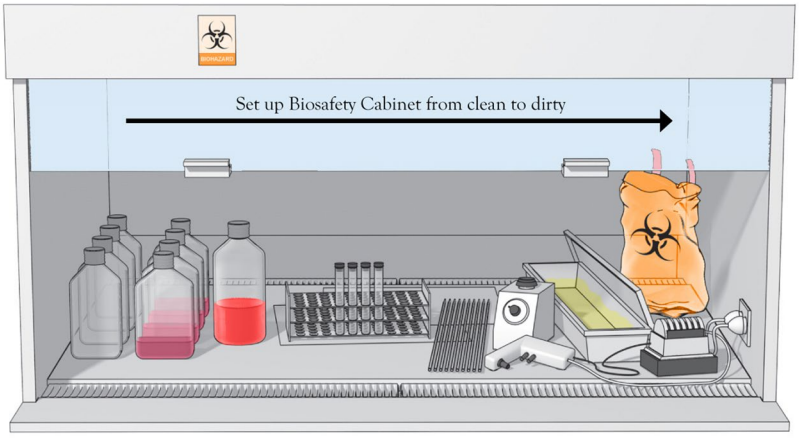


Figure 4-2: A typical layout for working "clean to dirty". Clean cultures (left) can be inoculated (center) and contaminated pipettes can be discarded in the shallow pan (right). Other contaminated materials can be discarded in the biohazard bag. (adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

All personnel working in biosafety cabinets must complete the web- based biosafety cabinet training module. PIs must provide hands- on biosafety cabinet training regarding laboratory specific procedures

Special Considerations

Vacuum System Protection (see Figure 4-3)

Aspirator bottles or suction flasks (A) should be connected to an overflow collection flask (B) containing an appropriate disinfectant. In addition, a hydrophobic filter (C) must be installed before the vacuum line (D). This combination will provide protection to the central building vacuum system as well as to personnel who service this equipment.

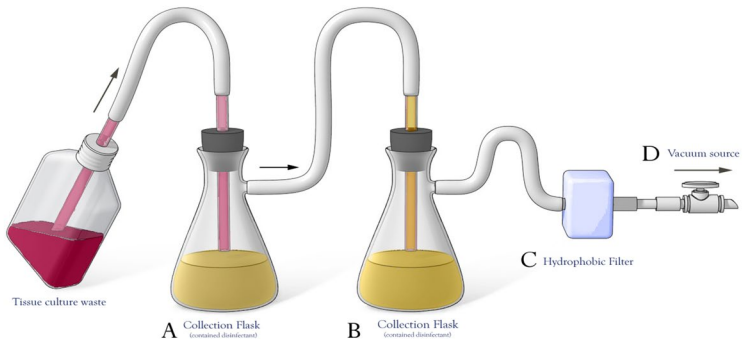


Figure 4-3: Vacuum System Protection (adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

Ultraviolet (UV) Lights

UV lights are not recommended to decontaminate the BSC. They are only effective if cleaned regularly AND checked periodically to ensure proper wavelength emission. If UV lights are installed, they MUST be turned off when the cabinet is in use.

Open Flames inside a BSC

DO NOT use continuous open flames (e.g. Bunsen Burners) inside a biosafety cabinet, as they are not needed in the near microbe-free environment of the BSC. Flames create temperature variations resulting in turbulence which disrupts the delicate air flow patterns inside the BSC compromising containment. The heat generated may damage the HEPA filters or cause a fire. If a flame is needed, consider alternative sterilizers shown in Figure 4-4.

Location of BSCs in Labs

The cabinet's air curtain is very delicate and is the only barrier between the inside air (potentially infectious aerosols) and the outside air. Therefore, air-flow turbulence from both inside and outside of the cabinet risks breach of containment. *Cabinets should be located away from doors, high traffic areas, and any building HVAC systems.* Contact your building administrator and EHRS to ensure proper installation and placement of a biosafety cabinet in your laboratory space.

Figure 4.4 Alternatives to use of flames inside a biosafety cabinet

Certification, Maintenance, and Repair

NSF/ANSI Standard 49:

This document outlines the certification standards a Class II biosafety cabinet must meet in order to work safely with risk group 1, 2, and 3 agents. It includes certification test specifications, decontamination procedures, construction criteria, etc. Penn uses this standard to certify Class II cabinets located on campus. BSCs must be tested and certified annually or if:

- A new cabinet is being installed
- A cabinet has been moved
- A cabinet is in need of repairs

BSC Service Provider

ALL certification, maintenance, and repairs must be conducted by **Technical Safety Services (TSS)**, the university's contracted service provider. Other service providers must be approved by a biosafety officer. **NEVER attempt your own repairs or modifications to any biosafety cabinet.** This may void your cabinet's warranty and poses a safety risk to lab personnel and the community.

Cabinet and HEPA Filter Decontamination

BSCs and their filters must be decontaminated:

- Before disposal
- Before certain repairs may be made to the unit

Technical Safety Services (TSS) uses chlorine dioxide gas (ClO_2) or vaporized hydrogen peroxide as the method of decontaminating biosafety cabinet filters, which is an NSF/ANSI Standard 49 approved decontamination method.

Consult the Biosafety Cabinets section of the EHRS website for current **Technical Safety Services (TSS) contact** information and how to prepare the Biosafety Cabinet for certification and/or repair.

Remember! BSCs will only protect you, your products, and the environment **if used properly**!

DO :	DO NOT:
• follow practices and procedures outlined in this section • complete the Biosafety Cabinet training available in WorkDay@Penn • call a Biosafety Officer at EHRS with any questions concerning BSC selection or use	• use if BSC is out of certification • cover front and back grills with any items • overcrowd cabinet to prevent proper air flow • put your head inside the cabinet • disrupt airflow with quick movements • make any repairs or modifications to the BSC yourself use an open flame inside a BSC

Section 4.2 – COMMON LAB EQUIPMENT

It is important to understand the hazards and maintenance needs of common lab equipment. It is the Principal Investigator's (PI) responsibility to ensure that all lab personnel receive proper training to safely handle material and operate equipment available in the laboratory.

Equipment may generate certain hazards. Equipment must be used safety to ensure there is no exposure or injury when using the equipment. See Table 4.3 for examples of different equipment associated with various hazards.

Pipetting

Laboratory-associated infections have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger and inhalation of aerosols. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when cultures are mixed by pipetting, or when the last drop of an inoculum is blown out. Regular maintenance and calibration of pipettes ensures they are accurate and reduces the need to blow out trace amounts of inoculum.

Key Safety Practices:

- **Never mouth pipette**. Always use a pipetting aid.
- Minimize aerosol production.
- Dispose of all pipet tips segregated from the regular lab trash. See Section 8.2 for additional information.
- Discard contaminated disposable pipettes in an appropriate sharps container.

Syringes

The use of needles and syringes should be restricted to procedures for which there is no alternative. Blunt cannulas should be used as alternatives to needles wherever possible (e.g., procedures such as oral or intranasal animal inoculations).

Key Safety Practices:

- **Never recap needles.**
- Use disposable safety-engineered needle- locking syringe units whenever possible.
- Used disposable needles and syringes must be placed in appropriate sharps disposal containers and discarded as infectious waste. (See section 8.3 on sharps waste management)
- The use of needle-nipping devices is prohibited.

Cryostats

Freezing tissue does not necessarily inactivate infectious agents. Safety glasses, lab coat, and disposable gloves should be worn during preparation of frozen sections. Cut resistant (Kevlar or stainless-steel mesh) gloves are recommended when changing or adjusting blades.

Key Safety Practices:

- **Consider the contents of the cryostat to be contaminated and decontaminate it frequently with appropriate disinfectants.**

- Ensure the handwheel is locked and the blade is guarded before placing tools inside the chamber.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant immediately after use with tissue known to contain bloodborne pathogens, *M. tuberculosis,* or nonhuman primate samples.
- Defrost and decontaminate the cryostat at least once a week to help to ensure that the equipment will continue to operate safely and to eliminate any buildup of material.
- Handle microtome blades with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Use tweezers or other tools to pick up blades that have fallen into the cryostat. Never leave blades that have fallen into the cryostat so they may not injure the next user. Never use a bare hand to clean up surfaces inside the chamber.
- Staining solutions used with potentially infected frozen tissue sections should be considered contaminated and disposed of as infectious waste.

Loop Sterilizers and Bunsen Burners

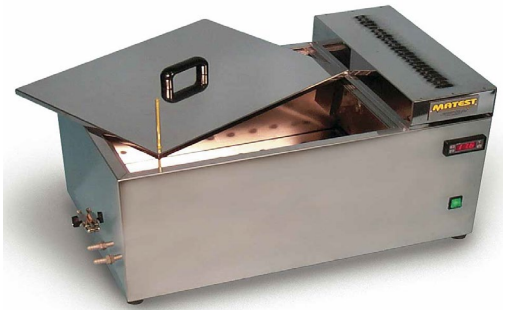
Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms.

Key Safety Practices:

- **The use of gas burners in a BSC is not allowed because burners can produce turbulence which disrupts the airflow in a BSC. (see Section 4.1 for alternatives)**
- Electric incinerators minimize aerosol production.
- Disposable plastic loops do not require sterilization.

Water Baths

The use of sterile water in water baths does not prohibit the growth of microorganisms, such as bacteria or fungi, but merely slows it down. Therefore, water should be changed on a regular basis to avoid the transfer of unwanted microorganisms via tissue culture bottles or other material into the biosafety cabinet and cell lines that are worked on within.



Key Safety Practices:

- Frequently empty water, wipe down with appropriate disinfectant, and use water to rinse out water bath
- Refill the water bath with sterile water
- Use an appropriate antimicrobial treatment for water bath to minimize unwanted growth
- Do not use azides as an antimicrobial treatment as they are toxic and cannot go into the sewer system.

Tissue Culture Incubator

This can be a main source of contamination of microbes that are easily transferred to the biosafety cabinet and other tissue culture samples. It is important to keep incubators clean, using good lab practices.

Key Safety Practices:

- Wipe down tissue culture flasks, plates, and other labware with appropriate disinfectants before placing into

the incubator

- Do not use disinfectants that may have toxic effects on the cells to wipe down the incubator
- Check the cleanliness of the incubator and discard old items
- Disinfect the incubator immediately after spills occur
- Keep areas around the incubator, such a benchtop and sink, clean and dust free
- Never mix bacterial culture and tissue culture in the same incubator
- After the occurrence of contamination, autoclave shelving and other removable parts and wipe down the inside of the incubator with an appropriate disinfectant

Autoclaves

Autoclave use can pose physical hazards that include heat, steam and pressure. Care must be taken when opening the autoclave after a run and handling the content, which may still be extremely hot.

Key Safety Practices:

- Contact equipment vendor to training specific to that model
- Use heat resistant glove when handling the autoclave rack and content
- DO NOT overfill bags or autoclave chambers because this decreases its effectiveness.
- Segregate autoclave loads since cycle times and temperature needs vary with different load types (e.g. infectious waste, liquid, or labware).

Procedures

BEFORE Autoclaving

- Review the operator's manual for instructions as different makes and models of autoclaves have different controls.
- Wear appropriate PPE while loading and unloading the autoclave, including heat resistant gloves, lab coat, and eye protection. A face shield should be worn if a splash hazard is present.
- Leave autoclavable bags unsealed to allow for steam penetration.
- Fill liquid containers only ½ full and loosen caps or use vented closures.
- DO NOT autoclave liquid and dry material together.
- Use autoclavable polypropylene / polyethylene biohazard bags ONLY. **Other bags may melt!**
- Use a heat-resistant secondary container to retain any leakage that may occur.

DURING Autoclaving

- Use appropriate cycle times for the items you will be autoclaving: (adapted from the *Guideline for Disinfection and Sterilization in Healthcare Facilities*, 2008, CDC)
  - Sterilizing Clean Materials: 30 min. at 121°Cand 15 psi
  - Decontaminating Waste: minimum 45 min. at 121°C and 15 psi
  - Dense Loads: lengthen running time
  - Liquids: use slow exhaust
  - Glassware: use fast exhaust

DO NOT leave autoclaved material in autoclave overnight!

AFTER Autoclaving

- **HOT, HOT, HOT!** - Allow materials to cool down for 15-20 minutes prior to their removal.
- ALWAYS make sure the pressure has gone to ZERO before opening the door!
- Use extreme caution when opening an autoclave door – there still may be steam inside the chamber after the pressure has dropped to zero which can cause severe burns.
- Use orange, elbow-length heat-resistant gloves to remove items from the autoclave.

Centrifuges

Rotational energies involved with most centrifuges can generate two serious hazards: mechanical failure, and dispersion of aerosols or droplets. This section describes general classes of centrifuges, and general operation and maintenance guidelines to minimize centrifuge hazards. (Figures 4-5 and 4-6)

Key Safety Practices:

Before centrifugation:

1. Use only rotors compatible with the centrifuge.
2. Check the expiration date for ultracentrifuge rotors.
3. Check tubes, bottles, O-rings, and rotors for cracks and deformities before each use.
4. Make sure that the rotor, tubes, and spindle are dry and clean.
5. Examine O-rings. Replace if worn, cracked, or missing.
6. Cap tubes before centrifugation.
7. Balance buckets, tubes, and rotors properly.
8. Check that the rotor is seated on the drive correctly, put lid on rotor, close the lid on the centrifuge, and secure it.
9. When using swinging bucket rotors, make sure that all buckets are hooked correctly and move freely.
10. Load and unload samples from the rotor in a biosafety cabinet when working under BSL-2 or BSL-3 conditions.

During centrifugation:

Keep the lid closed at all times during operation. Never open a centrifuge until the rotor has stopped.

1. Do not exceed safe rotor speed (Figure 4-7). Safety speeds will vary per rotor and may not be the same on different models by the same manufacturer. Always consult the manual and labels on the rotor before starting a run.
2. The operator should not leave the centrifuge until full operating speed is attained and the machine appears to be running safely without vibration
3. Stop the centrifuge immediately if an unusual condition (e.g., noise or vibration) begins and rebalance the load if needed.
4. If you suspect samples have spilled inside the centrifuge, either during or after a run, wait 30 minutes for aerosols to settle before opening the equipment

After centrifugation:

1. Allow the centrifuge to come to a complete stop before opening
2. Wear gloves to remove rotor and samples
3. Check inside of centrifuge for possible spills and leaks. Disinfect centrifuge and rotor thoroughly if necessary)
4. Wash hands after removing gloves

Centrifuge Maintenance Guidelines:

Moisture, chemicals, strong cleaning agents, and other substances can promote corrosion of centrifuge parts and cause centrifuge failure. Long-term centrifuge use may also cause centrifuge failure. The following are general maintenance recommendations:

Follow manufacturer instructions for maintenance and cleaning:

- Keep the centrifuge clean and dry.
- Clean up all nonhazardous spills immediately.
- Never clean rotors and associated parts with abrasive wire brushes.
- Store the rotor upside down in a dry place, with lids or plugs removed, to prevent condensation.
- Remove adapters after use. Inspect them for corrosion.
- Inspect rotors regularly. Remove rotors from use if they show any signs of defects. Report the defective rotors to a manufacturer's representative for inspection.
- To avoid rotor failure, record the length of time and speed for each high-speed rotor in a log book. Track and discard rotors according to the manufacturer's recommended schedule

Additional precautions when centrifuging **BIOHAZARDOUS MATERIAL**:

- Wear gloves when handling tubes and rotors
- Use sealed safety cups, safety buckets, or sealed rotors with O-rings as secondary containment.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or bucket
- Fill and open centrifuge tubes, rotors and accessories in a BSC.

Avoid the use of celluloid tubes with biohazards. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort when boiled and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them

Table 4-3: Equipment Hazards

Equipment Type	Hazards	Examples
aerosol generating	The diameter of aerosols generated from certain types of equipment will vary from 0.1 to 100 microns. • Bacterial cells and spores are 0.3 to 10 microns in diameter. • Viruses are 0.02 to 0.3 micron in diameter. • Biological particles generated from liquid or powder form particles that are 0.5 micron diameter.	• blender: 2 micron diameter particles • sonicator: 4.8 micron diameter particles • dropping bacterial flask: 3.5 micron diameter particles • dropping lyophilized culture: 10 micron diameter particles • pipette blow out: 4.9 micron diameter particles • vortex culture: 4.8 micron diameter particles • centrifuge: 4 micron diameter particles
chemical hazards	Chemical hazards vary and should always be looked up in the corresponding Safety Data Sheet for the material. Physical hazards can include explosive, flammable, oxidizing, or corrosive. Health hazards can include irritant, sensitizer, carcinogen, toxin, mutagenic.	• various dilutions of ethanol – flammable • dried picric acid – explosive • chronic Formaldehyde exposure – sensitizer • ethidium bromide – mutagen
cryogenic temperature	Cryogenic temperatures of -80°C are used to remove moisture from materials and contain low-temperature refrigerants. If protective equipment is not used, exposure to low temperature may cause cryogenic burns and frostbite.	• -80°C freezers • lyophilizers (freeze dryers) • dry ice • liquid nitrogen
high temperature	The use of heat to decontaminate or sterilize materials is widely used in the biological research laboratory. Physical injury from burns may occur from sudden accidental releases of heat sources or from the handling of hot items.	• dry heat sterilization temperatures range from 80°C to 200°C • autoclaves utilized wet heat to sterilize materials and can range between 80°C to 500°C • saturated steam operates at 121°C
high pressure	Compressed gas cylinders and pressurized equipment are commonly used in the laboratory. Injury may occur from rupture high-pressure lines.	• autoclaves operate at high pressures of 1,000 kilo Pascal (145 psi)
oxygen deficiencies	Oxygen deficiency environment may result from the displacement of oxygen by expanding gases (i.e., 700 parts of air to 1 part liquid nitrogen) or the linear displacement of oxygen from carbon dioxide(gas)	• liquid nitrogen or liquid carbon dioxide compressed gas cylinders or tanks • dry ice
rotational energies	Sudden release of such rotational energies can cause serious physical injury from unbalanced equipment or flying shrapnel.	• Tabletop unit – up to 5,000 rpm • ultracentrifuges unit – > 100,000rpm ○ 30 kg rotor weight
sharp objects	Any material having the potential to puncture. Such material used in the manipulation of infectious material carries a higher risk.	• needles with or without syringes • needles with vacutainers or attached tubing • blades (razors, scalpels, X-ACTO knives) • broken glass • serological pipettes and pipette tips
ultraviolet radiation (UV)	UV radiation is used for inactivating microorganisms. Its usefulness, however, is limited by a variety of factors (wave length, distance, exposure). The eyes and skin can be damaged by exposure to UV radiation.	• UV lights in Biosafety cabinets • Transilluminators • UV light boxes

Source: adapted from the Lawrence Berkeley National Laboratory Biosafety Manual 2010, Section 3 Table 7

Section 5.1 - INFECTIOUS AGENTS

Infectious agents encompass all microorganisms and agents that are known to be pathogenic to humans. Additional precautions may be taken with animal pathogens, when other animals are at risk for infection, such as in the animal facilities.

Types of Infectious Agents

When referring to infectious agents, we categorize them into these main types:

- Bacteria
- Parasites
- Prions
- Viruses
- Fungi

Although pure cultures of infectious agents may be generated in the proper media, human and animal materials, such as blood or tissues, may harbor such pathogenic agents as well. Further information on the use of Human Source Material in the lab can be found in Section 5.3 of this manual.

Many research laboratories at Penn focus their studies on a particular infectious agent. Some laboratories use infectious agents to support research in other fields of science. Therefore, live infectious agents are present in a vast array of labs at Penn.

Risk Assessment and How It Relates To Infectious Agents

A risk assessment must be made whenever an infectious agent is used in the laboratory. Please refer to Section 3.2 for detailed information on how to perform a risk assessment of infectious agents and the risk group categorization.

Penn Policy for Work with Infectious Agents

When working with infectious agents at Penn, each lab must employ certain practices and procedures to protect personnel and the public from infection. Therefore, biosafety levels are assigned to each laboratory based on a risk assessment of the infectious agents being used. Biosafety levels describe the practices and procedures that must be followed while working in the laboratory. See Section 3.3 for additional information on biosafety levels and animal biosafety levels.

Registration and Protocol Review

The biosafety staff reviews the following:

1. Animal Protocols: All animal protocols are reviewed for safety issues concerning the use of infectious agents, biohazardous material, and recombinant or synthetic nucleic acid (rsNA) in live animals.
2. Recombinant & Synthetic Nucleic Acids (rsNA): Work with rsNA is reviewed by the Institutional Biosafety Committee (IBC) in compliance with the *NIH Guidelines for Research Involving Recombinant NA Molecules*. Additional information can be found in Section 5.2 of this manual.
3. Biological Agents and Materials: Biological agents that are used or stored in Penn's laboratories are registered in the digital BioRaft system. Every lab must complete this registration and update it annually or when additional agents are introduced in the lab.
4. Biosafety Level 3 (BSL-3) Work: The Institutional Biosafety Committee (IBC) must review and approve all BSL-3 work and grant access to the BSL-3 laboratory. Additional information can be found in Section 3.3 of this manual or in the BSL-3 manual.
5. Select Agents: EHRS provides the oversight required by federal laws and regulations for research performed with Select Agents. Additional information can be found in Section 5.5 of this manual.

6. Research Grants: If you have a research grant that requires approval for use of infectious agents or other biohazardous materials, please contact a biosafety officer.

University of Pennsylvania Biological Registration

The Office of Environmental Health & Radiation Safety (EHRS) developed an electronic Biological Registration to establish a database of biological agents at Penn. Information collected by the Biological Registration process will be used by the biosafety staff and the research community to conduct risk assessments to mitigate risks associated with working with the infectious agents by selecting the appropriate biosafety levels, engineering controls, and safety practices to safeguard the University of Pennsylvania.

The Biological Registration is an on-line form that must be completed by the Principal Investigator (PI) or by a lab member appointed by the PI. Guidance to complete the Biological Registration can be found on the EHRS website.

The Biological Registration must be updated on an annual basis or amended when changes are made pertaining to the laboratory and experimental procedures. You will be notified when it is time to update your information. All information from your previous entry is saved in the database so that only information relevant to changes in your research, personnel or space must be entered.

If assistance is required to complete this form, please call EHRS at 215-898-4453 and ask to speak with a biosafety officer.

Section 5.2 - RECOMBINANT & SYNTHETIC NUCLEIC ACIDS (rsNA)

In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:
1. Molecules that:

a) are constructed by joining nucleic acid molecules and

b) that can replicate in a living cell, i.e., recombinant nucleic acids

2. Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or

3. Molecules that result from the replication of those described in 1. or 2. above.

Institutional Biosafety Committee (IBC)

The University of Pennsylvania's Institutional Biosafety Committee (Penn IBC) is responsible for risk assessment and ensuring compliance with the National Institutes of Health (NIH), Office of Science Policy (OSP) guidelines (NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules). The IBC establishes, reviews, and ensures compliance with policies relevant to university-related use or transfer of rsNA or, cells, organisms, and viruses containing rsNA. The IBC reviews and approves all work being done with rsNA.

Mandatory Compliance with the NIH Guidelines

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* detail safety practices and containment procedures for basic and clinical research involving recombinant or synthetic nucleic acid molecules, including the creation and use of organisms and viruses containing recombinant or synthetic nucleic acid molecules. For institutions that receive any NIH funding for rsNA research, all research at that institution using recombinant material must comply with the NIH Guidelines

Types of rsNA Work reviewed by the IBC

All projects involving recombinant or synthetic nucleic acid molecules and/or gene editing technology must be reviewed and approved by the Penn IBC. Work requiring IBC review and registration includes but is not limited to:

- Use and/or generation of viral vectors (e.g. lentivirus, retrovirus, adenovirus, adeno-associated virus, baculovirus)
- Use of CRISPR or other gene editing technologies
- Use and administration of modified cells in animals
- Use and administration of modified microorganisms in animals
- Expression of foreign genes in animals via plasmid
- Use of recombinant viruses and/or other recombinant pathogenic microorganisms (Risk Group 2-4 [RG2-4]) and experiments with them in animals
- Experiments with recombinant influenza virus (Section III.D-7 of the NIH Guidelines)
- Gene transfer experiments in companion and privately-owned animals
- Generating transgenic animals (e.g., rodents, ants, fish, nematodes, frogs, fruit flies, other insects)
- Crossing transgenic animals, except for mice
- Experiments with non-pathogenic (RG1) microorganisms that are not exempted by Section III.F, Appendix C of the NIH Guidelines (e.g., use of baculovirus for protein expression, experiments with

bacteriophage for phage display, use of Pichia pastoris for protein expression)

- Experiments with modified cells that contains greater than one-half of a viral genome
- Experiments with a defective virus in the presence of a helper virus (Section III.D-3 of the NIH Guidelines)
- Experiments with genes from RG3 or RG4 agents (in prokaryotic or lower eukaryotic cells, or as mRNA vaccine)
- Cloning and expression of toxin molecules (also requires NIH-OSP approval if meets requirements in Section III-B of the NIH Guidelines)
- Large-scale production of recombinant material (equal or greater than 10L in a vessel)
- Human Gene Transfer experiments
- Use and/or Generation of transgenic plants
- Crossing transgenic plants
- Use and/or administration of modified organisms in plants

Examples of exempt work include:

- Modified cells for tissue culture work only
- Plasmids expressed in tissue culture only
- Modified E. coli K-12 strains
- siRNA, shRNA, miRNA

*Please consult the *NIH Guidelines* for specifics or the graphic below. Contact a biosafety officer with additional questions or request for guidance.

Registration Review Process at Penn

An electronic document describing work being done with rsNA, including the type and source of material, target recipient(s) of rsNA materials, who will be performing and supervising research with rsNA materials, and any other relevant experimental details must be submitted for review. The electronic registration system, Penn IBC Electronic Registration System (PIERS) can be found at https://apps.research.upenn.edu

- Log on to the **Penn IBC Electronic Registration System** (PIERS) at ***https://apps.research.upenn.edu***
- A biosafety officer will pre-review your IBC registration prior to presenting it to the Penn IBC.
- Registrations are reviewed by the Penn IBC once a month. Submission deadlines and IBC meeting dates can be found on the EHRS website. The IBC is not permitted to review registrations outside of a fully convened meeting.
- Submit an amendment to the registration, through PIERS, to add personnel, genes, target recipients, to change the containment level, or any other relevant changes to the original registration.

IBC Approval: After the IBC approves a registration, the PI and those lab staff identified in the registration with editing capabilities will receive an email noting the approval, including:

- The IBC registration number (ex. #24-095)
- The assigned biosafety level (BSL) AND/OR

The assigned animal biosafety level (ABSL)

Registration Renewal: Registrations expire after 3 years. To stay in compliance, renew a registration within PIERS prior to the end of the month in which the registration expires.

rDNA registration documents WILL NOT be approved until ALL training is complete

Training Requirements

All lab members working with recombinant material must be listed on the registration, including the PI. The PI and their lab members must complete Recombinant or Synthetic Nucleic Acid Guidelines training in WorkDay@Penn before approval of the IBC registration can be granted or work with rsNA material is initiated.

Training is required every 3 years.

PIs are responsible for all hands-on training of their laboratory members. A knowledge-based training module for work with rsNA can be found online in WorkDay@Penn. **ALL** personnel who are listed on a registration document must complete the online training module, including:

- Principal Investigator
- Anyone directly involved in handling rsNA material

When do I need to register my work with the Institutional Biosafety Committee?

The NIH Office of Science Policy compiles and maintains the NIH Guidelines for Researching Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2019) on behalf of the NIH Director. Compliance is required for all institutions receiving NIH funding. This list is meant to serve as a guide for researchers but is not comprehensive or authoritative. Please contact a Biosafety Officer at biosafety@lists.upenn.edu with any questions or reach out to NIH OSP (NIHGuidelines@od.nih.gov).

III-A: Requires NIH & IBC Approval **Before** Initiation	
Transfer of drug resistance to non-host microorganisms	If not known to acquire the trait naturally or could compromise the ability to control disease in humans, animals, or agriculture

III-B: Requires NIH & IBC Approval **Before** Initiation	
Cloning of toxin molecules	If recombinant work will result in biosynthesis of toxin molecules lethal to vertebrates at LD_{50} < 200 ng/kg body weight * *contact NIH OSP for more info re: E. coli K12 specific approvals

III-C: IBC Approval **Before** Initiation	
Human gene transfer	Recombinant DNA or RNA that do one of the following: contains >100 nucleotides, can integrate into the genome, replicate in a cell, be translated or transcribed

III-D: IBC Approval **Before** Initiation	
Genetically-engineered plants or plant-associated insects or microbes	Exotic infectious agents, cloned genomes that may reconstitute via complementation, cloning vertebrate toxins *in planta,* recombinant microorganisms infecting small animals and insects associated with plants
Use of Risk Group 2, 3, & 4 or restricted agents	Viruses, host-vector systems, DNA encoding the above, helper virus systems (infectious or defective) in tissue culture
Whole animals	Genome editing (except rodents), modified microorganisms, modified cells
Influenza Viruses	Human H2N2 (1957-1968),H1N1 (1918) Avian H5N1 (highly pathogenic strains)
Large scale experiments (> 10 L culture)	Viral vector production, bacterial growth, tissue culture, eg

III-E: Requires IBC Approval **Simultaneous** with Initiation	
Tissue culture	Culturing a single family of eukaryotic viruses containing less than <2/3rds of viral genome
Whole plants & plant-associated microbes	Noxious weeds, introducing complete genomes of non-exotic infectious agents, modified non-exotic microbes, modified arthropods or small animals associated with plants
Creation of transgenic rodents	Gene editing (eg CRISPR/Cas9), introduction of recombinant or synthetic nucleic acid into the germline of rodents (ABSL 1 only)

III-F: Exempt from NIH Guidelines* *other state/local standards may apply			
Breeding of transgenic rodents	If both parents are housed at ABSL1 and their genomes do not contain more than 50% of a eukaryotic virus genome or transgenes under control of a gammaretroviral LTR and If transgenic offspring are not expected to contain more than 50% of an exogenous viral genome		
Non-replicable RNA/DNA	If it cannot replicate or generate nucleic acids, integrate into DNA, or code for a lethal toxin; will not be used in humans	**Non-permeable RNA/DNA**	If it cannot replicate or generate nucleic acids, integrate into DNA, or code for a lethal toxin; will not be used in humans
Naturally occurring elements	Exact sequence from a single source Prokaryotic plasmids/viruses propagated in same/similar species host DNA commonly exchanged between species	**Transposable elements**	If they do not contain recombinant DNA

Section 5.3 - HUMAN SOURCE MATERIAL

Human Source Materials: Cells, blood, serum, tissues, feces, or other potentially infectious material (OPIM) (sputum, urine, saliva, etc.) originating from humans.

Bloodborne Pathogens: Refers to pathogenic microorganisms that are present in human blood and can cause disease in humans, including but not limited to Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV).

Exposure Control Plan: A written document required to be completed by the research lab and reviewed annually by each lab employee working with human source material and other potentially infectious material (OPIM). The plan is designated to describe procedures to eliminate or minimize employee exposure to human material.

Universal Precautions: An approach to infection control, where all human source material is handled as if known to be potentially infectious. All human material must be handled under BSL-2 practices and procedures.

Risks

Working with human source materials carries a risk of being exposed to any infectious agents present in the material. Therefore, all human materials are considered potentially infectious regardless of whether they are primary materials or commercially available.

Some pathogens potentially found in human source material and that have documented cases of laboratory acquired infections (LAIs) include:

Blood

- Hepatitis B Virus (HBV)
- Hepatitis C Virus (HCV)
- Human Immunodeficiency Virus (HIV)
- *Plasmodium* spp.

Lung Tissue or Sputum

- *Mycobacterium tuberculosis*
- *Influenza* Virus

Cerebral / Spinal Material

- Prions
- Prion-like proteins

Fecal Material

- *Escherichia coli* O157:H7
- *Salmonella* spp.
- *Shigella* spp.
- *Helicobacter pylori*
- *Giardia intestinalis*

(This is NOT an all-inclusive list. Examples are for illustrative purposes only.)

OSHA

Bloodborne Pathogen Standard

SECTION 5.3 – HUMAN SOURCE MATERIAL

The OSHA 29 CFR 1910.1030 Bloodborne Pathogen Standard stipulates that employers must train all employees who have occupational exposure to human source material. The Standard also covers employees who work in HIV and HBV research laboratories. Components of the BBP standard include:

- Development of a laboratory specific Exposure Control Plan
- Completion of annual BBP training
- Offer vaccination against HBV at no cost to the employee
- Implementation of Universal Precautions
- Implementation of engineering and work practice controls
- Containment and disposal of infectious waste

Exposure Control Plan

Every laboratory working with human source material must complete an Exposure Control Plan. This document outlines laboratory specific procedures designed to limit employee exposure to bloodborne pathogens. The following must be included in the plan:

- Job classifications
- PPE requirements
- Engineering and work practice controls
- Decontamination and spill clean-up procedures
- Emergency exposure procedures

University of Pennsylvania's ***Exposure Control Plan*** can be found on the EHRS website. *Appendix C* of the plan must be completed by each individual lab. The plan must be reviewed and signed annually by all lab members working with this material, including the Principal Investigator.

Training

All students and Penn employees working with human source material must complete **Bloodborne Pathogen Training** annually. Information on training can be found on the EHRS website. Training is available in WorkDay@Penn.

Vaccination

Vaccination against HBV is offered free of charge to all Penn employees who may be occupationally exposed to BBPs. Employees may obtain the vaccine from Occupational Medicine and students from Student Health Services. Further information on HBV vaccination is outline in the Penn's Exposure Control Plan and on the EHRS Website. See the EHRS Website for the Occupational Medicine request letter.

Personal Protective Equipment (PPE)

All lab staff working in the laboratory space must wear long pants and closed-toed shoes. Shorts, most skirts, and open toed shoes are prohibited as lab attire while working in the laboratory space.

The following PPE is required to be worn when manipulating any human source material:

- Lab coat: A washable, front-button lab coat or disposable lab coat/gown must be worn.
- Nitrile gloves: Disposable nitrile gloves (minimum thickness of 4mm) must be worn.
- Eye protection: Eye protection, such as goggles, safety glasses, and a face shield may be required depending on the risk of a splash. (Figure 1)

When working inside a biosafety cabinet, there is a *minimal splash risk*. Therefore, eye protection may not be

necessary.

Figure 5.1 eye protection

Practices and Procedures

Biosafety Level 2 (BSL-2) practices and procedures must be used when manipulating any human source material. A detailed explanation ofBSL-2 criteria can be found in the *CDC's Biosafety in Microbiological and Biomedical Laboratory (BMBL), 6th Edition*. Some key practices include, but are not limited to the following:

1) Biosafety Cabinet Use

   The manipulation of all human materials that may create aerosols MUST be performed inside a certified biosafety cabinet. If a biosafety cabinet is not available, only procedures that will not create aerosols may be performed on an open bench. Adequate eye protection from splashes must be worn during all procedures on an open bench.

2) Reduction / Elimination of Glass and / or Sharp Objects

   Whenever possible, work with non-glass and non-sharp objects to prevent cutaneous injury. Safety-engineered scalpels, butterfly needles, and syringes are also appropriate devices to use when using human source material (Figure 5-2).

3) Surface Disinfection

   The biosafety cabinet or bench top surfaces must be disinfected when work has been completed. An appropriate surface disinfectant is a solution of bleach diluted 1:10 with water. Wipe down surfaces with this solution and, in a biosafety cabinet, follow with 70% ethanol to prevent corrosion.

4) Waste Disposal

   All research materials and PPE used in manipulating human materials must be disposed of through the infectious waste stream. Any objects that may poke through a bag (e.g. pipette tips, serological pipettes, syringes, needles, razors, glass) must be disposed of in a hard-sided, leak-proof sharps container. PPE and non-sharp objects may be placed in red/orange autoclavable bags. See Section 8 for details on Infectious Waste and Sharps management. Penn requires the autoclaving of waste in some locations. See Resources for Biohazardous Waste Disposal for additional guidance on autoclaving requirements by school and center.

5) Transport

   To transport between laboratories, human materials must be placed in a leak-proof, closeable container labeled with the universal biohazard sticker.
   Shipping of human materials must be in accordance with IATA regulations and shippers must have completed

training. Please see the University of Pennsylvania's ***Shipping Manual for Infectious Substances and Biological Materials*** for more information.

Emergency Procedures

All workers with potential exposure risk to human source materials must comply with the Occupational Safety and Health Administration's Bloodborne Pathogen Standard **(OSHA 29 CFR 1910.1030).**

If an exposure to a mucous membrane (i.e. Splash to eyes, nose, or mouth) occurs:

- Irrigate the exposed area with running water at an eyewash station for **15 to 20 minutes**.

If a penetrating wound (e.g. cut, puncture, needle-stick) occurs:

- Thoroughly wash the injured area with soap and water for **15 to 20 minutes**

Emergency contact information can be found at the beginning of this manual and on the EHRS website.

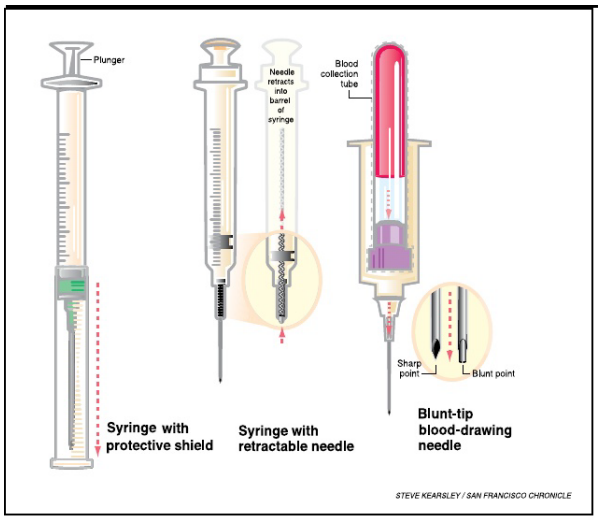


Figure 5.2 Examples of syringes with safety features

Section 5. 4 - NON-HUMAN PRIMATE MATERIALS

Non-human primate (NHP): Refers to species ranging from apes to monkeys. Specifically, old world macaque monkeys, including rhesus macaques, pig-tailed macaques, and cynomolgus monkeys, are of concern as they are known to carry **Macacine alpha herpesvirus 1** (also known as Cercopithecine herpesvirus 1, Herpesvirus simiae, Herpes B virus, or B virus).

NHP source material: Cells, blood, serum, tissues, feces, and body fluids (sputum, urine, saliva, etc.) originating from non-human primates.

Risks of Zoonotic Agents

Due to their genetic similarities, humans and non- human primates are susceptible to many of the same diseases. Therefore, all NHP materials are considered potentially infectious regardless of whether they are primary materials or commercially available. Laboratory acquired infections from handling NHP materials have been well documented.

Some NHP zoonotic agents are as follows: (Fleming, Diane O., and Debra L. Hunt, eds. *Biological Safety Principles and Practices*. 5th ed. Washington, DC: ASM,2017.).

Viruses

- Hepatitis A and B virus
- Macacine alpha herpesvirus 1
- Poxviruses
- Respiratory syncytial virus
- Rotavirus
- Simian hemorrhagic fever virus
- Simian Immunodeficiency viruses
- Simian retrovirus type D
- Simian T-cell leukemia virus
- Simian virus 40

Bacteria

- *Campylobacter* spp.
- *Mycobacterium tuberculosis*
- *Shigella flexneri*
- *Streptococcus pneumoniae*

Parasites

- *Balantidium coli*
- *Entamoeba histolytica*
- *Strongyloides spp*

Macacine alpha herpesvirus 1

- Macacine alphaherpesviru1 (or B virus) has an approximately 70% mortality rate in humans when the exposure is not immediately treated.
- B virus may be present in materials from macaque, including saliva, feces, urine, tissues or fluids. Therefore, cell cultures derived from infected monkeys may contain this virus.
- It is essential to handle all NHP material while wearing proper PPE and using correct containment practices. If an exposure should occur, emergency procedures must be implemented immediately.

Personal Protective Equipment (PPE)

The following PPE is required to be worn when manipulating any NHP source material:

- Long pants and closed toed shoes: Shorts, skirts, and open toed shoes are prohibited.
- Lab coat: A washable, front-button lab coat or disposable lab coat/gown must be worn.
- Nitrile gloves: Disposable nitrile gloves (minimum thickness of 4mm) must be worn.
- Eye protection: Eye protection, including goggles/safety glasses with a face shield, must always be worn when working with ***live NHPs***.
  Goggles/safety glasses must be worn when working with NHP material, including when working within a biosafety cabinet.
- Examples of eye protection:
  - UVEX Genesis safety glasses
  - Hybrid safety glasses/goggles, 3M Maxim 2x2 Goggles
  - Disposable full-length face shield

Figure 5.3 eye protection

Practices and Procedures

Biosafety Level 2 (BSL-2) practices and procedures must be used when manipulating any NHP material. A detailed explanation of BSL-2 criteria can be found in the CDC's ***Biosafety in Microbiological and Biomedical Laboratory (BMBL), 6^{t} Edition***. Some key practices include, but are not limited to the following:

- Biosafety Cabinet Use

  The manipulation of all NHP materials that may create aerosols MUST be performed inside a certified biosafety cabinet.

- Reduction/Elimination of Glass and/or Sharp Objects

  Whenever possible, work with non-glass and non-sharp objects to prevent cutaneous injury.

- Surface Disinfection

  The biosafety cabinet or bench top surfaces must be disinfected when work has been completed. An appropriate surface disinfectant is a solution of bleach diluted 1:10 with water. Wipe down surfaces with this solution and follow with 70% ethanol to prevent corrosion.

- Waste Disposal

  All research materials and PPE used when manipulating NHP materials must be disposed of through the infectious waste stream. Any object that may poke through a bag (i.e. pipette tips, serological pipettes, syringes, needles, razors, glass, etc.) must be disposed of in a hard-sided, leak-proof sharps container. PPE and non-sharp objects may be placed in red/orange autoclavable bags. All waste is autoclaved before final disposal.

- Transport

  To transport between laboratories, NHP materials must be placed in a leak-proof, closable secondary container labeled with the universal biohazard symbol.

Transport of live animals must be performed in accordance with ULAR policy.

Shipping of NHP material must be in accordance with IATA regulations. ALL persons shipping Dangerous Goods or certain biological material must complete IATA shipping training. See WorkDay@Penn for training session information.

Emergency Procedures

If an <u>exposure to a mucous membrane</u> (i.e. splash to eyes, nose, or mouth) occurs:

Irrigate the exposed area with sterile saline or running water at an eyewash station for 15 to 20 minutes.

If a <u>penetrating wound</u> (i.e. cut, puncture, needle-stick, etc.) occurs:

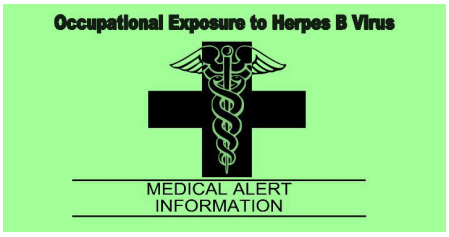
Locate the "bite kit" in the room. Thoroughly clean the injured area with povidone- iodine, chlorhexidine, or detergent and water for 15 to 20 minutes.

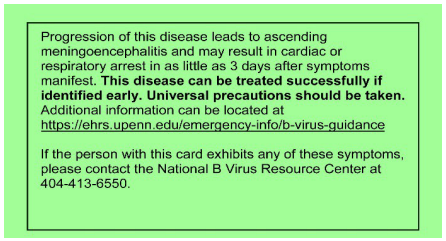
Report immediately to Occupational Medicine or the ER for medical care. Bring the GREEN Exposure Card to the attending physician. The Hospital of Pennsylvania Emergency Room will have additional guidance available to collect samples and treat the injury.

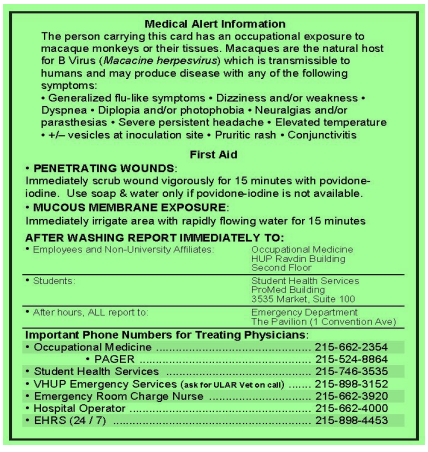
IMMEDIATELY REPORT FOR MEDICAL EVALUATION AFTER ANY EXPOSURE or possible exposure:

Emergency contact information can be found at the beginning of this manual and on the EHRS website.

<u>Green NHP Exposure Cards:</u>
Wallet-sized "Green Cards," which describe procedures to follow in case of an exposure to NHP material, are available from ULAR. These cards should be carried by all researchers working with NHP material and should be presented to a health care professional if an exposure occurs or herpes B symptoms develop (see below).







Section 5.5 - SELECT AGENT PROGRAM

The purpose of the University of Pennsylvania's Select Agent policy is to ensure that all federally regulated select agents on Penn's campuses are handled safely, secured properly, and registered with The National Select Agents Registry Program (NSAR). The Federal Select Agent Program (FSAP) is jointly administered by the Division of Select Agents and Toxins (DSAT) within the Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and the Division of Agricultural Select Agents and Toxins (DASAT) within the United States Department of Agriculture, Animal Plant Health Inspection Service (APHIS).

Terms to Know

Select Agents: Biological agents and toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products.

Responsible Official (RO): The Executive Director of EHRS performs this role at Penn. All activities involving registration with federal agencies, intramural or extramural transfers, disposal and exclusion or exemption from regulation must be coordinated through EHRS and reviewed and approved by the RO. The RO submits all applications to DSAT and/or DASAT.

Alternate Responsible Official (ARO): The ARO assumes the responsibilities and duties of the RO when the RO is unavailable. The Institutional Biosafety Officer performs this role at Penn.

Background

On June 12, 2002, President Bush signed the "Public Health Security and Bioterrorism Preparedness Response Act of 2002" (The Act; Public Law 107-188). The Law's purpose is to improve the ability of the United States to prevent, prepare for, and respond to bioterrorism and other public health emergencies. The Law requires that all persons possessing select biological agents or toxins deemed a threat to public health, animal or plant health, or animal or plant products register with the appropriate federal agency.

On March 18, 2005, final rules (42 CFR Part 73, 7 CFR Part 331, 9 CFR Part 121) were published in the Federal Register by the Departments of Health and Human Services (HHS) and Agriculture (USDA) governing the possession, use, and transfer of select agents and toxins. All provisions of these final rules superseded those contained in the interim final rules and became effective on April 18, 2005.

Registration of Select Agents

A Principal Investigator (PI) may not possess or use, receive from outside the United States, or transfer from within the United States, any biological agent or toxin listed as a select agent by Departments of Health and Human Services (HHS) and Agriculture (USDA) until they have been approved to use the biological agent or toxin by the Office of Environmental Health & Radiation Safety (EHRS) and have been granted a certificate of registration through the National Select Agent Registry.

Registration Process:

1. Complete Penn's Select Agent Registration form.
2. Complete the appropriate agency packet in coordination with EHRS.
3. Submit a proposal for use of select agents and, if necessary, a registration document for use of recombinant nucleic acids or recombinant organisms to Penn's Institutional Biosafety Committee (Penn IBC) for approval.
4. After all local documents are completed and approved, the Penn's RO will sign the agency registration packet and submit to the appropriate agency.

If the PI proposes the use of a Select Agent in animals, approval from the Institutional Animal Care and Use Committee (IACUC) and EHRS is required.

List of Select Agents

Please see the Select Agents and Toxins List from FSAP, here in **Appendix B,** for a current list of select agents.

Exclusions from Select Agent Policy

Certain attenuated strains of select agent microorganisms are excluded from the federal select agent policy. In addition, certain select agent toxins are not regulated if the total amount under the control of a Principal Investigator *does not exceed, **at any time**, the amount permissible by the select agent regulations.*

List of Excluded Microorganisms

For a current list of the exclusions, please visit the Select Agents Exclusions Website. (https://www.selectagents.gov/sat/exclusions/index.htm).

Permissible Toxin Amounts:

The University of Pennsylvania recognizes the critical importance of maintaining the highest standards of safety, security, and due diligence in handling and storing permissible toxin amounts, as stipulated by the Federal Select Agent Program (FSAP) in the United States. The University of Pennsylvania's Permissible Toxin Amounts policy aims to ensure that labs and individuals adhere to standards governing the possession, use, and storage of permissible amounts of select agents and toxins

Permissible quantities of select agent toxins are those that, in aggregate, **DO NOT EXCEED** the following amounts:

HHS Toxins [42 CFR 73.3(d)(7)]	Amount
Abrin	1,000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha Conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Figure 5.5 HHS Permissible Toxin Amounts

Compliance with Permissible Toxin Amounts

- **Inventories:** Labs and individuals are mandated to maintain up-to-date inventories of all permissible select agents and toxins in their possession. These inventories must be regularly reviewed and verified to guarantee strict compliance with permissible toxin amounts.
- **Security Measures:** To comply with security requirements, select toxins must be stored in designated, secure locations.
- **Permissible Amounts:** The University emphasizes strict adherence to the permissible toxin amounts outlined by the FSAP. Labs must conduct regular internal audits to ensure that these amounts are never exceeded, and immediate corrective actions must be taken if any discrepancies are identified.

- **Transfer and Destruction:** To transfer a Select Toxin, a "Request to Transfer Exempt Amounts of a Select Agent" form must be submitted to EHRS for review. To destroy a Select Toxin, a "Notification of the Proposed Destruction of Select Agents" form must be submitted to EHRS for review. Documents can be found on the EHRS website.

Training and Awareness: All personnel involved in handling and storing select agents and toxins must be up to date on their Lab safety training to enhance their awareness of safety protocols and emergency procedures.

Compliance Monitoring

The University is committed to proactive compliance monitoring to identify and rectify any potential issues. Regular inspections will be conducted by Biosafety Officers to ensure that all labs and individuals are following the established protocols and guidelines.

For any questions regarding the **SELECT AGENTS PROGRAM** at the University of Pennsylvania please contact EHRS at 215-898-4453 and ask to speak with the RO or ARO.

Possession of select agent toxins above permissible quantities requires registration with the CDC Select Agent Program

It is a criminal offense to possess quantities above the permissible levels without registration with the CDC. Offenders may be penalized with up to five years in prison and/or $500,000 in fines. *(Public Health Security & Preparedness Response Act of 2002,Sec. 231 (c))*

Section 6 - THE BIOLOGICAL RESEARCHLABORATORY

There are several components that need to be in place for employees to work safely in a research lab environment. These elements are not only in place for the individual but also for everyone else working within this space, such as housekeeping and facilities personnel. Following the practices and guidelines listed below help to create a safe working environment in the laboratory.

Training

Training programs are required by the Occupational Safety & Health Administration (OSHA) for all employees who work with hazardous substances including: chemicals, human blood, blood products, fluids, and human tissue specimens. Training requirements based on job duties and responsibilities are determined for each employee by completing the Penn Profiler. Dates for live training are published in WorkDay@Penn. All web-based trainings are accessible through WorkDay@Penn. For more information on WorkDay@Penn and how to use it, see the WorkDay@Penn toolbox (https://www.workday.upenn.edu/home/toolbox) or contact EHRS at traininghelp@ehrs.upenn.edu or 215- 898-4453.

- Laboratory and Biological Safety at Penn – required for any *NEW* PENN employee who works in a research lab (includes Bloodborne Pathogen training).
- Laboratory Safety Update – required for any PENN employee who works in a research lab. Course is assigned one year after completing Laboratory and Biological Safety at Penn and every 2 years thereafter.
- Bloodborne Pathogen Safety at Penn – OSHA training required for any PENN employee who works with or come in contact with human material, including blood, tissue, cells, cell culture lines, or secretions. Required annually.
- Recombinant or Synthetic Nucleic Acid Guidelines – required for any PENN employee who conducts research using recombinant or synthetic nucleic acids and is listed on an IBC Registration. Required every 3 years.
- Biosafety Cabinet Operation - required for any PENN employee who conducts research in a Biosafety Cabinet. Must only be completed once.
- Shipping of Hazardous Materials and Dangerous Goods for Laboratory Staff – IATA training required for any PENN employee who prepares and packages infectious and biological substances (classified as Dangerous Goods) for shipping. First time shippers must complete the instructor led training. Required every 24 months.
- Training for Personnel Who Collect & Ship Infectious Waste – DOT training required for any PENN employee who packages infectious waste and/or signs off on shipping manifest for infectious waste. Shipping Infectious Waste Update can be taken 3 years after the instructor led training is complete. Required every 3 years.

Immunizations

Immunizations may be suggested for PENN employees who work with certain hazardous biological agents, such as Vaccinia, Rabies, or human source material. Immunizations are free of charge to the employee that requires them. Immunizations do not replace engineering controls, proper work practices for the use of PPE. Post- exposure prophylaxis should also be considered after exposure to certain hazardous biological agents, such as vaccinia or HIV.

Annual Lab Inspections

Laboratory Inspection are performed annually or more frequently as needed. Inspections assess the condition of a lab space from a safety standpoint, confirm that proper lab practices are being followed, and ensure that local and federal regulations are being followed.

Inspection reports contain recommendations to improve safety in the lab and are sent to the PI and their lab staff. It is the PI's responsibility to take action on and review the report with their lab staff. The PI or Lab Safety Coordinator must finalize the inspection report in BioRaft, indicating resolution of the findings listed in the report .

A Biosafety Officer performing the inspection will address many of the points covered in this manual and listed on the Laboratory Safety Self-Assessment Tool posted on the EHRS website.

Additional lab safety concerns may also be addressed depending on the work being done in the lab.

Room Signs

Room signs provide safety information to visitors and service personnel (Figure 6-1).

Room sign information includes:

Section One

- Department, building name and room number

Section Two

- Principal Investigator(s)
- Other researchers using this space

Section Three

- Hazard Labels (Biological, Chemical, Radioactive, etc.)

Section Four

- Name and contact number for after-hour emergencies University of Pennsylvania main emergency numbers

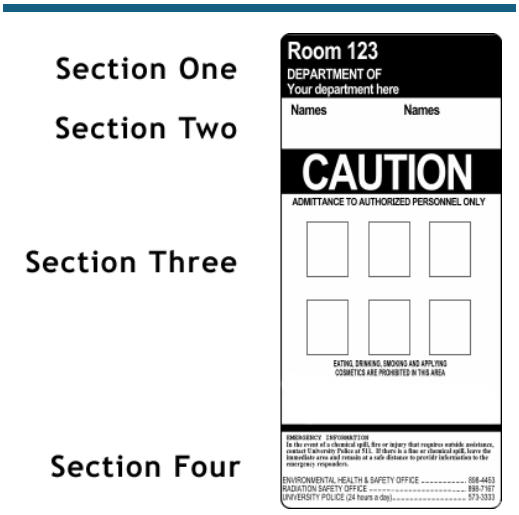


Figure 6-1: Laboratory room sign

See the EHRS website for a room sign request form for most buildings on campus. For Perelman School of Medicine buildings, request the room sign from Space Planning & Operations (PSOM SPO).

Hazard Labels

All laboratory spaces in which biohazardous material is used and all equipment in which biohazardous material is manipulated or stored (centrifuges, water baths, cryogenic freezers, incubators, etc.) must be posted with the biohazard warning sign (Figure 6-2).

Personal Protective Equipment

Personal Protective Equipment (PPE) is worn by researchers to protect their body from hazardous agents and materials. The PI is responsible for determining what PPE is required and providing it to their employees. EHRS is available to assist PI's with the selection of PPE. At a minimum lab coats and safety glasses are required while working in the lab. Additional PPE may include face, eye, hand, foot, body, and respiratory protection.

A. Face and Eye Protection

- Face and eye protection is used by researchers to protect from splashes, splatters, or debris from biohazardous materials.
- **Safety glasses must be worn while working in the laboratory.** Goggles, face mask, or face shield must be worn when procedures may produce splashes, sprays, splatters, or droplets of infectious or other hazardous materials.
- Call EHRS for assistance in selection of appropriate face and eye protection.

B. Hand Protection

Gloves protect the researchers' hands, fingers, and nails from becoming contaminated with biohazardous material. This prevents infection via breaks through the skin. The following should be followed for glove selection, use, and disposal.

- Gloves must NOT be worn outside of the laboratory!

- Gloves **must** be worn when working with biological hazards, including: organisms containing recombinant material, recombinant experimental animals, RG2 pathogens, BBP materials or surfaces and items contaminated with any of these biological hazards.
- Use of standard nitrile examination gloves (minimum 4mm thickness) is considered adequate for handling most biological materials. EHRS discourages the use of latex gloves.
- Change gloves when contaminated, when their integrity has been compromised, or when otherwise necessary.
- Do not wash or reuse gloves.
- Remove gloves after use and dispose of as appropriate. Wash hands immediately after removing gloves. See Figure 6.2 and 6.3 for proper procedures.

C. Foot Protection

- **Closed-toe shoes must be worn at all times in the laboratory.** Open-toe shoes and sandals are not permitted.

D. Body Protection

- **Long pants are required when working in a laboratory**. Long sleeves are also suggested. Shorts are not permitted.
- Additional PPE includes lab coats, gowns, smocks, or uniforms designed to protect street clothes and exposed skin from contamination by biological materials or exposure to other hazards.
- **Lab coat must be worn while working in the laboratory.** Lab coats made of 100% cotton are strongly recommended.
- **DO NOT WEAR LAB CLOTHING IN NONLABAREAS** (i.e., elevator, stairwell, lounge, administrative offices, bathrooms). Laboratory clothing must be removed and left in the lab before exiting.
- Laundry – All laboratory clothing must either be disposed of or laundered after it has been contaminated and prior to using again. Personnel are not permitted to launder laboratory clothing at home. Search

"**Apparel Rental and Laundry Services**" at Penn Procurement Services to find current vendors that launder lab coats.

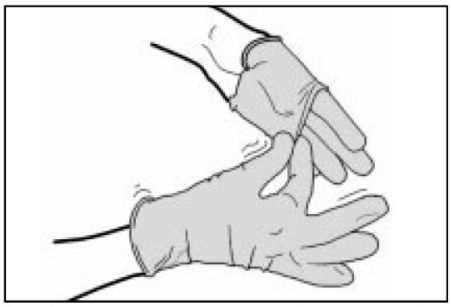
E. Respiratory Protection

- The University of Pennsylvania has determined that certain employees are exposed to respiratory hazards during routine operations. Penn's respiratory protection program will ensure that all University of Pennsylvania employees and students are protected from exposure to these respiratory hazards.
- Employees who are required to wear respirators must pass a medical exam before being permitted to wear a respirator on the job. Employees are not permitted to wear respirators until a physician has determined that they are medically able to do so. An employee refusing the medical evaluation will not be allowed to work in an area requiring respirator use.
-

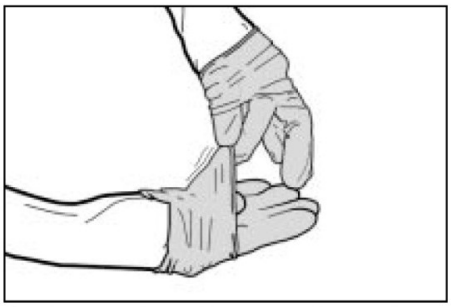
For more information and contact information visit the EHRS website's Respiratory Program

Removal of Gloves Technique

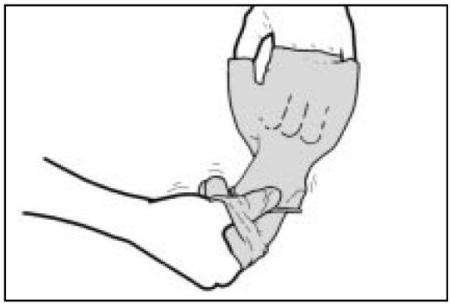
1. Use the following pictures as a guide to help you remove gloves safely
2. Avoid touching the outside of the gloves. Only touch the inside
3. Wash hands after removing and disposing of gloves in a sealable bag



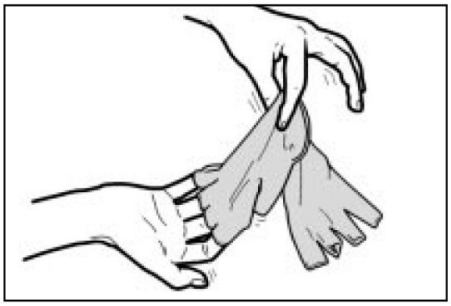
1. Grasp one glove at wrist and pull down to knuckles.



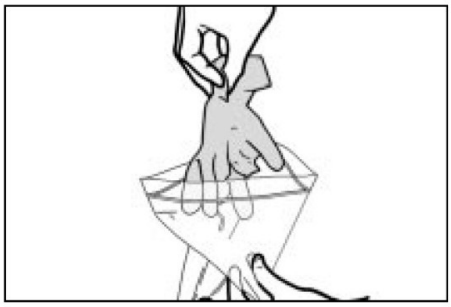
2. Grasp other glove at wrist and pull down to knuckles.



3. Grasp wrist end of one glove and pull it off completely.



4. Remove other glove in similar way touching only the inside of gloves.



5. Dispose of gloves in an appropriate container.

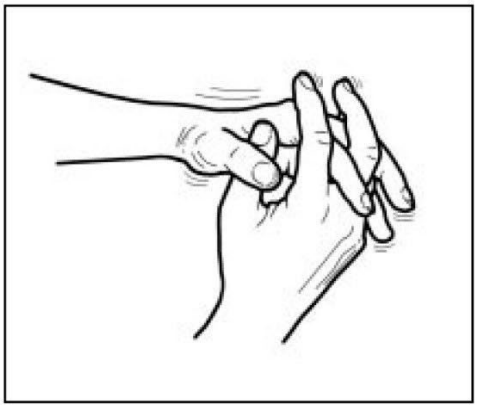
6. Wash hands after removing and disposing of gloves.

Figure 6-2: Removal of Gloves Technique (The following instructions were adapted from the Lawrence Berkely National Laboratory Biosafety Manual 2010)

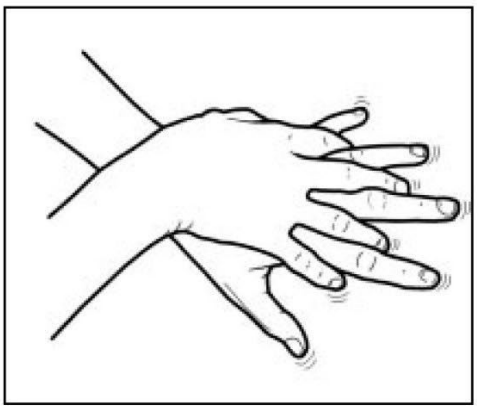
Hand Washing Technique

1. Use soap and water
2. Vigorously wash hands for 20 to 30 seconds, using the following pictures as guides
3. Rinse hands with water
4. Dry hands thoroughly

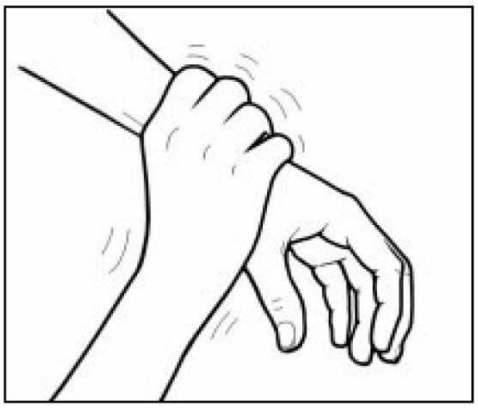
1. Wash palms.



2. Wash between fingers.



3. Wash back of hands.



4. Wash wrists.

Figure 6.3 Handwashing Technique (The following instructions were adapted from the *Lawrence Berkely National Laboratory Biosafety Manual 2010*)

Section 7 - DECONTAMINATION

Decontamination describes the process or treatment of medical devices, instruments, or environmental surfaces which makes them free of pathogens. A decontamination procedure can range from sterilization to simple cleaning with soap and water. Sterilization, disinfection and antisepsis are all forms of decontamination.

Sterilization is the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.

Disinfection eliminates virtually all pathogenic non-spore-forming microorganisms but not necessarily all microbial forms on inanimate objects (work surfaces, equipment, etc.).

Antisepsis is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms. It includes swabbing an injection site on a person or animal and hand washing with germicidal solutions. Manufacturers' recommendations for appropriate use of germicides should always be followed.

Methods of Decontamination

There are four main categories of physical and chemical means of decontamination:

- Wet Heat
- Dry Heat
- Liquid Disinfection
- Vapors and Gases

Wet Heat

This is the most dependable method of sterilization and can be achieved by autoclaving. See Section 8.5 Infectious Waste: Autoclaving for more details.

Use wet heat for the decontamination of organic material and consumable waste.

Dry Heat

Dry heat is less efficient than wet heat and requires a longer exposure time to higher temperatures in order to achieve sterilization.

Use dry heat for decontamination of nonorganic material (i.e. glass). Sterilization of glassware by dry heat can be accomplished at 160-170°C for periods of 2-4 hours. Use of biological indicators strips (*B. subtilis (globigii)*) can help to ensure proper decontamination.

Liquid Disinfection

Liquid disinfectants are practical for surface decontamination. Liquid disinfectants, at sufficient concentration, may also be used for liquid waste prior to final disposal in the sanitary sewer.

Liquid disinfectants must be a Selected EPA-Registered Disinfectant (www.epa.gov) and must be shown to be effective against the target organism(s) to be deactivated. Always follow EPA recommendations and contact time.

Liquid disinfectants are divided into the following categories:

- Aldehydes
- Halogen-based biocides
- Quaternary Ammonium Compounds

- Phenolics
- Acids/Alkalis
- Heavy Metals

Alcohols

See Table 1 below for examples, activity and information on the efficacy of each category of liquid disinfectants. See Table 2 for examples of liquid disinfectants and use guidance.

Vapors and Gases

A variety of vapors and gases possess decontamination properties. Vapors and gases are primarily used to decontaminate biological safety cabinets and associated systems, bulky or stationary equipment not suited to liquid disinfectants, instruments or optics which might be damaged by other decontamination methods, and rooms, buildings and associated air handling systems. Agents included in this category are glutaraldehyde and formaldehyde vapor, ethylene oxide gas, peracetic acid and hydrogen peroxide vapor. When used in closed systems and under controlled conditions of temperature and humidity, excellent disinfection can be obtained. Great care must be taken during use because of the hazardous nature of many of these compounds. Contact EHRS for monitoring requirements if these compounds are to be used.

Radiation

Although ionizing radiation will destroy microorganisms, it is not a practical tool for laboratory use.

Non-ionizing radiation in the form of ultraviolet radiation (UV) is used for inactivating viruses, bacteria and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces or in the presence of products of unstable composition that cannot be treated by conventional means.

Limitations of UV decontamination:

- Because of the low penetrating power of UV, microorganisms inside dust or soil particles, or under solid objects will be protected from its action, limiting its usefulness.
- Because UV can cause burns to the eyes and skin after a short exposure period, proper shielding must be maintained when it is in use.
- Because UV lamp intensity or destructive power decreases with time, it should be checked with a UV meter yearly. Frequent cleaning every few weeks is necessary to prevent accumulation of dust and dirt on the lamp that also reduces its effectiveness drastically.
- Use UV light must be done in areas that are not occupied by personnel.
- For more information, see the *"Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets"*, Applied Biosafety, 11(4) pp. 228- 230, ABSA 2006.

Disinfectants:

Disinfectants are substances that can destroy or inactivate bacteria, fungi, viruses, and sometimes spores. Different types of disinfectants can work on either hard non-porous surfaces or in liquid with organic load. The EPA regulates disinfectants and how they are used to ensure efficacy and safety. Users should always read and understand manufacture guidance when using any disinfectant. Some disinfectants may also have to be collected as chemical waste if sewer disposal is not appropriate.

- **Table 1** shows disinfectants by group. Please see the **Selected EPA-Registered Disinfectants** on the EPA website, for a complete list of products available which are effective against common pathogens.
- **Table 2** lists commonly recommended disinfectants for non-porous high touch surfaces in the lab. Contact a Biosafety Officer for additional guidance on selecting an appropriate disinfectant.

Table 7.1: Disinfectant Groups

Disinfectant Group	Activity	Efficacy	Disadvantages	Examples
Aldehydes	Coagulates cellular proteins. Good for bacterial, fungal, TB, viral, and spores.	Surface and space decon as a gas and liquid. Relatively non-corrosive. Works on material that can't be autoclaved.	Not stable in solution. An alkaline solution. Inactivated by organic material. Skin and respiratory irritant. Sensitizer. Toxic.	Calgocide 14, Cidex, Vespore, Formaldehyde
Chlorine Compounds	Free available chlorine combines with the microorganism to react and cause death. Good for human body fluids, wide bacterial spectrum, fungicidal, sporicidal, viral (lipid and non-lipid viruses).	Kills a wide variety of pathogens. Penetrates organisms and cells well. Short contact time. Tuberculocidal with extended contact time. Needs 50-5000ppm in solution.	Will compete for chlorine ion, making is organic load dependent. Increase alkalinity will decrease efficacy on bacteria. Solution degrades over time and exposure to UV/sun. Corrosive. Odorous. Irritant.	Chlorine solutions, Clidox S Base, Purerox
Iodophors (Iodine with a carrier)	Quickly penetrates cell wall of microorganism; disrupts protein and nucleic acid structure and synthesis. Carrier helps penetrate soil/fat. Good for bacterial, fungal, and viral. Acts as oxidizing agent.	Kills broad range of organisms. Low tissue toxicity. Short contact time. Safe for food prep surfaces. Not affected by hard water. Need 30 to 50 ppm in solution.	Iodine alone is unstable. Antiseptics should not be used as surface disinfectants. Corrosive on metal and may stain. Irritant. Toxic.	Iodine, Wescodyne, Povidone-iodine
Quaternary Ammonium Compounds (QUATS)	Cationic detergents. Biostatic action causes membrane damage and leakage, followed by denaturation. Most effective against gram positive bacteria. Good for water baths.	Activity is reduced in the presence of heavy organic matter loads. Good for water baths, incubators, and applications where halideor phenolic residues are not desired.	Generally ineffective against viruses, spores, and *Mycobacterium tuberculosis*.	Coverage Plus NPD, End-Bac II Spray, Lysol, Vindicator+, Virex II
Phenolics	Gross protoplasmic poison, disrupts cell walls. Precipitates cell proteins. Low concentrations can inactivate enzyme systems. Good for vegetative bacteria, fungal, TB, lipid containing viruses.	Effective against vegetative bacteria and lipid-containing viruses. Commonly used as an antiseptic.	Not as effective in present of alkaline/hard water, natural soap or organic matter. Can be absorbed through latex gloves. Penetrates intact skin. Toxic. Skin & eye irritant. Sensitizer. Corrosive. Odorous.	Vesphene III, LpH III, Hil-Phene (Hillyard)
Alcohols	Disruption of cellular membranes, stabilization of lipids and denaturation of proteins, cell lysis. Active against vegetative bacteria and lipid-containing viruses.	Water is required for effective disinfection process. No residue. Best when used to immerse/soak items, such as surgical instruments.	**Not recommended as primary disinfectant!** Not effective with most viruses. Evaporate quickly, interferes with long contact time. Not effective with organic matter. Flammable. Irritant. Toxic.	Ethanol and isopropyl alcohol at 70 to 80% aqueous solutions, Cavicide

Table 2: Liquid Disinfectants

Product Name	Formulation: Ready to Use or Concentrate	Use dilution (if applicable) and contact time	Appropriate for decontamination of liquid waste*
Alcohols with additional active ingredients (e.g. quaternary amines or phenolics)			
Cavicide	Ready to use (RTU)	RTU;3-minute contact time	No*
CaviWipes	Ready to use	RTU;3-minute contact time	No*
Lysol® Disinfectant Spray	Ready to use	RTU;10-minute contact time	No*
Sodium hypochlorite			
Bleach	Concentrate	500ppm; 1:10 v:v; 5-minute contact time	Yes
Sani-Cloth Bleach Germicidal Disposable wipe	Ready to use	RTU;1-minute contact time	No*
Quaternary ammonium			
Sani-cloth Germicidal Disposable Cloth	Ready to use	RTU; 3-minute contact time	No*
Lysol® Brand All Purpose Cleaner	Ready to use	RTU; 10-minute contact time	No*
Virex™ II / 256	Concentrate	1:256 v:v; 10-minute contact time	Yes
Lysol® Disinfecting Wipes (All Scents)	Ready to use	RTU; 10-minute contact time	No*
Phenolics			
LpH® IIIse Phenolic Disinfectant	Concentrate	1:128 v:v; 10-minute contact time	Yes
Vesphene IIIse Phenolic Disinfectant	Concentrate	1:128 v:v; 10-minute contact time	Yes
Hydrogen peroxide			
Peroxigard	RTU or Concentrate	Concentrate (1:16 v:v); 5-minute contact time	Concentrate only: Yes
Peridox	Concentrate	1:5 v:v; 3-minute contact time	Yes

* Disinfectants listed as not appropriate for decontamination of liquid wastes may be used on non-porous surfaces, but must not be used to decontaminate liquid wastes due to either their availability only as ready to use formulations or chemical waste disposal regulations, or both.

Adapted from University of Pittsburgh Environmental Health and Safety.

Section 8.1 - INFECTIOUS WASTE MANAGEMENT

Infectious waste refers to biological material (viral and bacterial cultures, human cells lines, tissue samples, organs, blood and blood products, live and attenuated vaccines, or any recombinant material) creating in research laboratories and requiring disposal.

Infectious waste must be disposed of through the **Infectious Waste Stream**. The Infectious Waste Stream refers to the segregation of different types of waste listed above that require disposal in a specific manner.

Infectious Waste Streams

There are three categories of infectious waste:

- infectious sharps waste
- infectious liquid waste
- infectious solid waste

Infectious Sharps Waste - Any device/item having corners, edges, or projections capable of cutting or piercing the skin or puncturing a waste bag.

Infectious Liquid Waste – Liquid material that is infected with biological agents, including human blood and body fluids, liquid culture media, viral supernatant, media from infected cells, or recombinant material.

Infectious Solid Waste – Other waste produced in the lab that may be potentially infected with biological material but is not categorized as infectious sharps waste, including non-sharp plastic consumables, agar gels, gloves, gowns, etc.

ALL syringes (with and without needles), scalpels, and razors, whether contaminated or not, are disposed of in an infectious waste sharps container.

Infectious Waste Management

Sharps Waste

See Section 8.2 for guidance on sharps waste management

Liquid Waste

Liquids must be decontaminated prior to disposal through the sanitary sewer system by either an appropriate disinfectant or autoclaving.

Solid Waste

1. Biological material must be disposed of directly into a red or orange biohazard waste bag.
2. Solid waste must be collected in autoclavable biohazard bags contained in a hard-sided, leak proof, lidded container.
3. All bags marked with the red or orange biohazard symbol, whether used or not, must be disposed

of as infectious waste. Figure 8-1

4. All infectious waste must be segregated properly at time of generation.

5. DO NOT autoclave biological waste contaminated with chemicals.
6. DO NOT discard any infectious waste through the regular waste stream.
7. DO NOT use the biohazard bag for any other purpose than that for which it is intended.

Procedures

1. All personnel handling infectious waste must complete **Management of Laboratory Waste** training, found in WorkDay@Penn**.**
2. Each generator is responsible for segregating their infectious waste. See the Laboratory Waste Disposal Guide and Know Where to Throw posters below and in Appendix D.
3. Dispose of all material with the Universal Biohazard Warning Sign through the infectious waste stream.
4. See the Biohazardous Waste Disposal Guides, found on the EHRS website, specific to your school, center, or building for more details on collection and disposal of all laboratory waste.
5. AUTOCLAVING: Infectious Waste may be required to be autoclaved prior to disposal in the infectious waste stream, depending on the location of the lab. See the Biohazardous Waste Disposal Guides on the EHRS website specific to your school or center for more details on collection and disposal of infectious waste red bags.
6. Infectious waste needs to be properly packaged for removal and incineration by the University's infectious waste hauler.
7. An Infectious Waste Manifest documents the transfer of waste from the University of Pennsylvania to the contracted waste hauler. ONLY trained personnel may sign off on the Federal Document: **Infectious Waste Manifest.** Contact a biosafety officer with EHRS to receive **Training for Personnel Who Collect & Ship Infectious Waste**.

Sharps Waste

1. Sharps containers must be used with their lid in place.
2. Sharps containers must be closed and discarded when they are ¾ full.
3. Disposable sharps containers marked with "Chemical Contaminated Sharps DO NOT AUTOCLAVE" are placed directly into the collection bin for the infectious waste stream.
4. Sharps containers marked with RADIOACTIVE must not be put into the infectious waste stream. Contact Radiation Safety for more information on how to dispose of Mixed Waste.
5. Reusable sharps containers are picked up by the vendor at their location.

Liquid Waste

1. Liquid infectious waste in volumes greater than 20 cc must be segregated and decontaminated, either by autoclaving or by adding an appropriate disinfectant.
2. Decontaminated liquids can be disposed of into the sanitary sewer system with large amounts of water.
3. Reusable liquid containers must be washed and autoclaved prior to reuse.

Solid Waste

1. See the Biohazardous Waste Disposal Guides on the EHRS website specific to your school or center for more details on collection and disposal of infectious waste red bags.

2. Solid waste must be collected directly in autoclavable biohazard bags. Both the bags and their containers must be labeled with the universal biohazard symbol.

3. DO NOT allow infectious waste to accumulate. Infectious waste must be disposed of on a regular basis.

Solid Infectious Waste MUST NOT be discarded in the regular trash!!!

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Environmental Health & Radiation Safety

Rev. 01292025

Know Where to Throw Lab Waste

Sharps Containers

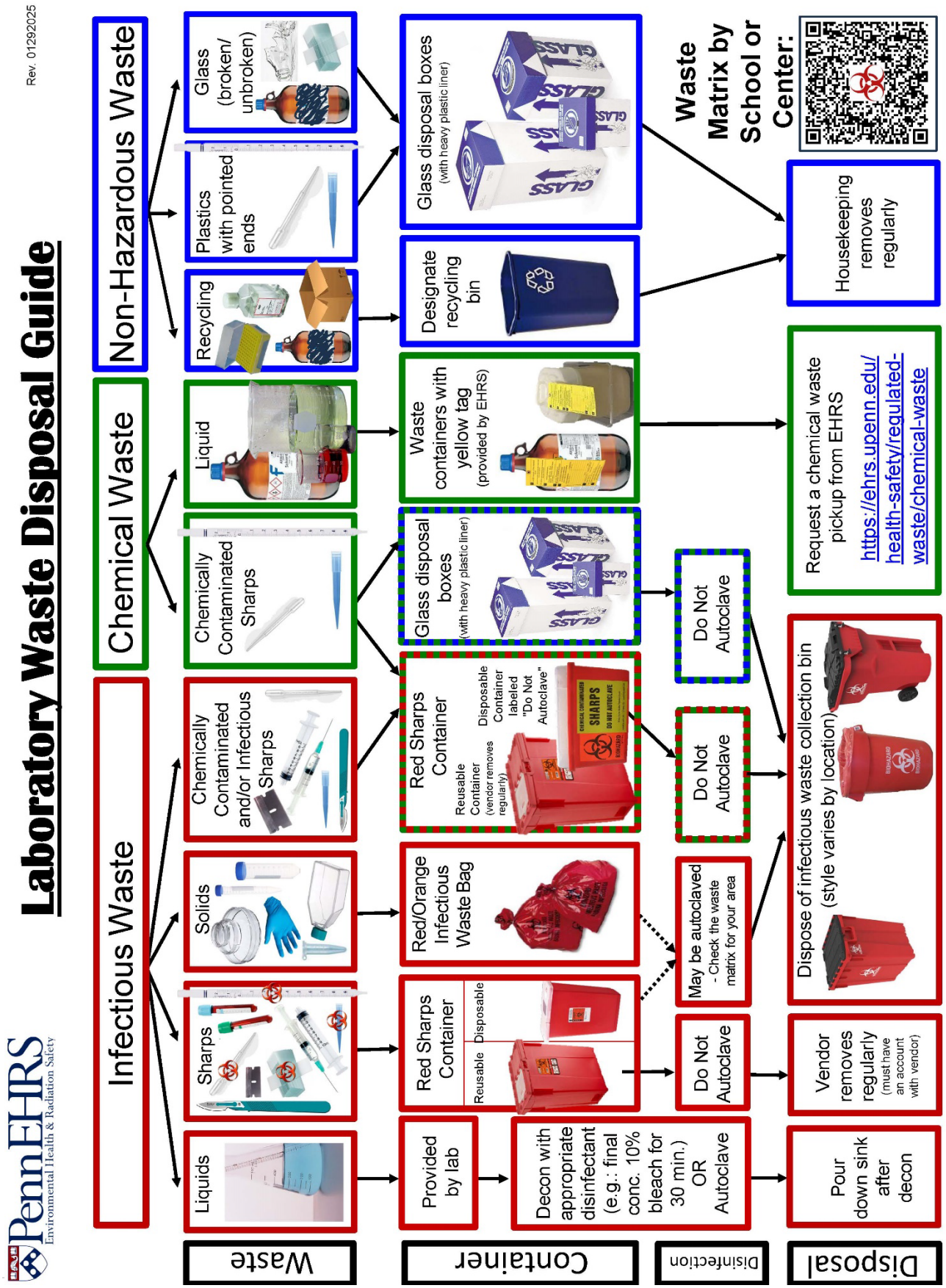
- **ALL needles, syringes (with/without the needle), and blades**
- Infectious (or potentially infectious) pipette tips, serological pipettes, blood vials, slides/cover slips, other glass
- Nominal contamination with chemicals

Biohazard Bags

- Infectious (or potentially infectious) disposable items
- culture flasks, dishes, centrifuge tubes, specimen bags, PPE,
- Items with a biohazard symbol

Glass Disposal Box

- Non-infectious/Non-hazardous items
- Pipette tips, serological pipettes, broken/unbroken glass, slides/cover slips, other pointy items, and empty chemicals bottles (triple rinsed)
- Nominal contamination with chemicals


Laboratory Waste Disposal Guide
Rev. 01292025
PennEHRS
Environmental Health & Radiation Safety
Infectious Waste
Chemical Waste
Non-Hazardous Waste
Waste
Container
Disinfection
Disposal
Liquids
Sharps
Solids
Chemically Contaminated and/or Infectious Sharps
Liquid
Chemically Contaminated Sharps
Recycling
Plastics with pointed ends
Glass (broken/ unbroken)
Provided by lab
Red Sharps Container
Reusable
Disposable
Red/Orange Infectious Waste Bag
Red Sharps Container
Reusable Container (vendor removes regularly)
Disposable Container labeled "Do Not Autoclave"
Glass disposal boxes
(with heavy plastic liner)
Waste containers with yellow tag
(provided by EHRS)
Glass disposal boxes
(with heavy plastic liner)
Designate recycling bin
Decon with appropriate disinfectant (e.g.: final conc. 10% bleach for 30 min.) OR Autoclave
Do Not Autoclave
May be autoclaved
- Check the waste matrix for your area
Do Not Autoclave
Do Not Autoclave
Pour down sink after decon
Vendor removes regularly
(must have an account with vendor)
Dispose of infectious waste collection bin
(style varies by location)
Request a chemical waste pickup from EHRS
https://ehrs.upenn.edu/health-safety/regulated-waste/chemical-waste
Housekeeping removes regularly
Waste Matrix by School or Center:

Section 8.2 - INFECTIOUS WASTE SHARPS

Sharps include any object that can potentially puncture a waste bag or cause injury to personnel, including syringes (with and without needles), scalpels, razors, pipette tips, serological pipettes, plastic transfer pipettes, blood vials, slides, cover slips and other broken or unbroken glass or plasticware.

Infectious Waste Sharps refer to those sharps that have been used in the manipulation of infectious agents, animal procedures, and human patient care or treatment. Infectious Waste Sharps must be disposed of in a red puncture resistant, leak proof, lidded container, marked with the biohazard symbol and labeled with the word "SHARPS"

These include: (Figure 8-2)

- hypodermic needles
- needles with attached tubing
- suture needles
- syringes (with or without the attached needle)
- scalpels
- razor blades
- Pasteur pipettes or plastic transfer pipettes
- pipette tips
- serological pipettes
- blood vials
- slides and cover slips
- other broken or unbroken glass or plasticware that has been used in the manipulation of biological material, animal procedures, or human patient care or treatment

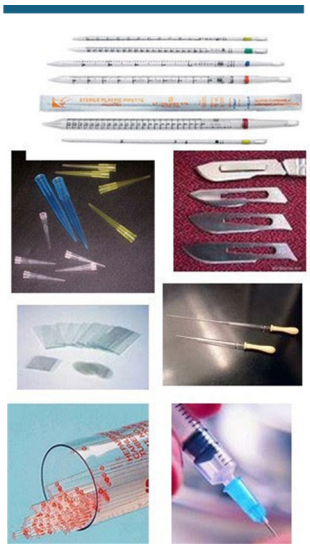


Figure 8.2 Examples of Sharps

Infectious Waste Management

1. Sharps must be disposed of directly into a sharps container. Figure 8-4
2. Contaminated sharps must not be reused
3. Syringes are not to be recapped prior to disposal.

DO NOT discard sharps through the regular waste stream.

4. DO NOT discard sharps in any bag.
5. DO NOT autoclave chemically contaminated sharps. Instead, place these contaminated sharps in a Reusable Sharps Container OR in a disposable container, label with "**Chemical Contaminated Sharps DO NOT AUTOCLAVE**", close when the container is ¾ full, and dispose of through the infectious waste stream. Figure 8-3.
6. ALWAYS keep the lid to a sharps container closed
7. Stop using and seal the lid when the container is ¾ full

Figure 8-3: Sharps container for chemical contaminated sharps

Procedures

SECTION 8.2 – INFECTIOUS WASTE SHARPS

1. Each generator is responsible for segregating their sharps waste. See the Proper Disposal of Sharp Objects posters below and in Appendix D for additional guidance or consultation with a Biosafety Officer.
2. See the Biohazardous Waste Disposal Guides on the EHRS website, specific to your school or center for more details on collection and disposal of sharps containers.
3. Sharps containers must be used with their lids in place
4. Sharps containers must be closed and discarded when they are ¾ full.
5. Sharps containers that are autoclaved (except for those marked "Chemical Contaminated Sharps DO NOT AUTOCLAVE") are placed into the collection bin for the infectious waste stream.
6. Sharps container marked with "Chemical Contaminated Sharps DO NOT AUTOCLAVE" are not autoclaved and are placed directly into the collection bin for the infectious waste stream. Contaminated disposables with TRACE AMOUNTS of chemical can go into the reusable sharps containers
7. Sharps containers marked as RADIOACTIVE **MUST NEVER** be put into the infectious waste stream. Contact the Radiation Safety team for more information on disposal of mixed waste.

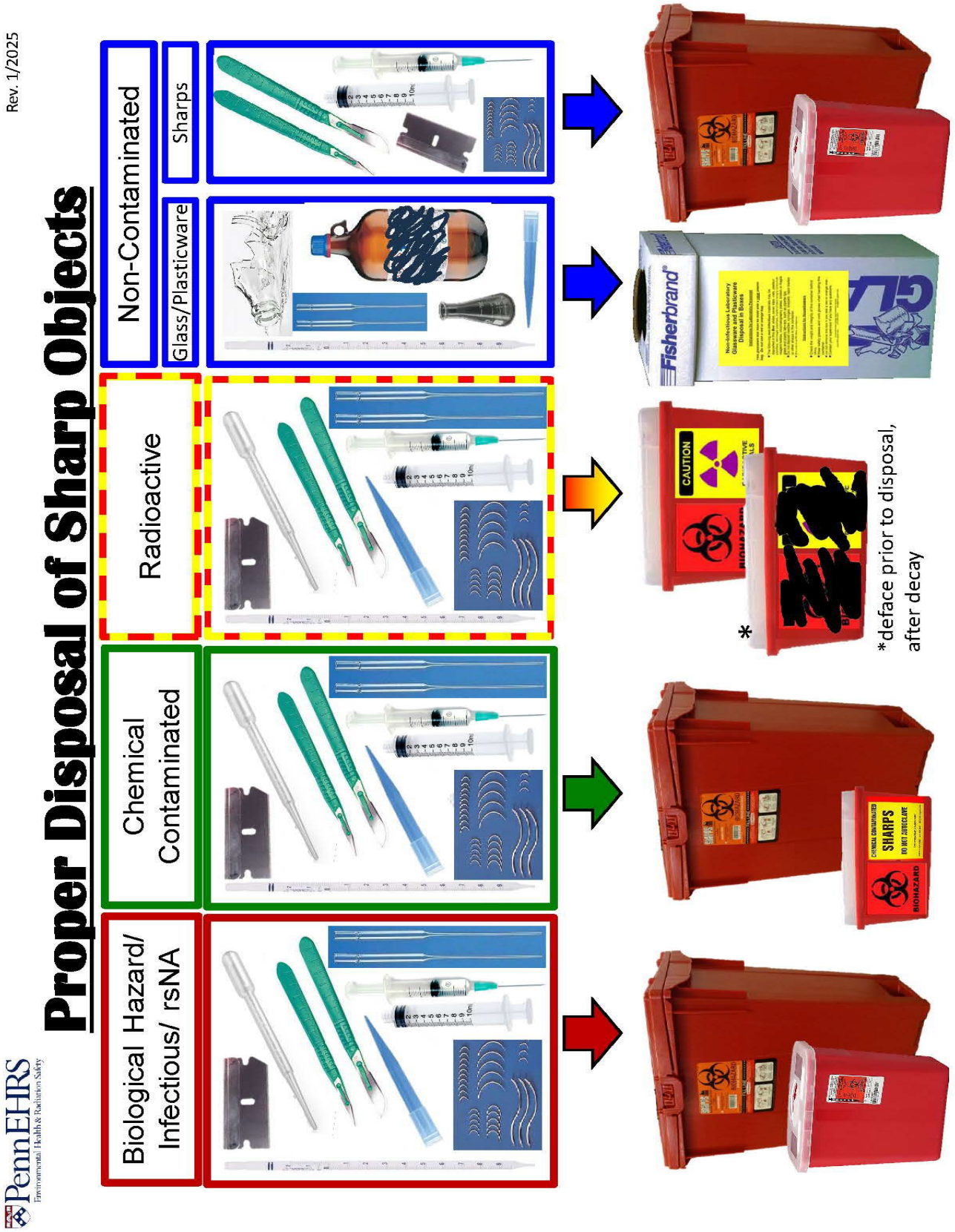
Reusable Sharps Container Program

The Reusable Sharps Container Program is available to all University of Pennsylvania laboratories for the disposal of biohazardous sharps waste.

Sharps containers are provided by the infectious waste vendor. The cost is for the weight of the waste in the container only, which average 17 pounds per container. Utilizing the Reusable Sharps Container program helps with safety and compliance with the disposal of sharps waste. Full containers are removed by the infectious waste vendor regularly.

New Account: Submit the UPenn Client Information Sheet directly to Sharps Medical Waste Services or contact a Biosafety Officer with questions. See the Biohazardous Waste section on the EHRS website for additional details and contact information.

Figure 8-4: Single Use and 17-gallon Reusable Infectious Waste Sharps Container


Rev. 1/2025
PennEHRS
Environmental Health & Radiation Safety
Proper Disposal of Sharp Objects
Biological Hazard/ Infectious/ rsNA
Chemical Contaminated
Radioactive
Non-Contaminated
Glass/Plasticware
Sharps
CAUTION
Fisherbrand
CHEMICALLY CONTAMINATED
SHARPS
DO NOT AUTOCLAVE
BIOHAZARD
*deface prior to disposal, after decay

Section 8.3 - MIXED WASTE

Mixed waste refers to liquid or solid waste that is contaminated with radioactive material AND either biohazardous agents and/or chemicals.

Chemical Contaminated Infectious Waste refers to liquid or solid waste that is contaminated with one or more chemicals.

Infectious Waste Management

1. DO NOT autoclave infectious waste that may be contaminated with either radioactive material or chemicals.
2. Decontaminate infectious material with appropriate disinfectant prior to placing mixed waste (radioactive) in the radiation bin. **Radiation Safety WILL NOT collect infectious material or waste in red bags or marked with the biohazard symbol.**
3. DO NOT put radioactive material in the infectious waste stream!
4. Use appropriate receptacles, such as red biohazard bags or properly labeled sharps containers, to collect chemical contaminated infectious waste.

Procedures

Provisions must be made for proper disposal of mixed waste or chemical contaminated infectious waste **PRIOR** to its creation.

Contact the Radiation Safety team regarding the disposal of mixed waste or chemical contaminated infectious waste.

Radioactive Mixed Waste

1. Decontaminate infectious material with an appropriate disinfectant.
2. Deface all “biohazard” and “regulated medical waste” symbols and markings from decontaminated material before disposing of as radioactive waste
3. Collect decontaminated radioactive mixed waste in the appropriately labeled radiation bin.
   * See the Radiation Safety Researcher's User Guide available on the EHRS website for more information.

Chemical Contaminated Waste

1. Segregate infectious waste that is contaminated with chemicals into appropriate collection devices (biohazard bag or sharps container) and label with “Chemical Contaminated - DO NOT AUTOCLAVE”.

2. Dispose of chemical contaminated infectious waste through the infectious waste stream without autoclaving.

Section 8.4 - ANIMAL WASTE MANAGEMENT

Waste that is created from the use of research animals, including animal carcasses, body parts, blood, blood products, secretions, excretions and bedding, that were known to have been exposed to zoonotic infectious agents or non-zoonotic human pathogens during research (including research in veterinary schools and hospitals), production of biologicals, or testing of pharmaceuticals. All of this material must be treated as infectious waste and disposed of through the infectious waste stream.

Infectious Waste Management

1. All non-radioactive animal carcasses are to be returned to University Laboratory Animal Resources (ULAR) to be disposed of through the infectious waste stream. Each vivarium has a morgue freezer for the disposal of carcasses. Animals from barrier facilities must be collected in external morgue freezers available to researchers.
2. Radioactive animal carcasses are collected by EHRS for decay and then disposed of through the infectious waste stream.
3. Disposal of any animal carcasses, whether infectious or not, in the general waste stream is PROHIBITED!!!

Procedures

1. Contact your vivarium manager for complete details on disposal of animal waste
2. Proper PPE, including gloves, safety glasses, and surgical mask should be worn when disposing of animal carcasses and bedding

Animal waste **MUST NEVER** be discarded in the regular trash

Section 8.5 - AUTOCLAVING INFECTIOUS WASTE

Autoclaves are specialized pieces of equipment designed to deliver steam generated heat under pressure to a sealed chamber with the goal of decontaminating or sterilizing its contents. (Figure 8-5).

Figure 8.5 Autoclave

Infectious Waste Management

1. All cultures and stocks of infectious agents, consumables used in the manipulation of said cultures, recombinant and synthetic nucleic acids (rsNA), human and infectious animal tissue waste, cages and bedding of animals contained in ABSL-2 and ABSL-3, and reusable lab ware must be disposed of appropriately through the infectious waste stream. See the Waste Disposal Segregation Guide on the EHRS website for additional guidance as waste inactivation prior to disposal (i.e. autoclaving waste) may not be required in your building or center.
   - DO NOT AUTOCLAVE items contaminated with solvents, volatile or corrosive chemicals, or items containing carcinogens, mutagens or teratogens.
2. Contact a Biosafety Officer for guidance on how to dispose of non-autoclavable items properly.
3. Various devices can be used to indicate proper function of an autoclave, ranging from the least reliant (autoclave tape) to Chemical and Biological Indicators. Figure 8-6.
   - Autoclave tape gives you a visual indication that the item has passed thru steam sterilization. Autoclave tape does not provide assurance of sterility.
   - Chemical Integrator Strips consists of a steam and temperature sensitive chemical pellet, enclosed in a paper/foil envelope, which melts and migrates when exposed to autoclave conditions. The distance of migration depends on the exposure to steam, time, and temperature.
   - Biological Indicators (BIs) provide the best assurance of sterility by challenging the sterilizer with quantifiable and highly heat resistant bacterial (*B. stearothermophilus*) spores. BIs must be incubated from 48-72 hours to obtain results, usually defined by a color change.

EHRS recommends that a service contract be established for each autoclave and maintenance be performed annually and when needed. Penn does not have an institutional policy mandating that autoclaves supporting research laboratories undergo a periodic validation or be included in an autoclave validation program.

Figure 8-6: Devices to indicate proper function of an autoclaved

Figure 8-7: Biohazard bags placed in autoclavable secondary containers prior to autoclaving

Section 9 - TRANSPORT AND SHIPPING OF BIOHAZARDOUS MATERIAL

For a detailed discussion of IATA Dangerous Goods regulations, requirements, and Penn policies regarding shipping of biological hazards consult Penn's *Shipping Manual for Infectious Substances and Biological Materials*.

Intramural Transport

When transporting biohazardous materials on Penn's campus take precautions to communicate the hazard to those around you and follow best practices to prevent an accidental spill. Transport all biohazardous materials (tissues, blood samples, contaminated supplies, etc.) in a rigid, securely sealed, watertight primary container, contained within a second rigid, sealed, watertight container. Add sufficient absorbent to the second container to take up contents of the first container in case of leakage. Label the outer container with the universal biohazard symbol.

EHRS must approve the transport of experimentally infected animals that are removed from the animal facility. When transporting infected animals between the animal facility and the laboratory, place them in cages fitted with filter bonnets and transport them on carts with sides. Outer containers and/or animal cages must be labeled with the universal biohazard symbol.

Extramural Transport

The packaging and shipping of biological materials for extramural transport must comply with federal and international Dangerous Good regulations. It is the intent of the regulations that biological material, which may contain infectious agents, will be packaged and shipped in such a way that the contents will not leak and will arrive in good condition. The shipper (i.e., person with direct knowledge of what is being shipped) must be trained every 24 months to IATA standards and be familiar with the most current packaging and shipping requirements. Consult Penn's *Shipping Manual for Infectious Substances and Biological Materials* for guidance in the packaging and shipping of diagnostic specimens, biological and infectious substances, material on dry ice, import and export of biological materials, live organisms, and resources for appropriate forms and supplies.

Permits

Importing Infectious Agents

The following items will require an import permit from CDC:

Etiologic materials

An import permit is required for any infectious agent that may cause disease in humans. This includes but is not limited to bacteria, bacterial toxins, viruses, fungi, rickettsiae, protozoans, and parasites.

Biological materials

Unsterilized specimens of human and animal tissues (such as blood, body discharges, fluids, excretions or similar material) containing an infectious or etiologic agent require a permit in order to be imported.

Host and vectors:

1. Animals: Animals known or suspected of being infected with any disease transmissible to man, the importation of turtles less than 4 inches in shell length and all nonhuman primates requires an importation permit issued by the CDC, Division of Global Migration and Quarantine. Animals known or

suspected of being infected with any disease transmissible to livestock or poultry required an importation or interstate permit issues by the USDA-APHIS.

2. Biological materials: Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions, or similar material, when known or suspected to be infected with disease transmissible to humans requires an importation permit issued by the CDC.
3. Insects: Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to humans. Also, if alive, any fleas, flies, lice, mites, mosquitoes, or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.
4. Snails: Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either CDC or the Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.
5. Bats: All live bats require an import permit from the CDC and the U.S. Department of Interior, Fish and Wildlife Services. Bat specimens require an import permit from the CDC.
6. Vectors, protein, and other biological materials: Many pathogenic vectors and various components there of may be regulated by different federal agencies for import into the US, including biological material made with or possibly contaminated with such materials. Confirm that no import permit is required prior to collaborating to have biological material imported into the US.

When an etiologic agent, infectious material or vector containing an infectious agent is being imported to the United States it must be accompanied by an importation permit issued by the US Public Health Service (USPHS).

Importation permits are issued only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by U.S. Customs.

The importer is legally responsible to assure that foreign personnel package, label, and ship material in accordance with CDC and IATA regulations. Shipping labels, permit number, packaging instructions and the permit expiration date are also issued to the importer with the permit. For more information consult Penn's *Shipping Manual for Infectious Substances and Biological Materials*.

Non-Infectious Material

If an importation permit is not issued from a federal agency, such as the CDC, because the materials have been judged to be no regulated, a formal statement letter MUST be composed describing the material by the shipper to accompany the shipment, as required by U. S. Customs and Boarder Protection. Examples of such items may be formalin fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine cerebrospinal fluid, and other tissues or materials of human origin when there is no evidence or indication that such materials contain an infectious agent. A TEMPLATE of such a letter statement is available in the Shipping section of the EHRS website.

7. Application to any federal agency for an importation permit should be made 4-6 weeks in advance of the shipment date to allow time for processing, issuance and delivery of the permit and shipping labels to the permittee. See the Import/Export section of the EHRS website for additional information on federal agencies issuing import permits or connect with U. S. Customs and Boarder Protection for additional guidance.

Animal, Plant and Other Permits:

U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required to import or interstate transport of infectious agents of livestock and poultry. Biological materials containing animal material, particularly that derived from, but not limited to, livestock or poultry. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U. S. Applications for USDA/APHIS permits may be obtained from eFile at APHIS Veterinary Services (VS). Further information may be obtained by contacting USDA/APHIS at APIE@usda.gov.

Export Controls and Permits

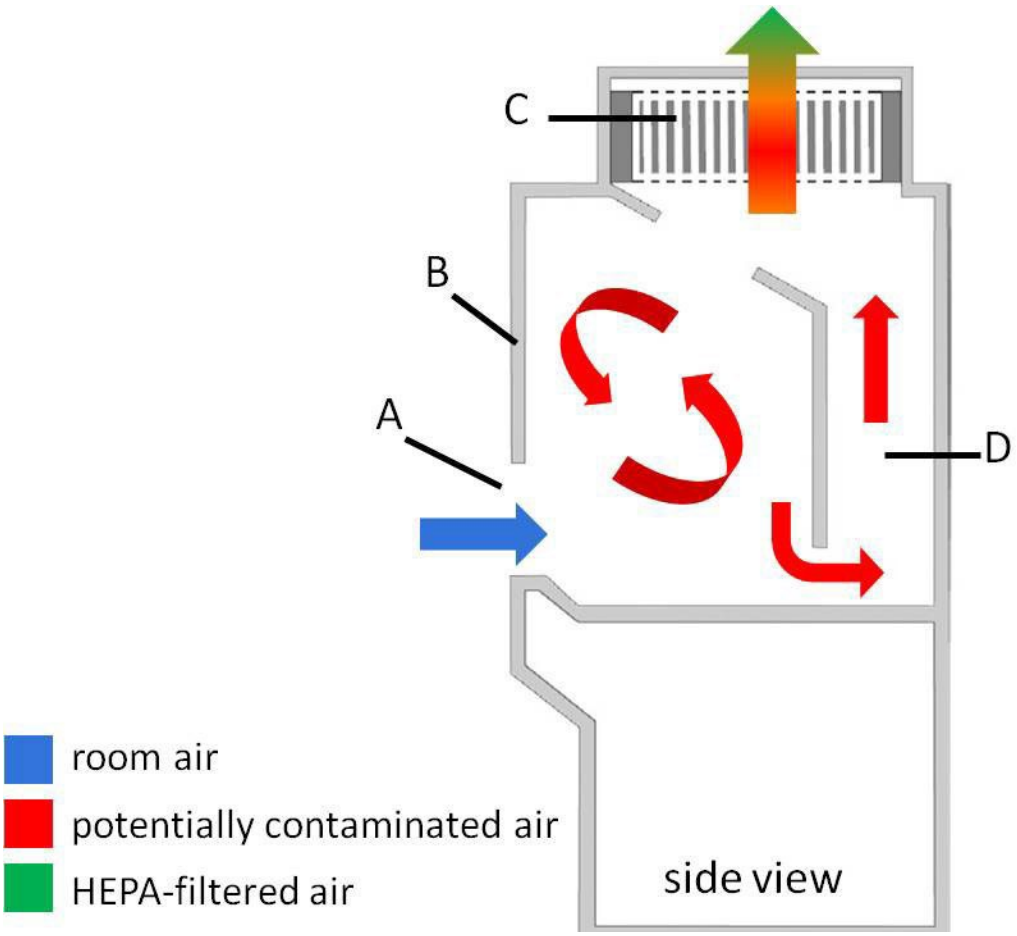
Depending on the nature of the shipment, a U.S. export permit may be required when sending your package. Additionally, an import permit may be required into the country where the package is being shipped. If your shipment requires an export permit, it must be completed and approved by the appropriate government agency **prior** to shipment. If you have questions about Export Controls and Permits, please contact Penn Export Compliance. If the receiver also needs an import permit, they will need to reach out to their safety or shipping support team or local agency overseeing that permit.

APPEDICIES

Biosafety Cabinets

Biosafety Cabinet Air Flow: Class I

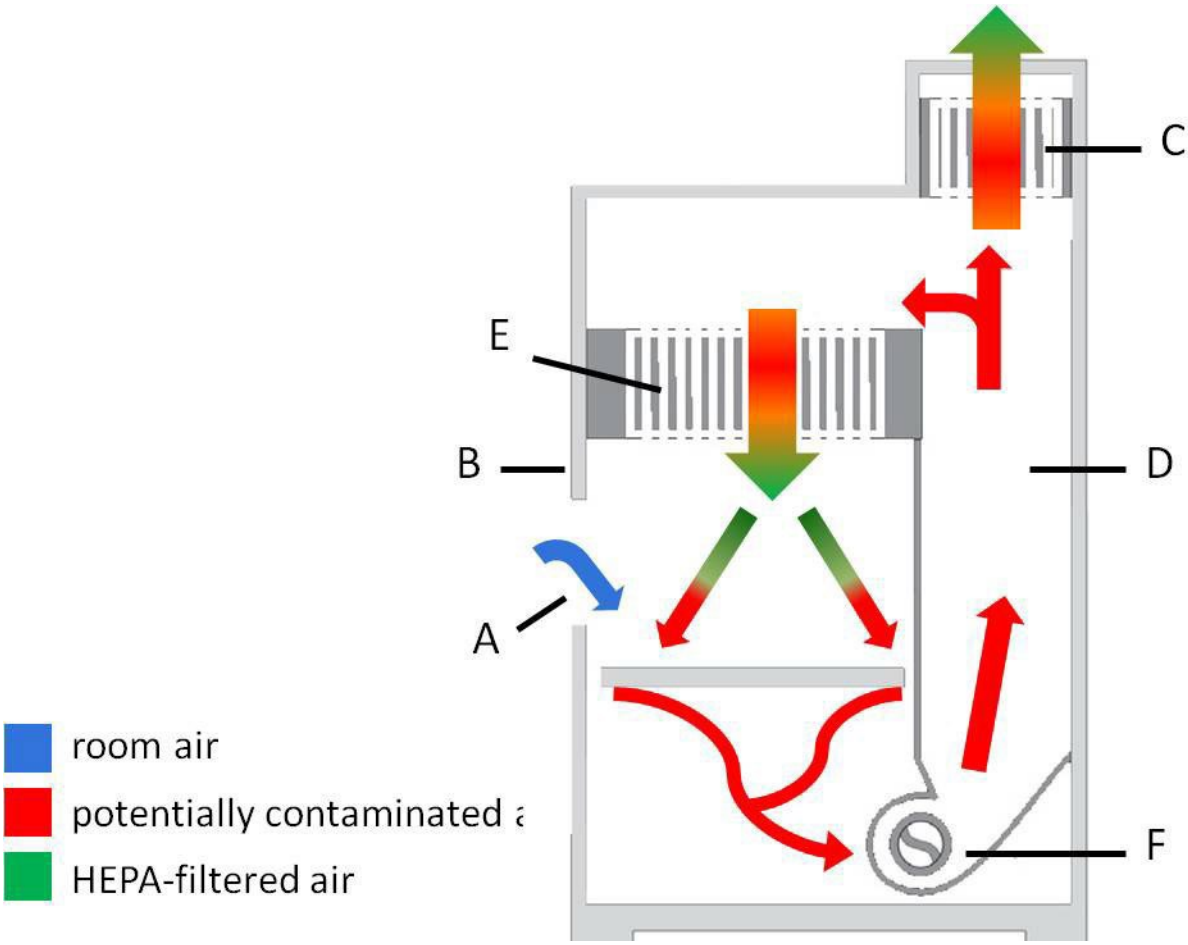
The Class I BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) exhaust plenum. Note: The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used



(adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

Biosafety Cabinet Air Flow: Class II A1

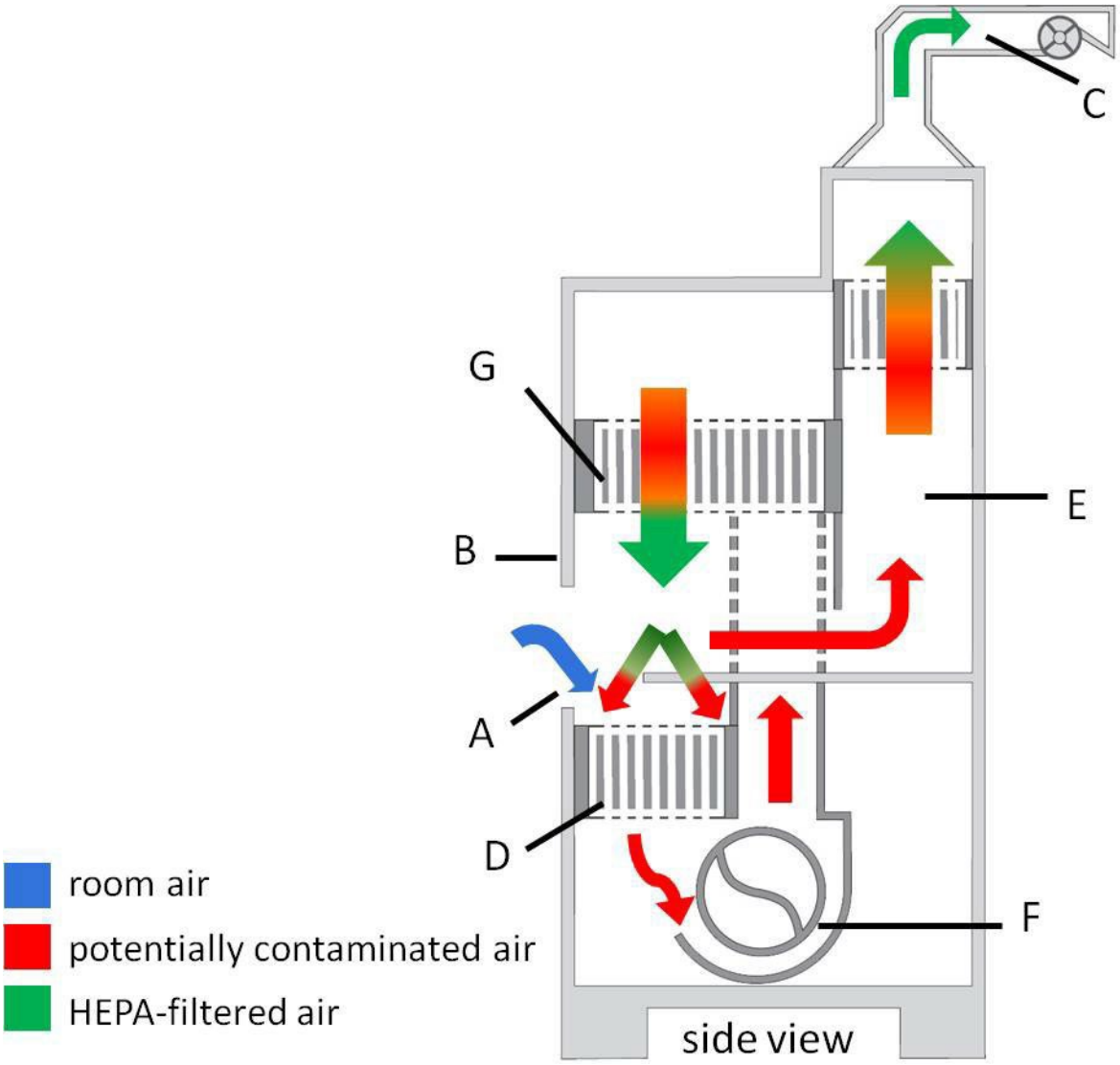
The Class II, Type A1 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D)supply HEPA filter; (E) common plenum; (F) blower.



(adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

Biosafety Cabinet Air Flow: Class II B1

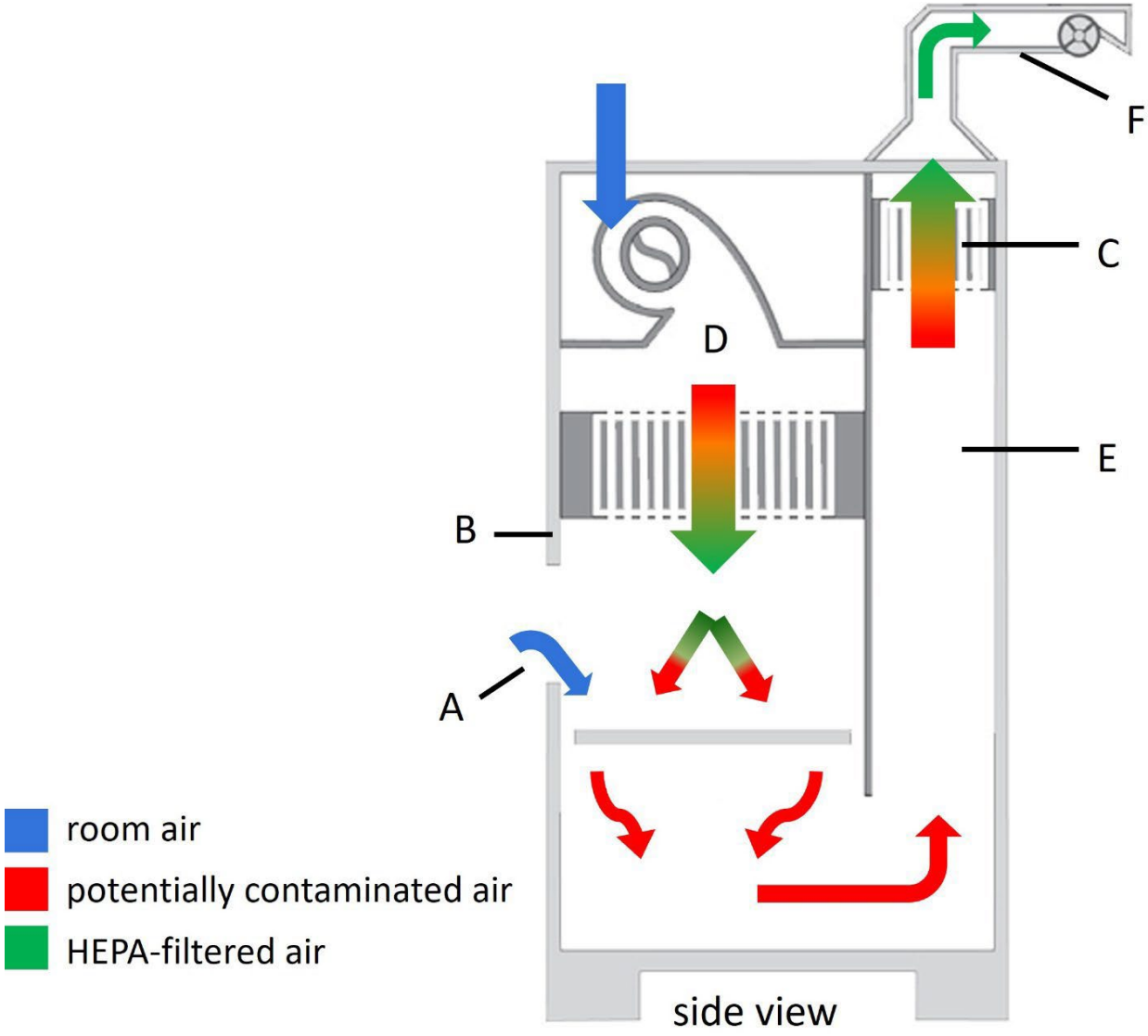
The Class II, Type B1 BSC (classic design) (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. Note: The cabinet exhaust needs to be hard connected to the building exhaust system.



2(adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

Biosafety Cabinet Air Flow: Class II B2

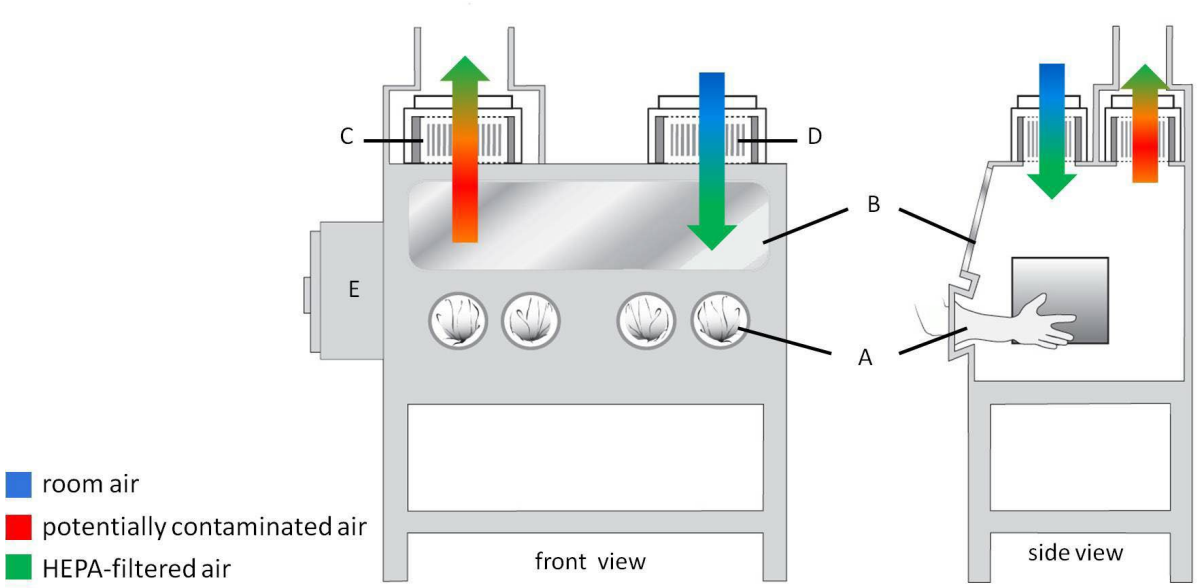
The Class II, Type B2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D)supply HEPA filter; (E) negative pressure exhaust plenum. Note: The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.



(adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

Biosafety Cabinet Air Flow: Class III

The Class III BSC "Glove Box" (A) glove ports with O-ring for attaching arm- length; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double- ended autoclave or pass-through box. Note: A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to the building exhaust system.



(adapted from Biosafety in Microbiological and Biomedical Laboratories; BMBL 6th Edition, 2020)

Select Agents & Toxins List

(HHS and USDA Select Agents and Toxins 7CFR Part 331, 9CFR Part 121, and 42 CFR Part 73)

HHS SELECT AGENTS AND TOXINS

1. Abrin
2. *Bacillus cereus* Biovar *anthracis**
3. Botulinum neurotoxins*
4. Botulinum neurotoxin producing species of *Clostridium**
5. Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X1CCX2PACGX3X4X5X6CX7)[1]
6. *Coxiella burnetii*
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol
9. Eastern Equine Encephalitis virus[3]
10. Ebola virus*
11. *Francisella tularensis**
12. Lassa fever virus
13. Lujo virus
14. Marburg virus*
15. Monkeypox virus[3]
16. Reconstructed replication competent forms of the1918 pandemic influenza virus
17. Ricin
18. *Rickettsia prowazekii*
19. SARS-associated coronavirus (SARS-CoV)
20. Saxitoxin

South American Haemorrhagic Fever viruses:

21. Chapare
22. Guanarito
23. Junin
24. Machupo
25. Sabia
26. Staphylococcal enterotoxins A,B,C,D,E subtypes
26. T-2 toxin
27. Tetrodotoxin

Tick-borne encephalitis complex (flavi)viruses:

29. Far Eastern subtype
30. Siberian subtype
31. Kyasanur Forest disease virus
32. Omsk hemorrhagic fever virus
33. Variola major virus (Smallpox virus)*
34. Variola minor virus (Alastrim)*
35. *Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

36. *Bacillus anthracis**
37. *Bacillus anthracis* Pasteur strain
38. *Brucella abortus*
39. *Brucella melitensis*
40. *Brucella suis*

41. *Burkholderia mallei**
42. *Burkholderia pseudomallei**
43. Hendra virus
44. Nipah virus
45. Rift Valley fever virus
46. Venezuelan equine encephalitis virus[3]

USDA SELECT AGENTS AND TOXINS

47. African horse sickness virus
48. African swine fever virus
49. Avian influenza virus[3]
50. Classical swine fever virus
51. Foot-and-mouth disease virus*
52. Goat pox virus
53. Lumpy skin disease virus
54. *Mycoplasma capricolum*[3]
55. *Mycoplasma mycoides*[3]
56. Newcastle disease virus[2,3]
57. Peste des petits ruminants virus
58. Rinderpest virus*
59. Sheep pox virus
60. Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS ANDTOXINS

61. *Coniothyrium glycines* (formerly *Phomaglycinicola* and *Pyrenochaeta glycines*)
62. *Peronosclerospora philippinensis(Peronosclerospora sacchari)*
63. *Ralstonia solanacearum*
64. *Rathayibacter toxicus*
65. *Sclerophthora rayssiae*
66. *Synchytrium endobioticum*
67. *Xanthomonas oryzae*

**Denotes Tier 1 Agent*

Quick Reference Sheets

QUICK REFERENCE: HUMAN SOURCE MATERIAL

Version 2.1 2021

Quick Reference: Human Source Material

Penn EHRS Environmental Health & Radiation Safety

OSHA BLOODBORNE PATHOGEN STANDARD

BLOODBORNE PATHOGENS (BBP)

Requirements:

- **Annual BBP Training**
  - Available in WorkDay
  - Required for all employees working with or have potential exposure to human materials
- **Exposure Control Plan**
  - Review and sign annually!
  - Each lab must complete Appendix C for lab-specific procedures
  - Necessary documents are available on the EHRS website.
- **Engineering and workplace controls**
  - Reduce sharps/glass usage
  - Transport samples in leak-proof, secondary container labeled with the biohazard symbol (training required to SHIP human samples)
  - PPE, including lab coat, nitrile gloves, eye protection. Also long pants and close-toed shoes.
  - Decontamination procedures: disinfect BSCs, lab benches, tools, etc. often with appropriate disinfectant, such as 10% Bleach
  - Hepatitis B vaccination is offered to all Penn employees at no cost
- **Standard Precautions:**
  - Treat ALL human materials as potentially infectious
  - Use Biosafety Level 2 practices and procedures (see the Penn Biosafety Manual or CDC's Biosafety in Microbiological and Biomedical Laboratory (BMBL) 6th Edition for details)
- **Containment & disposal of infectious waste**
  - All research materials and PPE used with human materials must be disposed of as INFECTIOUS WASTE (see the Penn Biosafety Manual for details on infectious waste disposal)

All workers with potential exposure risk to human source materials must comply with the Occupational Safety and Health Administration's Bloodborne Pathogen Standard **(OSHA 29 CFR 1910.1030)**

What is Human Source Material?

Any tissue, cell lines, blood, or other potentially infectious material (OPIM) originating from humans.

What are Bloodborne Pathogens?

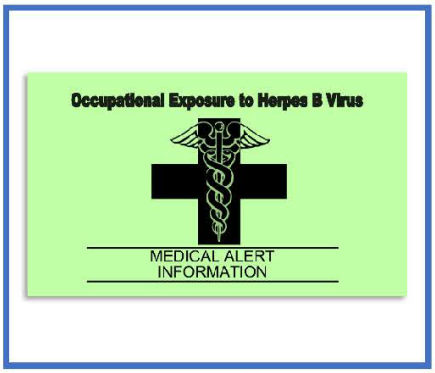
Pathogenic organisms that are present in human blood and can cause disease in humans. They can include but are not limited to: Hepatitis B virus, Hepatitis C virus, Human Immunodeficiency Virus.

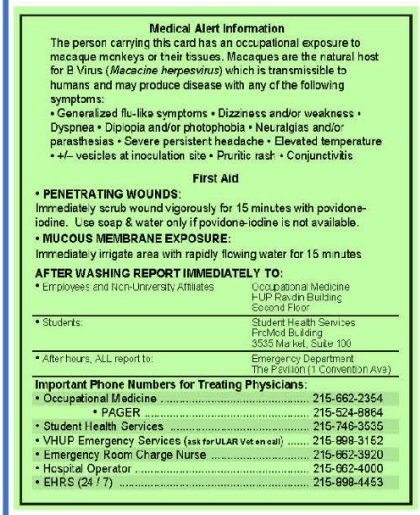
Exposure Control Plan (ECP)

Each employer having an employee(s) with occupational exposure shall establish a written ECP designed to eliminate or minimize employee exposure.

EMERGENCY RESPONSE

- Wash exposed area with water and soap for 15 minutes.
- For eye exposure, use eye wash for 15 min
- Immediately seek medical attention
  - Occ Med, Student Health, or ER
- Report injuries and exposures to your supervisor





Medical Alert Information

The person carrying this card has an occupational exposure to macaque monkeys or their tissues. Macaques are the natural host for B Virus (*Macacine herpesvirus*) which is transmissible to humans and may produce disease with any of the following symptoms:

• Generalized flu-like symptoms • Dizziness and/or weakness • Dyspnea • Diplopia and/or photophobia • Neuralgias and/or parasthesias • Severe persistent headache • Elevated temperature • +/– vesicles at inoculation site • Pruritic rash • Conjunctivitis

First Aid

• **PENETRATING WOUNDS:**
Immediately scrub wound vigorously for 15 minutes with povidone-iodine. Use soap & water only if povidone-iodine is not available.

• **MUCOUS MEMBRANE EXPOSURE:**
Immediately irrigate area with rapidly flowing water for 15 minutes

AFTER WASHING REPORT IMMEDIATELY TO:

• Employees and Non-University Affiliates	Occupational Medicine HUP Ravdin Building Second Floor
• Students:	Student Health Services ProMed Building 3535 Market, Suite 100
• After hours, ALL report to:	Emergency Department The Pavilion (1 Convention Ave)

Important Phone Numbers for Treating Physicians:

• Occupational Medicine	215-662-2354
• PAGER	215-524-8864
• Student Health Services	215-746-3535
• VHUP Emergency Services (ask for ULAR Vet on call)	215-898-3152
• Emergency Room Charge Nurse	215-662-3920
• Hospital Operator	215-662-4000
• EHRS (24 / 7)	215-898-4453

Quick Reference: Non-Human Primate Material

PennEHRS
Environmental Health & Radiation Safety

SAFETY PRACTICES AND PROCEDURES

- **Biosafety Level 2**
  - BSL-2 practices and procedures must be used when manipulating any NHP material
  - Handle all samples inside a certified biosafety cabinet
  - Reduce or eliminate the use of glass and sharps when possible
  - Wipe down all surfaces with appropriate disinfectant
  - Dispose of all NHP contaminated waste through the infectious waste stream
- **Exposure Control Plan**
  - Review and sign annually!
  - Each lab must complete Appendix C for lab-specific procedures
- **PPE Requirements**
  - Long pants and closed toed shoes
  - Disposable gown and gloves
- **Emergency Procedures:**
  - For an exposure to a mucous membrane (splash to eyes, nose, or mouth) irrigate the exposed area with running water at an eyewash station for 10-15 minutes.
  - For a penetrating wound (cut, puncture, needle-stick) wash injured area with povidone-iodine solution found in **Exposure Kit**
  - Immediately report ALL exposures to a healthcare provider:
    - Occupational Medicine (for Penn employees) HUP RAVDIN 2nd floor, 34th & Spruce Streets.
    - Student Health (for Penn students) ProMed Building, Suite 100, 3535 Market St.
    - HUP or Presbytarian Hospital ER (ALL after work hours and non-university affiliates)
- **Green Exposure Cards**
  - Wallet sized "Green Exposure Cards" (shown above), which describe procedures to follow in case of an exposure to NHP material, are available from EHRS and ULAR. These cards should be carried by all researchers working with NHP material or animals and should be presented to a health care professional if an exposure occurs.

DEFENITIONS

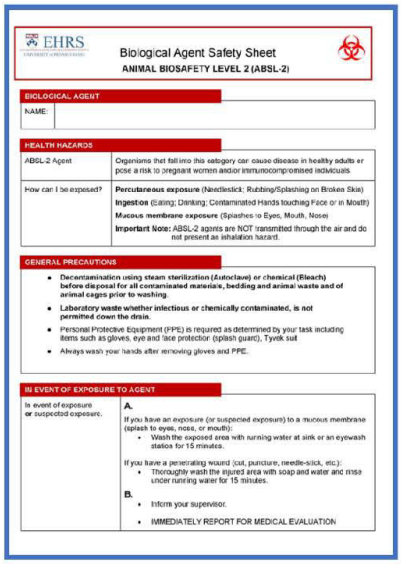
Refer to the Penn Policy for Laboratory Work with Non-Human Primate Materials for full details

What is Non-Human Primate Material?

Non-human primate (NHP) source material includes cells, blood, serum, tissues, feces, and body fluids (sputum, urine, saliva, etc.).

Why is this material a potential risk?

All NHP materials are considered potentially infectious regardless of whether they are primary materials or commercially available. Materials from macaque monkeys, a genus of Old World monkeys, may harbor Herpes B virus (*Macacine herpesvirus*). Herpes B virus has an approximately 70% mortality rate in humans when not immediately treated. Various other zoonotic pathogens may be present depending on the type of NHP material being manipulate.


Biological Agent Safety Sheet
ANIMAL BIOSAFETY LEVEL 2 (ABSL-2)

BIOLOGICAL AGENT
NAME:

HEALTH HAZARDS
ABSL-2 Agent — Organisms that fall into this category can cause disease in healthy adults or pose a risk to pregnant women and/or immunocompromised individuals.
How can I be exposed? — Percutaneous exposure (Needlestick; Rubbing/Splashing on Broken Skin)
Ingestion (Eating; Drinking; Contaminated Hands touching Face or in Mouth)
Mucous membrane exposure (Splashes to Eyes, Mouth, Nose)
Important Note: ABSL-2 agents are NOT transmitted through the air and do not present as inhalation hazard.

GENERAL PRECAUTIONS
- Decontamination using steam sterilization (Autoclave) or chemical (Bleach) before disposal for all contaminated materials, bedding and animal waste and of animal cages prior to washing.
- Laboratory waste whether infectious or chemically contaminated, is not permitted down the drain.
- Personal Protective Equipment (PPE) is required as determined by your task including items such as gloves, eye and face protection (splash guard), Tyvek suit
- Always wash your hands after removing gloves and PPE.

IN EVENT OF EXPOSURE TO AGENT
In event of exposure or suspected exposure:
A.
If you have an exposure (or suspected exposure) to a mucous membrane (splash to eyes, nose, or mouth):
- Wash the exposed area with running water at sink or an eyewash station for 15 minutes.

If you have a penetrating wound (cut, puncture, needle-stick, etc.):
- Thoroughly wash the injured area with soap and water and rinse under running water for 15 minutes.

B.
- Inform your supervisor.
- IMMEDIATELY REPORT FOR MEDICAL EVALUATION

Quick Reference: Working at Animal Biosafety Level 2

Penn EHRS
Environmental Health & Radiation Safety

SAFETY PRACTICES AND PROCEDURES

- **Best Practices**
  - Perform cage changes and other animal procedures in a biosafety cabinet following proper procedures.
  - Dispose of all waste from an ABSL-2 room through the infectious waste stream.
  - All ABSL-2 rodents must be housed in filter-top rodent cages in the ULAR facility and in satellite housing. Cages must be opened only in a biosafety cabinet. Empty cages must be returned to the facility of origin.
- **Training**
  - Access to ABSL-2 rooms is limited to those researchers and ULAR staff who have been adequately trained. Contact ULAR for more information on training requirements for ABSL-2 spaces.
- **Emergency Procedures:**
  - Know what to do and where to go after a potential exposure or injury.
  - Irrigate exposed mucous membrane with running water for 15 minutes.
  - Wash out wounds with soap and water for 15 minutes.
  - Report exposure or injury to your supervisor and immediately seek medical attention: Occupational Medicine, Student Health, HUP, or Presbytarian Hospital ER
- **Signage & Hazard Information**
  - See the image above for proper ABSL-2 room signage. Contact your ULAR facility manager to request ABSL-2 signage.
  - It is the PI's responsibility to inform ULAR staff of the hazards and any additional procedures required for their animals housed at ABSL-2
  - PIs must complete a Biological Agent Safety Sheet prior to beginning work with the ULAR facility.
  - PIs are responsible for the hands-on training of their research staff regarding the hazards of working with specific infectious agents.

DEFENITIONS

Refer to the Biosafety Manual for details to work in an ABSL-2 space.

What is Animal Biosafety Level 2 (ABSL-2)?

Animal Biosafety Levels 2 (ABSL-2) refers to the practices and producers required to work with animals infected with agents associated with human disease.

Why is this material a potential risk?

Agents used at ABSL-2 usually fall into Risk Group 2 and have a moderate risk associated with them. Direct exposure to these agents through ingestion, percutaneous injury, or mucous membrane exposure can cause illness.

QUICK REFERENCE: DISPOSAL OF TRANSGENIC ANIMALS

Animal Research Subject to NIH Guidelines

All creation, crossing, and use of **TRANSGENIC ANIMALS** is subject to the ***NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*** (rsNA Molecules) and requires review and approval by the Institutional Biosafety Committee (IBC). rsNA includes molecules that are constructed by joining natural or synthetic nucleic acid segments. Plasmids and CRISPR/Cas9 are included among common examples of tools used to create transgenic animals.

What are TRANSGENIC ANIMALS?

TRANSGENIC ANIMALS are animals possessing a modified genome. These animals may be purchased through a vendor, received from a collaborator, custom-made by a core or created in your lab. **TRANSGENIC ANIMALS** used at Penn include but are not limited to insects, nematodes, fish, amphibians, and rodents.

NIH Guidelines must be followed when disposing of dead transgenic animals or animals treated with rsNA

As required by the *NIH Guidelines*, all dead **TRANSGENIC ANIMALS** or animals treated with rsNA must be disposed of as biohazardous waste to prevent the release of such animals or rsNA material into the environment. Disposal of any **TRANSGENIC ANIMAL** or animal part is not allowed in regular trash or sewer, with the exception of embryos!

DISPOSAL POLICY FOR TRANSGENIC ANIMALS

- **ARTHROPODS** (flies, mosquitos, ants, and other insects)
  - Dispose of insects, captured or killed in ethanol or oil, as chemical waste. Cap waste when not in use. Request pick-up using online Chemical Waste Pickup Request form.
  - Place dead insects (free of chemicals) in a biohazard bag labeled "Do Not Autoclave" and leave in designated infectious waste collection bins.

- **NEMATODES** (*C. elegans*, *Strongyloides spp.*, and others)
  - Autoclave culture dishes containing agar and worms. Dispose autoclaved dishes in designated infectious waste containers.
- **FISH & AMPHIBIANS** (Zebra fish and frogs)
  - Place dead adult fish or amphibian in a biohazard bag labeled "Do Not Autoclave" and leave in designated carcass cooler within ULAR facility.
  - Bleach-inactivated fish or amphibian embryos are disposed of as liquid waste. Embryos are not subject to IACUC regulation.
- **OTHER ANIMALS** (mice, rats, and other macrofauna)
  - Place dead animals in biohazard bag and leave in designated carcass cooler within ULAR

All transgenic animals must be rendered non-viable prior to disposal. Alternate methods of disposal must be approved by the Penn IBC.
Contact EHRS for details: ehrs@ehrs.upenn.edu or 215.898.4453.

QUICK REFERENCE: SHARPS

Quick Reference: Sharps

Penn EHRS
Environmental Health & Radiation Safety

DISPOSAL

SHARPS AND SAFETY

Biohazardous Sharps

- All sharp materials that have come into contact with human materials, pathogens, recombinant or synthetic nucleic acids, or other potentially infectious materials must be disposed of in red, leak-proof, biohazardous sharps containers.
- **ALL needles, razor blades, scalpels, and syringes** (with and without needles attached) must ALWAYS be disposed of in a sharps container, regardless of their use.

Proper sharps disposal is essential to keeping Penn workers, the environment, and the public safe.

Non-Infectious Sharps

- Any broken glass, serological pipets, pipet tips or other materials that have not come into contact with infectious or hazardous materials but have the potential to puncture a bag.
- Must be disposed of in leak-proof sturdy containers.

REUSEABLE SHARPS CONTAINER

This program is available for all labs on campus. Advant-Edge Solutions provides reusable sharps containers to labs with a scheduled pick-up service. This program saves labs time and money while improving safety to workers.

What are sharps?

Sharps are defined as any material that is capable of puncturing through a bag.

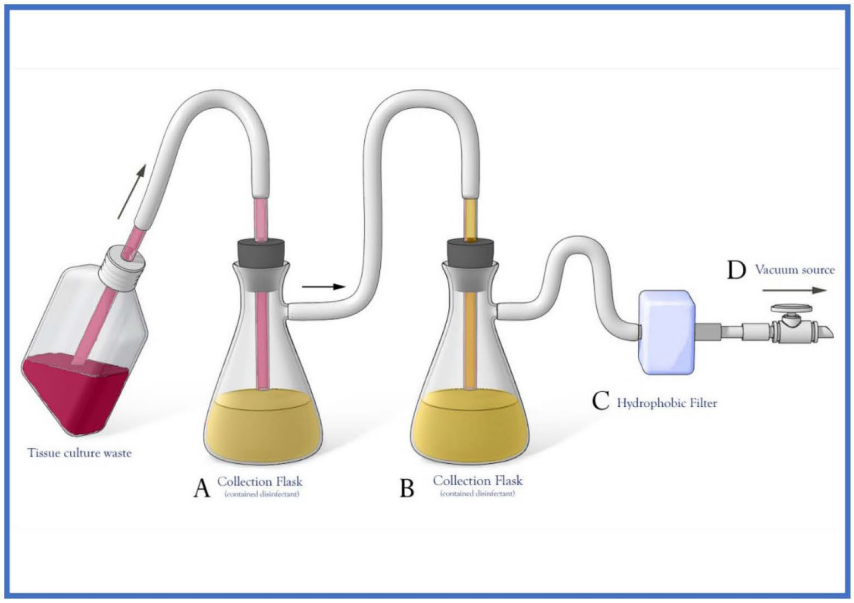
Sharps includes:
Needles, syringes (with and without needle attached), razor blades, pipet tips, serological pipets, pasture pipettes, etc.)

Points to remember

- Disposed of sharps container when 2/3 full.
- DO NOT rearrange waste after placing it in sharps or glasswaste container
- Do not overfill glasswaste boxes
- Handle all sharps with care
- NEVER put sharps in the regular landfill waste

MIXED WASTE

For those laboratories generating chemically contaminated or radioactively contaminated sharps waste, please see the biosafety manual or consult EHRS for proper waste disposal.



Quick Reference: Protection of Vacuum Systems

PennEHRS
Environmental Health & Radiation Safety

FILTER OPTIONS

Filter Options: Use in-line, hydrophobic, filters made of PTFE with 0.3µ particle retention. Filters are available from most scientific vendors. (some suggestions are listed below)

Name	Properties	Dimensions	Part Number	
Vacushield * Vent Device, Pall* Life Sciences	Hydrophobic PTFE membrane filter, 0.3µm particle retention, autoclavable	Stepped hose barbs 6.4 - 12.7 mm diameter	Pall 4402	
Whatman HEPA-Vent Filter	mildly hydrophobic, 0.3µm particle retention, autoclavable	Inlet/outlet: 1/4 to 3/8 inch stepped barb	Whatman 6723-5000	
Millipore Millex Vacuum Line Protection	Hydrophobic PTFE membrane filter, autoclavable	Variety of inlet/outlet combinations, including stepped hose barb & 1/8 NPTM	Millipore SLFH050 10 SLFG750 10	

Vacuum lines must be protected with filters to protect building central vacuum systems. Filters must be replaced as needed.

SET-UP AND DISPOSAL

Set-up:

- Aspirator is connected to the collection flask and overflow collection flask (A & B*).
- Flasks should contain appropriate disinfectant
- In-line filter (C) is connected to protect eh vacuum line (D).

*secondary collection flask (B) is not required but recommended for high workflow labs

Decontaminate aspirated waste:

- Use appropriate disinfectant in the collection flasks
- Decontaminated liquid can be poured down the drains
- Collection flask must be stored in a secondary container while in use

Penn Policies

Penn Policy for working with Animal Biosafety Level 2 Containment

PennEHRS Environmental Health & Radiation Safety

Penn Policy for working in Animal Biosafety Level 2 Containment

I. DESCRIPTION

Animal Biosafety Level 2 (ABSL-2) refers to the practices and procedures required to work with animals infected with agents associated with human disease. These infectious agents are typically moderately hazardous and can be contracted by direct exposure through ingestion, percutaneous injury, and mucous membrane exposure.

II. SIGNAGE

A sign that incorporates the universal biohazard symbol (pictured right) must be posted on the door of every ABSL-2 room. This sign must include the following information:

1. The universal biohazard symbol.
2. The Animal Biosafety Level (ABSL-2).
3. Personal Protective Equipment (PPE) requirements.
4. Principal Investigator(s) (PIs) responsible for the project.
5. Laboratory contact person(s) and emergency contact number(s).
6. Infectious agent(s) used in the room (on second page).

To request ABSL-2 signs, please contact your ULAR manager.

III. TRAINING

Access to ABSL-2 rooms is limited to researchers and support staff who have been adequately trained.

ULAR Training: All ULAR training requirements must be completed before access to ABSL-2 labs is granted. Please visit the ULAR training website for more information.

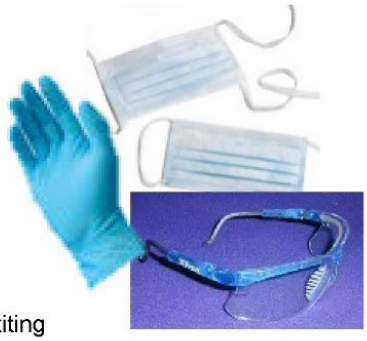
EHRS Training: All research personnel must take the Introduction to Laboratory Safety Training and subsequent annual updates before working in ABSL-2 containment. Additional EHRS training may be required depending on the agents used. Please visit the EHRS training website for more information.

It is the PI's responsibility to inform ULAR staff of the hazards and any special procedures required for work with their animals housed at ABSL-2. PI's are also responsible for the hands-on training of their research staff regarding the hazards of working with specific infectious agents.

Contact ULAR Management to schedule a HAZARD BRIEFING prior to working with Risk Group 2 organisms or human material.

Penn Policy for working in Animal Biosafety Level 2 Containment

IV. ROOM ENTRANCE & EXIT PROCEDURES



Before entering any ABSL-2 room, the following PPE must be worn:

- 2 pairs of foot covers
- 2 pairs of gloves
- 1 surgical gown, securely tied in the back
- 1 face mask covering both the mouth and nose
- Eye protection if there are procedures generating a splash risk

After all work is complete, the following steps must be taken before exiting individual ABSL-2 rooms:

- Remove the outer shoe covers and the outer pair of gloves (in that order).
- Step through door to main hallway.
- Wash hands with soap and water OR with an alcohol based hand sanitizer prior to leaving facility or performing other task.
- Put on a new second pair of gloves and foot covers before entering another animal room.

When preparing to exit the facility, remove all PPE except for the initial pair of shoe covers, which should be worn to exit the facility and then removed and thrown out.

V. EQUIPMENT

Animal Cages: All rodents housed in ABSL-2 rooms must be kept in filter-top cages. These cages may only be opened inside a functioning biosafety cabinet. These rodents must remain in ABSL-2 designated containment at all times, including transport. Larger animals may be housed in regular cages, however additional PPE may be required to work in the room.

Biosafety Cabinets: Biological Safety Cabinets must be used for all manipulations of small animals housed in ABSL-2 rooms, including the following procedures:

- Opening rodent cages
- Changing rodent cages
- Transferring rodents to new cages
- Injecting small animals with infectious agents
- Any other procedure that may generate infectious aerosols

Biosafety Cabinets must be certified annually and after being moved. ***Do not*** use the cabinet if it is out of certification or not functioning properly. Notify the facility manager to schedule any needed maintenance and repairs. Maintenance and repairs may only be performed by a designated service provider identified by the university.

For more information regarding biosafety cabinets, including maintenance/repair information, contact a biosafety officer at EHRS (215-898-4453) or visit the EHRS website.

Note: Animal cage change stations and clean benches are ***never*** appropriate to use when manipulating animals housed at ABSL-2. This equipment does not protect personnel or the environment from infectious agents.

Penn EHRS Environmental Health & Radiation Safety

Penn Policy for working in Animal Biosafety Level 2 Containment

VI. WASTE

All waste generated in the manipulation of animals housed in ABSL-2 rooms is considered biohazardous. All animal carcasses must be bagged and returned to the ULAR facility for disposal.

Biohazards waste is divided into three categories:

1. Biohazardous liquid waste: All infectious liquid waste must be disinfected in one of two ways:
   - Add bleach the liquid for a final volume of 10% bleach and let sit for 30 min. Pour down drain.
   - Autoclave the waste for the appropriate time and temperature. Pour down drain.

2. Biohazardous sharps waste: Sharps include razors, scalpels, needles, syringes (with or without needle attached), broken glass, broken plastic, pipette tips, serological pipettes, Pasteur pipettes, and anything else that could poke through a bag. Any of these items that have been used in research involving infectious agents must be disposed of in a hard sided, lidded container labeled with the universal biohazard symbol (pictured right).

3. Biohazardous "red-bag" waste: Any non-liquid or non-sharp waste such as PPE, rodent bedding, etc. must be thrown away in red or orange biohazard bags.

All infectious waste removed from the ABSL-2 rooms must be autoclaved and subsequently disposed of through the infectious waste stream. Autoclaves must be monitored by ULAR for proper function by using biological indicators.

NEVER dispose of sharps containers, red/orange biohazard bags, or any other biohazardous materials in the regular trash!!!

VII. RODENT CAGE CHANGING PROCEDURES

Rodent cages must be changed inside a certified biosafety cabinet using these procedures:

1. Turn on the biosafety cabinet and fluorescent light.
2. Let the cabinet run for 5-10 minutes before use to ensure adequate air exchange.
3. Wipe down inside surfaces with an appropriate disinfectant.
4. Load the cabinet with only a few animal cages at a time. Over-crowding the cabinet may disrupt airflow and expose you to infectious agents.
5. DO NOT cover the front and back air grilles. Blocking these air grilles may disrupt airflow and expose you to infectious agents.
6. Dirty cages containing used bedding should be removed from the cabinet and stacked upright inside a red biohazard bag.
7. When all cages have been changed, remove all materials from the biosafety cabinet.
8. Wipe down all inside surfaces with an appropriate disinfectant.

9. Let the biosafety cabinet run for 5-10 minutes to remove suspended particles.

10. Turn off the cabinet and the fluorescent light.
11. The biohazard bag containing the dirty cages must be loaded onto a cart in an upright position and taken to the autoclave for decontamination of bedding.
12. After all cages have been autoclaved, the bedding and cages are no longer considered infectious and can be handled according to standard procedures.

VIII. ANIMAL TRANSPORT

All rodents transported outside of the ABSL-2 room or the animal facility must be transported in filter-top rodent cages. If cages are changed in a lab outside of the animal facility, the same cage change procedures outlined above must be followed. Empty cages must be returned to the facility of origin.

IX. EMERGENCY PROCEDURES

If an exposure to a mucous membrane (i.e. splash to eyes, nose, or mouth) occurs:

- Irrigate the exposed area with running water at an eyewash station for 15 to 20 minutes.

If a penetrating wound (i.e. bite, cut, puncture, needle-stick, etc.) occurs:

- Thoroughly wash the injured area with soap and water for 15 to 20 minutes.

Immediately report ALL exposures or possible exposures to the facility manager and proceed to:

- Occupational Medicine (for Penn employees ONLY)
  Hospital of the University of Pennsylvania RAVDIN Building, 2nd floor

- Student Health (for Penn students)
  ProMed Building, 3535 Market St., Suite 100
  see website for hours (http://www.vpul.upenn.edu/shs/)

- **Hospital of the University of Pennsylvania Emergency Department** (ALL after work hours and non-university affiliates)
  The Pavilion, 1 Convention Avenue
  Penn Presbyterian Hospital, Myrin Building, 51 N 39th Street

X. ADDITIONAL RESOURCES

Non-human primates (NHPs): NHPs are housed in ABSL-2 containment. For additional information concerning work with NHPs, please see the ULAR Policy and EHRS Policy for work with non-human primates.

ABSL-2 Practices and Procedures: *The Biosafety in Microbiological and Biomedical Laboratories*, 6th Edition outlines further details on features of an ABSL-2 laboratory.

Penn Policy on Aerosol-Generating Hazards

Penn EHRS
Environmental Health & Radiation Safety

Life Sciences Safety (BIOSAFETY)
& Compliance Programs
3160 Chestnut Street, Suite 400
Philadelphia, PA 19104-6287
215-898-4453

Policy Statement: Aerosol-generating hazards

The University of Pennsylvania recognizes the potential hazards associated with the use of sonicators or homogenizers in spaces such as cold rooms or lab benches without appropriate containment.

Compliance and Protocols:

Using a sonicator or homogenizer in a cold room, or open lab does not ensure containment of aerosolized recombinant materials, tissue/cell particles, or bacterial endotoxin and therefore must be conducted inside a biosafety cabinet or fume hood due to the potential for significant aerosol generation during use. In addition, it is a requirement of the NIH Guidelines that "All procedures are performed carefully to minimize the creation of aerosol," regardless of the biosafety containment level (BSL-1 or BSL-2) of recombinant materials (e.g., transformed bacterial cell lines). Given the nature of cold rooms, this issue is compounded by recirculating air and lack of supply/exhaust, which will expose the operator or others using the space. Ice can be used to maintain temperature requirements during sonication or homogenization of biological hazards and recombinant materials conducted inside a biosafety cabinet or fume hood.

Compliance Monitoring:

The University is committed to proactive compliance monitoring to identify and rectify any potential issues. Regular inspections will be conducted by Biosafety Officers to ensure that all labs and individuals are following the established protocols and guidelines.

Please contact a Biosafety Officer (Biosafety@lists.upenn.edu) for questions and concerns or if additional guidance is needed.

This policy statement is effective immediately upon issuance.

Penn Policy on Permissible Toxin Amounts

PennEHRS
Environmental Health & Radiation Safety

Life Sciences Safety (BIOSAFETY)
& Compliance Programs
3160 Chestnut Street, Suite 400
Philadelphia, PA 19104-6287
215-898-4453

Policy Statement:
Permissible Toxin Amounts https://www.selectagents.gov/sat/permissible.htm

The University of Pennsylvania recognizes the critical importance of maintaining the highest standards of safety, security, and due diligence in handling and storing permissible toxin amounts, as stipulated by the Federal Select Agent Program (FSAP) in the United States. This policy aims to ensure that labs and individuals adhere to standards governing the possession, use, and storage of permissible amounts of select agents and toxins.

Compliance and Protocols:

- **Inventories:** Labs and individuals are mandated to maintain up-to-date inventories of all permissible select agents and toxins in their possession. These inventories must be regularly reviewed and verified to guarantee strict compliance with permissible toxin amounts.
- **Security Measures:** To comply with security requirements, select agents and toxins must be stored in designated, secure locations.
- **Permissible Amounts:** The University emphasizes strict adherence to the permissible toxin amounts outlined by the FSAP. Labs must conduct regular internal audits to ensure that these amounts are never exceeded, and immediate corrective actions must be taken if any discrepancies are identified.
- **Training and Awareness:** All personnel involved in handling and storing select agents and toxins must be up-to-date on their Lab safety training to enhance their awareness of safety protocols and emergency procedures.

Compliance Monitoring:

The University is committed to proactive compliance monitoring to identify and rectify any potential issues. Regular inspections will be conducted by Biosafety Officers to ensure that all labs and individuals are following the established protocols and guidelines.

For questions and concerns, please reach out to your biosafety team (biosafety@lists.upenn.edu).

This policy statement is effective immediately upon issuance.

IBC Policy on Biosafety Containment and Downgrade Requests

Penn University of Pennsylvania Institutional Biosafety Committee	**IBC Policy: Biosafety Containment Requirements & Downgrades**

BACKGROUND

Although the risk of laboratory acquired infection (LAI) from working with cell cultures is low, risk increases when working with human cells. The CDC Biosafety in Microbiological and Biomedical Laboratory (BMBL, 6th ed.) cites reports of LAIs associated among laboratory staff working with primary human cells, tissues, and body fluids.

In 1991, the Occupational Safety and Health Administration (OSHA) passed the Bloodborne Pathogens (BBP) Standard to protect employees who have occupational exposure to human blood or other potentially infectious materials. The BBP Standard includes human cells lines unless the specific cell line has been characterized to be free of hepatitis viruses, HIV, Epstein-Barr virus, papilloma viruses and other recognized bloodborne pathogens. Additionally, the BMBL recommends that human cells be handled using Biosafety Level 2 (BSL-2) and Animal Biosafety Level 2 (ABSL-2) containment and practices.

Mammalian cells transduced with lentiviral vectors (derived from wild-type HIV) are common in biomedical research but add additional risks due to the potential generation of Replication-Competent Lentivirus (RCL) or oncogenesis as the result of integration of an oncogene or insertional mutations into an exposed researcher's genome. Classified as a Risk Group 2 agent, lentiviruses and work with lentiviral vectors is performed at BSL-2 and ABSL-2 containment and practices. Cells transduced with a lentiviral vector must also be used at BSL-2 and ABSL-2 unless the specific cell lines have been characterized to be free of Replication-Competent Lentivirus (RCL).

Human induced pluripotent stem cells (iPSCs) are cells reprogrammed from somatic cells to resemble embryonic stem cells. They can differentiate into any cell type in the body. Undifferentiated human iPSCs are generally resistant to viral infection due to high expression of interferon-stimulated genes (ISGs). Because these cells do not support the growth of viruses, they may be handled at BSL-1 and ABSL-1 if they come from a reputable vendor, a cell bank system is used, and cells are not perpetually passaged. Differentiated human cells derived from iPSCs *are* susceptible to viral infection and must be used at BSL-2 and ABSL-2.

POLICY

The University of Pennsylvania's Institutional Biosafety Committee (IBC) has adopted the following policy to define the requirements of containment related to work with human cells, human source material, cells transduced with lentiviral vectors, and other potentially infected material.

Human cells and tissue cultures must be handled in accordance with the OSHA BBP Standard and under BSL-2 containment and practices for cell culture

experiments and ABSL-2 containment and practices when these materials are used in animal experiments.

Mammalian cells that have been transduced with a lentiviral vector must be handled in accordance with the BMBL and the OSHA BBP Standard (when handling transduced human tissue or cells) and under BSL-2 containment and practices for cell culture experiments and ABSL-2 containment and practices when these materials are used in animal experiments. These experiments require IBC approval prior to initiation of work.

Certain human and transduced cells lines may be eligible for an exemption from this policy and be granted a downgrade to BSL-1 containment and practices for cell culture experiments and ABSL-1 containment and practices when used in animals under certain criteria.

EXEMPTION CRITERIA FOR HUMAN CELL LINES AND TISSUE

- Cell lines or tissues must be tested for common viral bloodborne pathogens. At minimum, this includes HIV, HBV, HCV, and EBV.
- Acceptable testing may include antigenic screening for viral markers, co-cultivation with indicator cells that allow contaminates to grow, or molecular techniques such as PCR or nucleic acid hybridization. Testing may be done by the lab, the source vendor, or an outside company.
- Documentation of testing must be submitted with a formal letter to the IBC requesting the downgrade and guaranteeing that the cells are free of bloodborne pathogens and other potential contaminants.
- The IBC will review the request and issue a decision letter.
- Testing must take place every three years OR include detailed documentation of the use of a cell bank system including a master cell bank (MCB) and working cell bank (WCB) upon initial submission of the exemption request.

EXEMPTION CRITERIA FOR LENTIVIRAL VECTOR MODIFIED CELL LINES AND TISSUE

- If human cell lines and tissues are used, they must meet the criteria listed above under the exemption criteria for human cell lines and tissue.
- A downgrade will be considered for the use of 3rd or 4th generation lentiviral vectors only. Cells transduced with a 2nd generation lentiviral vector will not be considered for a downgrade.
- Transduced cells must be passaged in vitro at least 3 times before administration to animals to be considered for a downgrade.

- Acceptable testing may include qRT-PCR of the cellular supernatant for the presence of the lentiviral vector (i.e., Lenti-X qRT-PCR titration kit), commercially available RCL testing, or negative HIV-1 and HIV-2 tests.
- Documentation of the testing must be submitted as an amendment to an existing IBC registration or included upon initial registration to the IBC. If submitting the results of a qRT-PCR, the following must be included in the downgrade request:
    - Detailed experimental design
    - Timeline of cell transduction and passaging prior to testing
    - Standard curve, negative and positive controls
- The IBC will review the request and issue a decision letter.
- Testing must take place every three years OR include detailed documentation of the use of a cell bank system including a master cell bank (MCB) and working cell bank (WCB) upon initial submission of the exemption request.

EXEMPTION CRITERIA FOR UNDIFFERENTIATED HUMAN INDUCED PLURIPOTENT STEM CELLS (iPSCs)

- Human iPSCs must be obtained from a reputable vendor with a documented quality assurance program. A certificate of analysis must be provided.
- For labs that generate iPSCs, a Standard Operating Procedure (SOP) must be provided for review. The SOP must clearly demonstrate the steps taken for Sendai virus (SeV) removal from the iPSC culture.
- Human iPSCs must be undifferentiated.
- Differentiated human cells derived from iPSCs are not exempt unless they meet the exemption criteria for human cell lines and tissue above.
- The use of a cell bank system, including a master cell bank (MCB) and working cell bank (WCB) must be demonstrated so that cells are not continuously passaged.
- A Certificate of Analysis or SOP must be submitted with a formal letter to the IBC requesting the downgrade.
- The IBC will review the request and issue a decision letter.

REFERENCES

- Biosafety in Microbiological and Biomedical Laboratories, 6th Edition
- OSHA Bloodborne Pathogens Standard
- OSHA Letter of Interpretation: Human Cell Lines
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2024
- Intrinsic Immunity Shapes Viral Resistance of Stem Cells
- The purpose of cell banking: Uses & Applications
- What exactly is cell banking?

Resources

University Offices

University of Pennsylvania

Penn Center for Innovation (CTT)

Institutional Animal Care and Use Committee (IACUC) Institutional Biosafety Committee (IBC)

Institutional Review Board (IRB)

University Laboratory Animal Resources (ULAR)
Office of Environmental Health and Radiation Safety (EHRS)

Office of Facilities, Real Estate Services (FRES)

Penn Medicine Clinical Research (OHR)

Office of Research Services (ORS)

Office of Export Compliance

Perelman School of Medicine Space Planning & Operations (PSOM SPO)

Office of the Vice Provost for Research

EHRS Biosafety Resources

Laboratory Inspection Program Penn Biological Safety Manual Biosafety Cabinet information

Environmental Protection Policy Statement (Penn Almanac October 7, 2008)

Bloodborne Pathogens Policy and Exposure Control Plan Warning Sign and Label Request Form

BSC Certification & Repair
Personal Protective Equipment Policy Radiation Safety User Guides

Respiratory Protection Program

Room Sign Request Form (See below for PSOM room sign request form)

Shipping Biological Materials and DG Shipping Manual

Biohazardous Waste Disposal Guides (by school or building)

University Resources

Penn Occupational Medicine

Room Sign Request Form for Perelman School of Medicine Laboratories

Training Resources

EHRS Workday@Penn Learning Selection Guide

WorkDay@Penn

Training Help eMail

Regulatory and Other Biosafety Resources

American Biological Safety Association Risk Group Database (OSP)

Biosafety in Microbiological & Biomedical Laboratories (BMBL)

Bureau of Industry and Security (BIS) Commerce Control List

Canadian Pathogen Safety Data Sheets for Infectious Substances

CDC Import Permit Program

EPA Registered Disinfectants Federal Select Agent Program

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

NIH Office of Science Policy (OSP)

Occupational Safety and Health Administration (OSHA) OSHA Bloodborne Pathogen Standard

Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets

"Public Health Security and Bioterrorism Preparedness Response Act of 2002"

USDA/APHIS Controlled Import Permits

WHO Biosafety Manual