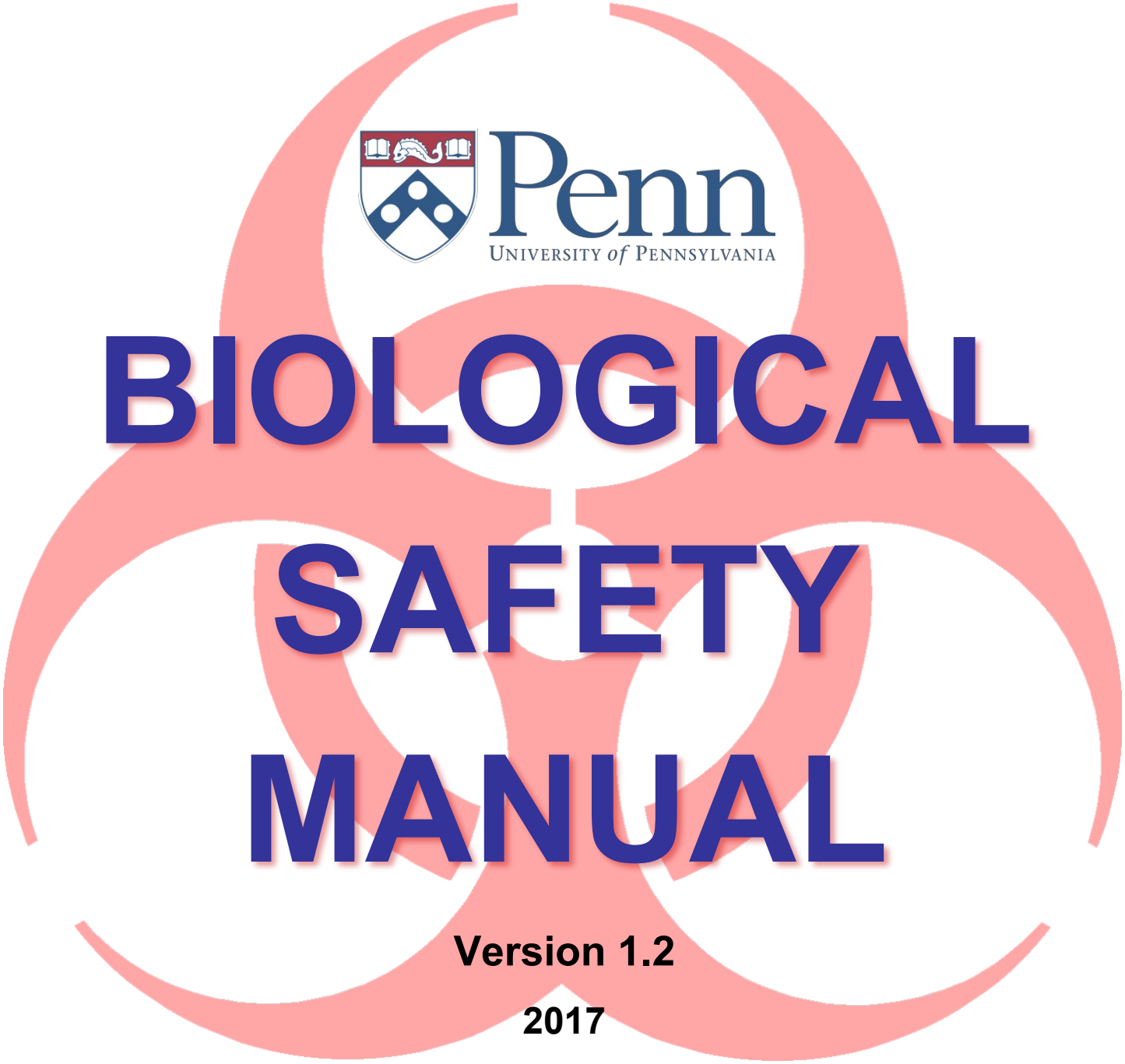

BIOLOGICAL SAFETY MANUAL

Version 1.2

2017

Environmental Health & Radiation Safety
3160 Chestnut Street, Suite 400
Philadelphia, PA 19104-6287
215-898-4453
ehrs@ehrs.upenn.edu

IF YOU ARE INJURED AND REQUIRE ASSISTANCE ON CAMPUS:

From a CAMPUS PHONE Call: 511

From Your PERSONAL PHONE Call: 215-573-3333

For medical assistance
DURING WORK HOURS (Mon – Fri 8AM - 4:30PM)

FACULTY AND STAFF report to:

HUP OCCUPATIONAL MEDICINE
Ravdin Building, 2nd Floor
3400 Spruce Street
215-662-2354

STUDENTS report to:

STUDENT HEALTH SERVICE
3535 Market Street, Suite 100
215-746-3535

For medical assistance AFTER WORK HOURS and HOLIDAYS:

ALL report to:

HUP EMERGENCY DEPARTMENT
Ground Floor Silverstein Pavilion
34th & Civic Center Blvd.
215-662-3920

ENVIRONMENTAL HEALTH AND RADIATION SAFETY

215-898-4453

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.

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 (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 changes in response to changes in existing regulations and inclusion of new regulations. 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. In order 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) works with researchers, centers, schools, and funders on the financial and contractual aspects of sponsored projects.
- Office of Regulatory Affairs (ORA) administers the University's program of compliance in the areas of human subjects (IRB) and the care and use of animals in research (IACUC).
- University Laboratory Animal Resources (ULAR) handles care and husbandry of laboratory animals.
- Office of Environmental Health and Radiation Safety (EHRS) performs risk assessments, provides awareness training, resources and regulatory guidance to ensure safe conduct of research. Oversees and reviews use of biological, chemical, and radiation hazards in the University's research programs.
- Center for Technology Transfer (CTT) obtains and manages patents, copyrights, and trademarks derived from the University's academic research enterprise.

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.

The Institutional Biosafety Committee

Research Compliance and adherence to EHRS guidelines for research at the University of Pennsylvania is mandated by the Environmental Protection Policy Statement (October 7, 2008). Compliance with University, federal, state and local regulations is a condition of acceptance of research funding from the NIH and various other granting agencies.

(IBC)

- The Institutional Biosafety Committee (IBC) has University-wide oversight as mandated by the National Institutes of Health (NIH) Office of Biotechnology Activities (OBA) and is 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, human gene transfer, and recombinant DNA.
- The IBC is responsible for review and approval of projects involving recombinant DNA research 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 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) is the operational arm of the Institutional Biosafety Committee (IBC). It 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 EHRS 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 reports 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 audits of research spaces where biological materials are used.
- BSOs review work requiring BSL-3 or ABSL-3 containment, pre-review rDNA registrations, maintain the Biological Agent Registration (BAR) and administer the Select Agent Program at Penn.
- BSOs will respond to and follow up on any major biological incident and/or spill as needed. BSOs should be called to assist with large spills containing infectious material. 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 to keep common laboratory spaces clean and in safe working condition. 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 DNA 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 a risk assessment to identify potentially hazardous procedures involving infectious agents, develop Standard Operating Procedures (SOPs), instruct and train all staff and students working in the lab on safe work practices, keep the lab space clean and up-to-date, and follow regulations for disposal of infectious waste. The PI must provide PPE to their staff.
- PIs must register research projects that require review by the IBC and/or EHRS, such as the generation and/or use of recombinant DNA, work requiring BSL-3 or ABSL-3 containment, Select Agents, and other work with infectious agents as needed.
- PIs must complete the Biological Agent Registration (BAR) 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 contact EHRS. Once the material has been contained, absorbed, and removed, housekeeping/facilities management should 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 are ultimately responsible to 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 Recombinant DNA 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 and 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 Knowledge Link.

SECTION 2.0 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 DNA?**

  rDNA (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 safety 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)

- **When should I be wearing gloves?**

  Biological Research Laboratory – Hand Protection (section 6)

- **I spilled my 50 ml tube of viral supernatant in the BSC. What do I do now?**

  Decontamination (section 7)

SECTION 2.0 FREQUENTLY ASKED QUESTIONS

- **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)

RISK ASSESSMENT

On the basis of the information ascertained during the risk assessment, a Biosafety Level 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 microorganism, their NIH established Risk Groups (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. Pathogenicity or Risk Group of microorganisms
2. Infectious dose needed to cause infection in a healthy person
3. Potential outcome of exposure
4. Natural route of infection (aerosol, ingestion, skin contact)
5. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestions); see Equipment Hazards below
6. Stability of the agent in the environment
7. Concentration of the agent and volume of concentrated material to be manipulated (tissue samples, blood, serum, etc.)
8. Origin of microorganism refers to geographic location, host (animal or human) or nature of source (potentially zoonotic, associated with a disease outbreak)
9. Presence of a suitable host (human or animal)
10. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
11. Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
12. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens (see Section 5.2: recombinant DNA)
13. Experience and skill level of at-risk personnel, including safety training or hands-on experience
14. Local availability of effective prophylaxis or therapeutic interventions (immunizations or post-exposure prophylaxis)
15. Medical surveillance program

> Bloodborne pathogen materials are designated RG-2 and the BMBL specifies BSL-2 containment practices for bloodborne pathogen materials in compliance with the OSHA Bloodborne Pathogens Standard

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 do a risk assessment of their laboratories in order to alert their staff to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and 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:

1. Capability to infect and cause disease in a susceptible human or animal host.
2. Its virulence as measured by the severity of disease.
3. Availability of preventative measures and effective treatment of the disease.
4. Probable routes of transmission of laboratory infection.
5. Capability of transmitting disease through respiratory exposure.
6. Infective dose.
7. Stability of agent in the environment.
8. Host range.
9. Endemic nature of the agent.
10. The use of laboratory animals.
11. 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 infectious diseases from foreign countries to the United States.
12. Genetically modified agents involve the same consideration for risk assessment as for wild-type organisms.

Identifying laboratory procedures

Working with biological agents may result in exposure and end with infection. 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.
2. Parenteral inoculation by a syringe needle or other contaminated sharp object including bites from infected animals and arthropod vectors.
3. Ingestion of liquid suspension of an infectious agent or by contaminated hand to mouth exposure.
4. Inhalation of infectious aerosols.

> Remember that the nature and severity of disease caused by a laboratory infection and the probable laboratory route of transmission of the infectious agent may differ from the route of transmission and severity associated with the naturally-acquired disease

Laboratory Acquired Infections (LAI)

Historical data on laboratory acquired infections (LAIs) is an indicator of laboratory procedure hazards that have resulted in disease. Historical data shows that past LAIs have occurred from:

- parenteral inoculation by a contaminated sharp or syringe needle,
- spills or splashes of contaminated materials directly onto the skin and mucous membranes,
- ingestion through mouth pipetting,
- animal bites and scratches, and
- inhalation of infectious aerosol

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
(see Appendix A for list and classification)

- Human Pathogens
- Plant & Animal Pathogens
- Biological Toxins
- Select Agents
- Prions
- Bloodborne Pathogens
- rDNA
- 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
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 BMBL 5th Ed., 2007.

Table 3-2: Equipment Hazards

Equipment Type	Hazards	Examples
aerosol generating	The diameter of aerosols generated from certain types of equipment will vary from 0.1 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
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) • 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 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 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 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 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, 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 and transilluminators.

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

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

Biosafety Levels are the levels of containment recommendations in 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 biosafety levels (Table 3-3).

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.

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: In order to protect those working in the laboratory, using primary barriers is important. Safety equipment such as biosafety cabinets and personal protective equipment are controls designated to remove or minimize exposures to hazardous biological materials.

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

+

Facility Design

=

CONTAINMENT

Biosafety Level I (BSL-1)

BSL-1 is appropriate for work done with well characterized strains of viable microorganisms not known to cause disease in healthy adult humans. 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

- 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

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

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None Required	Laboratory bench and sink required
2	Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials. PPE* : • Laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available. • BSL-2 Signage. • Negative airflow into laboratory • Exhaust air from laboratory spaces 100% exhausted, not recirculated is required at Penn.
3	Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequence	BSL-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 containment devices used for all open manipulation of agents PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease (**NOT ALLOWED TO BE USED AT PENN)**	BSL-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	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems

.*Source: adapted from the BMBL 5th Ed., 2007.*

Signage

- No special signage is required to designate a BSL-1 laboratory space.
- 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 Perlman 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 authorize 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.
- Eating, drinking, smoking, handling contact lenses, applying 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.
- Always preform procedures 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.
- 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.
- 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 involving agents that pose moderate hazards to personnel and the environment and where vaccines or post-exposure treatment is available.

Laboratory Design

- Restricted access to authorized personnel only.
- Separate laboratory work area from public spaces and eating areas
- Directional, single pass airflow is required for BSL-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 Perlman School of Medicine, through Space Planning Operations (SPO)

Training

- 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.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

Personal Protective Equipment (PPE)

- May include, but is 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 must be used in combination with BSCs and other devices that contain biohazardous agents, animals or materials. When it is impractical to work in BSCs, PPE forms the primary barrier between personnel and infectious materials. Examples include certain animal studies, animal necropsy, agent production activities and activities relating to maintenance, service or support of the laboratory facility.

Equipment

- Biosafety Cabinets are used for all work involving the manipulation of Risk Group 2 or higher agents.
- Safety devices must be used to contain potential aerosols created during processing of infectious material (see below).
- Autoclaves must be used to decontaminate all potentially infectious waste collected in red

The use of PPE is always the last step in mitigating a hazard!

bags or in red sharps containers.

For assistance in the selection of a BSC or other safety equipment, contact EHRS by phone (215-898-4453) or email: ehrs@ehrs.upenn.edu

- Eyewash must be readily available to lab personnel within a 10 sec walk from their location of work.
- Eyewashes must be tested weekly to ensure they are functioning properly.
- Safety Centrifuge Cups and Safety Blenders are enclosed containers designed to prevent aerosols from being released during centrifugation or homogenization of infectious material. These containers should only be opened in a Biosafety Cabinet

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.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.

Biosafety Level III (BSL-3)

BSL-3 is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents (i.e., West Nile virus, Chikungunya virus and Yellow fever virus) include auto-inoculation, ingestion and exposure to infectious aerosols. Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols.

(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.
- Laboratory floor, walls, and ceiling are sealed.
- 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, which is negative to the entrance anti-room to ensure unidirectional airflow.

  No air passing through the lab is recirculated but is passed thru a HEPA filter before being released into the environment.
- Doors remain locked and are self-closing.
- Donning and doffing of PPE is done in an anteroom.
- Hands-free handwashing sinks are provided.
- Floors are slip resistant and impervious to liquids.
- Walls, windows and ceiling are sealed and easily decontaminated.
- Only authorized personnel are allowed to enter these laboratories.
- Only one person may enter the facility at a time under their own black key access.
- All non-authorized personnel that need access to the facility (i.e. maintenance) must be escorted by designated personnel (i.e., EHRS BSL-3 coordinator, biosafety officer) and

supervised at all times.

Signage

- Restricted access
- PPE requirements

Training

- All personnel need to fulfill live and hands-on training by a Biosafety Officer prior to entry.

Laboratory Procedures

- 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

- All work is performed in containment devices or equipment, such as sealed centrifuge rotors or biosafety cabinets.
- All manipulations of materials are performed in the Biosafety Cabinet, where possible
- Safety devices must be used to contain potential aerosols created during processing of infectious material.
- Autoclaves must be used to decontaminate all waste created in the BSL-3 laboratory

Waste

- All waste is autoclaved out of 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** be brought to Penn's campus.

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).

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.

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.

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

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.

Animal Biosafety Level I (ABSL-1)

ABSL-1 is appropriate for work done with uninfected animals or with well characterized strains of viable microorganisms not known to cause disease in healthy adult humans. 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

- No special signage is required to designate an ABSL-1 facility space.
- 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.
- 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 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. 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.

Laboratory Design

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

Signage

- A room sign must be posted on the door of every ABSL-2 room that includes the following information:
  1. The universal biohazard symbol.
  2. Animal Biosafety Level 2 (ABSL-2).
  3. Personal Protective Equipment (PPE) requirements.
  4. Procedures for entering and exiting the room.
  5. Principal Investigator(s) (PIs) responsible for the project.
  6. Laboratory contact person(s) and emergency contact number(s).
  7. Infectious agent(s) used in the room.

- To request ABSL-2 signs, please contact a biosafety officer at EHRS (215-898-4453).

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 instructor led Introduction to Laboratory Safety Training and subsequent annual updates before working in an ABSL-2 laboratory. Additional EHRS training may be required depending on the agents used.

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
2 surgical gowns securely tied in the back
1 hair bonnet
1 face mask covering both the mouth & nose
Eye protection if there are procedures generating a splash risk

- After all work is complete, remove the outer gown, outer shoe covers and the outer pair of gloves before leaving the ABSL-2 room.
- 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.

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

> 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.

- 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

Rodent Cage Changes Procedures

- Rodent cages must be changed inside a certified BSC using the following procedure:

- See the ABSL-2 Policy fact sheet for more details.

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 must be monitored by ULAR for proper function by using biological indicators

Animal Biosafety Level III (ABSL-3)

ABSL-3 is applicable to work done in animals infected with indigenous or exotic agents with a

> 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 agents.

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

- All personnel need to fulfill instructor led and hands-on training by a Biosafety Officer prior to entry.

Laboratory Procedures

- 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

- All work is performed in containment devices or equipment, such as sealed centrifuge rotors or biosafety cabinets.
- All manipulations of animals are performed in the Biosafety Cabinet, where possible.
- Safety devices must be used to contain potential aerosols created during processing of infectious material.
- Autoclaves must be used to decontaminate all waste created in the BSL-3 laboratory

Waste

- All waste is autoclaved out.

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.

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

ABSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND 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 warning signs • "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 containment devices • 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 containment devices • 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 TO BE 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 5th Ed., 2007.

Biosafety Cabinet:	**Clean Bench:**	**Fume Hood:**
• Use for infectious agents. • Provides product, user, and environmental protection. • **DO NOT** use for volatile chemicals.	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.
		

BSCs are not clean benches or fume hoods!

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.

Biosafety Cabinet Design

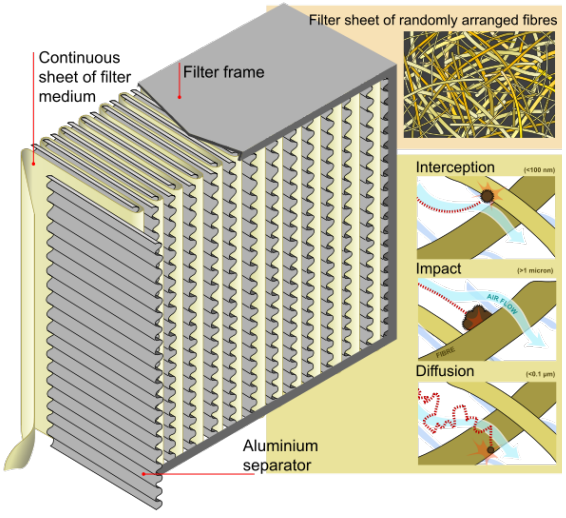
HEPA Filter

BSCs are equipped with High Efficiency Particulate Air (HEPA) filters in their exhaust and/or supply systems. These filters have a minimum efficiency of 99.97% 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. The main categories of cabinets are as follows:

- Class I cabinets exhaust HEPA filtered air back into 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.



- Class II Type B1 & B2 cabinets are 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
- Class III cabinets are completely enclosed glove boxes that are ducted to the building exhaust system. Air from the cabinet is 100% exhausted and passes through two HEPA filters. A separate supply HEPA filter allows clean air to flow onto the work surface.

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

TABLE 4-1: 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

Ducted Biosafety Cabinets

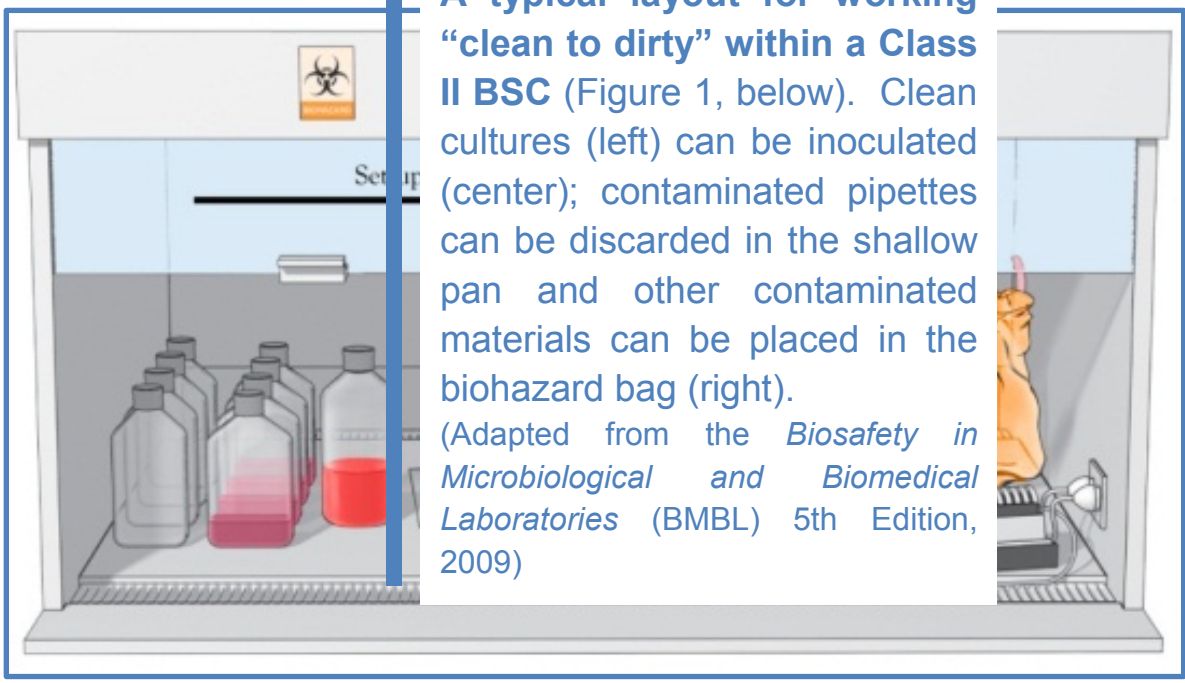
	Personnel Protection	Product Protection	Environmental Protection	Chemical Protection
Class II, Type B1	Yes	Yes	Yes	Yes (small amounts in back 1/2 of cabinet)
Class II, Type B2	Yes	Yes	Yes	Yes (small amounts)
Class III	Yes	Yes	Yes	Yes (small amounts)

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 and fluorescent light and let run for 5 to 10 minutes.
3) Wipe down the cabinet surfaces with an appropriate disinfectant.
4) Check magnehelic gauge for variations +/- minor scale divisions and report variations.
5) Transfer necessary materials (pipettes, pipette tips, waste bags, etc.) into the cabinet.

During Use:



A typical layout for working "clean to dirty" within a Class II BSC (Figure 1, below). Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right).

(Adapted from the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition, 2009)

1) Arrange work surface from "clean" to "dirty" from left to right (or front to back).
2) Keep front, side, and rear air grilles clear of research materials.
3) Avoid frequent motions in and out of the cabinet as this disrupts proper airflow balance and compromises containment.

1) Leave cabinet running for at least 5 to 10 minutes after use.
2) Empty cabinet of all research materials. The cabinet should never be used for storage.
3) Wipe down cabinet surfaces with an appropriate disinfectant.

After Use:

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

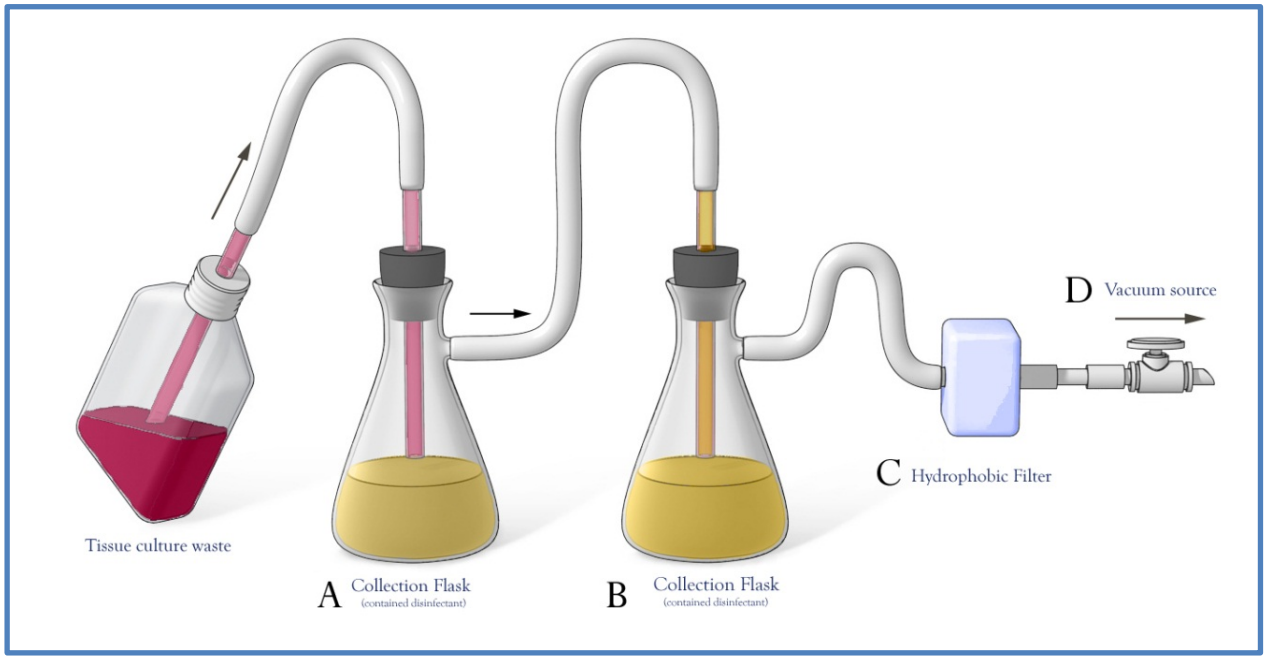
Special Considerations

Vacuum System Protection (see Figure 4-1)

Aspirator bottles or suction flasks (A) should be connected to an overflow collection flask (B) containing appropriate disinfectant. In addition, an in-line HEPA 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 4-1: Vacuum System Protection

(adapted from *Biosafety in Microbiological and Biomedical Laboratories*; BMBL 5th Edition, 2009)



Ultraviolet (UV) Lights

UV lights are not recommended to decontaminate the BSC. They are only effective if cleaned weekly to remove dust/dirt AND checked periodically with a meter to ensure proper wavelength emission. If UV lights are installed, they MUST be turned off when the cabinet is in use and whenever the room is occupied to protect eyes and skin.

Reference: Position paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. 2006. Applied Biosafety. (Appendix X)

Open Flames inside a BSC (see Figure 4-2)

DO NOT use continuous open flames (i.e. 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 disrupt 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 pictured below.

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.

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 all 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 troubleshooting or repairs

Figure 4-2: Alternatives to Use of Flames Inside a Biosafety Cabinet

BSC Service Provider

ALL certification, maintenance, and repairs must be conducted by **Micro-Clean, Inc.**, the university's contracted service provider. **NEVER attempt repairs or modifications to any biosafety cabinet.** This is against NSF/ANSI Standard 49, 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 moving to another location
- Before disposal
- Before certain repairs may be made to the unit

Micro-Clean, Inc. uses chlorine dioxide gas (ClO_2) as the method of decontaminating biosafety cabinet filters, which is an NSF/ANSI Standard 49 approved decontamination method. ClO_2 gas is non-carcinogenic. The decontamination process takes approximately four hours to complete and leaves no residue or residual odor on the cabinet.

Consult the Biosafety Cabinets section of the EHRS website for current **Micro-Clean, Inc.** contact information and how to prepare the Biosafety Cabinet for certification and/or repair.

Remember: BSCs will only protect you, your products, and the evnironment if used properly!

DO:

- follow practices and procedures outlined above
- call a Biosafety Officer at 215-898-4453 with any questions

DO NOT:

- use if out of certification
- clutter grilles
- overcrowd cabinet
- put head inside cabinet
- disrupt airflow with quick movements
- make any repairs or modifications to the BSC
- use an open flame inside a BSC

COMMON LAB EQUIPMENT

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

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

Key Safety Practices:

- **Never mouth pipette**.
  Always use a pipetting aid.
- Minimize aerosol production.
- Discard contaminated disposable pipettes in an appropriate sharps container.
- When possible, perform pipetting in a biosafety cabinet

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 (i.e., 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 on infectious waste)
- The use of needle-nipping devices is prohibited.

Cryostats

Freezing tissue does not necessarily inactivate infectious agents. Gloves should be worn during preparation of frozen sections.

Key Safety Practices:

- **Consider the contents of the cryostat to be contaminated and decontaminate it frequently with appropriate disinfectant.**
- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
- Handle microtome blades with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions used for staining potentially infected frozen tissue sections to be contaminated.

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 recommended because burners can produce turbulence which disrupts the airflow in a BSC. (see section 4.1)**
- If a gas burner must be used, select a touch-

plate burner with a pilot light. Appropriate hard piping from the house gas line must be used and an easily accessible shut-off valve must be placed outside the BSC.

- Electric incinerators minimize aerosol production.
- Disposable plastic loops do not require sterilization.
- See appendix "Alternatives to Bunsen Burners."

Water Baths

The use of sterile water in water baths does not prohibit the growth of microorganisms, such as bacteria or fungi, but merely slow 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 sterile 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 sterile using good lab practices.

Key Safety Practices:

- Wipe down tissue culture flasks, plates, and other labware with 70% ethanol before placing into the incubator
- Do not use disinfectants that may have toxic effects on the cells to wipe down the incubator
- Check sterility of incubator and discard old items
- Disinfect the incubator immediately after spills occur
- Keep areas around the incubator, such a bench tops 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

Aerosol Producing Equipment

Equipment / Procedure	Particle Size (diameter)
Sonicator	4.8 micron
Dropping liquid bacterial culture	3.5 micron
Dropping lyophilized culture	10.0 micron
Pipette blow out	4.9 micron
Vortexing culture	4.8 micron
Centrifuge	4.0 micron
Blender	0.2 micron

(Adapted from the *Lawrence Berkely National Laboratory Biosafety Manual 2010*)

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.

Key Safety Practices:

Before centrifugation:

- Use only rotors compatible with the centrifuge. Check the expiration date for ultracentrifuge rotors.
- Check tubes, bottles, and rotors for cracks and deformities before each use.
- Make sure that the rotor, tubes, and spindle are dry and clean.
- Examine O-rings. Replace if worn, cracked, or missing.
- Never overfill centrifuge tubes (do not exceed

three-fourths full).

- Cap tubes before centrifugation.
- Balance buckets, tubes, and rotors properly.
- Check that the rotor is seated on the drive correctly, put lid on rotor, close the lid on the centrifuge, and secure it.
- When using swinging bucket rotors, make sure that all buckets are hooked correctly and move freely.
- 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.
- Do not exceed safe rotor speed.
- The operator should not leave the centrifuge until full operating speed is attained and the machine appears to be running safely without vibration.
- Stop the centrifuge immediately if an unusual condition (e.g., noise or vibration) begins, and rebalance the load if needed

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.

Classes of Centrifuges

- **Low-speed centrifuges** that do not exceed 5,000 rpm are commonly made for bench top use
- **High-speed centrifuges** that do not **exceed** 25,000 rpm may include bench top or floor models.
- **Ultracentrifuges that may exceed** 100,000 rpm may include bench top or floor models

Additionally, when centrifuging **BIOHAZARDOUS MATERIAL** follow these precautions:

- 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.

After centrifugation:

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

(Information in this section was adapted from the University of Minnesota's "Bio Basics Fact Sheet: Centrifuge Safety" and the Lawrence Berkeley National Laboratory Biosafety Manual 2010)

INFECTIOUS AGENTS

Infectious agents encompass all microorganisms and agents that that are known to be pathogenic to humans.

Types of Infectious Agents

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

- Bacteria
- Parasites
- Prions
- Viruses

In addition, human and animal materials, such as blood or tissues, may harbor pathogenic agents. 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 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 DNA in live animals.

2. Recombinant DNA: Work with rDNA is reviewed by the Institutional Biosafety Committee (IBC) in compliance with the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Additional information can be found in Section 5.2 of this manual.

3. Biological Agents and Materials: The Biological Agent Registration (BAR) is an electronic registry of all biological agents that are used or stored in Penn's laboratories. 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 Agent Registry (Penn BAR)

The Office of Environmental Health & Radiation Safety (EHRS) developed an electronic Biological Agent Registry (BAR) to establish a comprehensive database of biological agents at Penn. Information collected by BAR will be used by the biosafety staff and the research community to conduct risk assessments *". . . that will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment and facility safeguards. . ."*[1] to mitigate

hazards.

BAR is an on-line form that may be completed by the Principal Investigator (PI) or by a lab member appointed by the PI. Initially, expect to devote at least one hour to complete this form depending on the extent of your work with biological agents. You may start the form, save information, and return at a later time to make changes. However, your form will not be submitted to EHRS until you have completed all steps and have clicked on "Submit to EHRS."

BAR must be updated on a yearly basis or when pertinent changes in the laboratory are made. 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.

[1] **Biosafety in Microbiological and Biomedical Laboratories** (5th Edition). U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and Prevention, National Institutes of Health.
HHS Publication No. (CDC) 21-1112 Revised December 2009.

RECOMBINANT DNA (rDNA)

Recombinant DNA (rDNA) includes molecules that are constructed outside living cells by joining natural or synthetic DNA segments of DNA molecules that can replicate in a living cell. They may also result from the replication of such construction DNA molecules. rDNA also includes synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g. a toxin or a pharmacologically active agent) and are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH guidelines.

NIH Guidelines for Research Involving Recombinant DNA Molecules established the recommendations for performing rDNA experiments in a manner safe for researchers and the public. These guidelines are applicable to **ALL** rDNA research at any institution that receives NIH support for rDNA research.

Institutional Biosafety Committee (IBC) – Functions to review, approve, and oversee projects in accordance with the responsibilities defined in the *NIH Guidelines*.

Types of rDNA Work

The following are considered types of work with recombinant DNA:

- Human gene transfer studies
- Creation of transgenic rodents
- Breeding of two different strains of transgenic rodents at ABSL-2 or higher
- Cloning genes using plasmids
- Generating and Use of vectors to deliver genes

Not all recombinant DNA work requires registration with Penn's IBC. Please see below for specifics regarding registration.

Compliance with the NIH Guidelines

Failure to comply with the NIH Guidelines can result in the suspension, limitation, or termination of NIH financial assistance for the non-compliant research project AND ALL other rDNA research at the institution.

Registration Requirements at Penn

Work that must be registered with the IBC ***simultaneous*** with the start of work*:

- CREATING transgenic rodents
- rDNA molecules with no more than 2/3 of the genome of a eukaryotic virus being manipulated and maintained in tissue culture

Work that must be registered with the IBC ***before*** work may begin*:

- GENERATION of rDNA constructs
- USE of rDNA constructs (including rDNA received from Vector Core, gifted, etc.)
- CROSSING two different transgenic rodent strains that:

  Are assigned to ABSL-2 or higher

  Contain more than 50% of an exogenous eukaryotic viral genome

  Carry a transgene under the control of a gamma retroviral promoter

Exempt experiments are those involving rDNA molecules that*:

- Are not in organisms or viruses
- Consist entirely of DNA from single non-chromosomal or viral DNA source
- Consist entirely of DNA from a prokaryotic host when propagated only in that host
- Consist entirely of DNA from an eukaryotic host when propagated only in that host (excluding DNA from viruses)
- Consist entirely of DNA from different species that exchange DNA by known physiological processes (list periodically updated in Appendices A-I through A-VI)
- Do not present significant risk to health or to the environment as determined by NIH Director with the advice of Recombinant DNA Advisory Committee (RAC)
- Crossing transgenic rodents requiring ABSL-1 containment

*Please consult the *NIH Guidelines* for specifics or obtain advice a biosafety officer.

Registration Review Process at Penn

Complete and Submit Registration Form: Penn's rDNA registration form can be found online at www.ehrs.upenn.edu. The PI must complete the form, sign it, and submit it to EHRS.

Pre-Committee Review: A biosafety officer will review the submitted registration document and contact you with any questions or necessary revisions.

Full IBC Review: The IBC reviews registrations once a month.

- The IBC is not permitted to review registrations outside of a fully convened meeting.
- Please be sure to submit registrations in a timely fashion. Penn's IBC meeting dates may be found online at the EHRS website.

IBC Approval: If the IBC approves your registration, you will receive an approval letter documenting:

- The IBC registration number (ex. #12-095)
- The assigned biosafety level (BSL)
- The assigned animal biosafety level (ABSL)

Registration Renewal: Registrations expire after 3 years. To renew a registration, submit a new registration document to the IBC.

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

Training Requirements

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

- Principal Investigator
- Anyone directly involved in rDNA experiments

Penn receives substantial NIH funding for projects involving rDNA research, therefore:

- **ALL researchers at Penn must comply with the *NIH Guidelines.***
- **Even those not directly receiving NIH funding must comply.**

Just one non-compliant researcher can jeopardize NIH grant funding for the entire university!

HUMAN SOURCE MATERIAL

Human Source Materials: Cells, blood, serum, tissues, feces, and body fluids (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 and reviewed annually by each lab working with human source material and other potentially infectious material (OPIM).

Universal Precautions: An approach to infection control, where all human source material is handled as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

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

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

The OSHA 29 CFR 1910.1030 Bloodborne Pathogen Standard (link) stipulates that employers must train all employees who have occupational exposure to human blood, human blood components, and products made from human blood. 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

Penn'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.

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.

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.

Personal Protective Equipment (PPE)

The following PPE is required to be worn when manipulating any human 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 may be required depending on the risk of a splash.

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

If there is a significant *splash risk*, such as when working on an open bench, goggles/glasses with a face mask or a face shield must be worn.

Practices and Procedures

Biosafety Level 2 (BSL-2) practices and procedures must be used when manipulating any human source material. A detailed explanation of BSL-2 criteria can be found in the *CDC's Biosafety in Microbiological and Biomedical Laboratory (BMBL), 5th 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 5-1).

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 follow with 70% ethanol to prevent corrosion.

4) Waste Disposal

All research materials and PPE used when manipulating human materials must be disposed of through the infectious waste stream. Any objects 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.

5) Transport

To transport between laboratories, human materials must be placed in a leak-proof, closable 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

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. cut, puncture, needle-stick, etc.) occurs:

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

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

- **Occupational Medicine** (215-662-2354)
  (Penn employees)
  Ravdin Building, 2nd Floor
  3400 Spruce St.
- **Student Health Service** (215-746-3535)
  (Penn students)
  3535 Market St.
  Suite 100
- **Hospital of the University of Pennsylvania Emergency Department**
  (ALL after work hours)
  Silverstein Pavilion 3400 Spruce St.

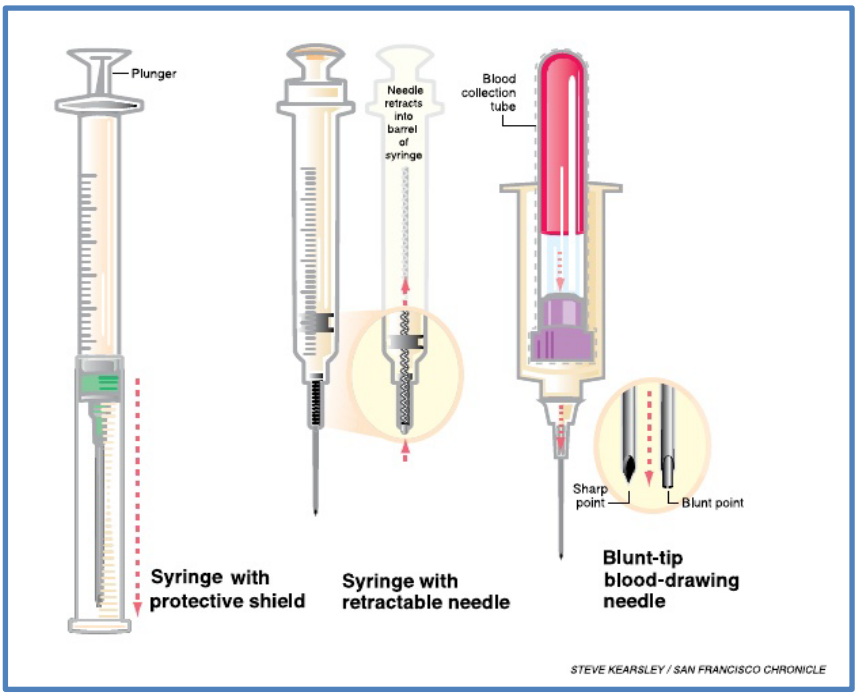


Figure 5-1: Examples of Syringes with Safety Features.
(NIOSH Hazard Review 2010)

NON-HUMAN PRIMATE MATERIALS

Non-human primate (NHP): Refers to species ranging from apes to monkeys. Specifically, old word macaque monkeys, including rhesus macaques, pig-tailed macaques, and cynomolgus monkeys, are of concern as they are known to carry Herpes B virus (see below).

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*. 4th ed. Washington, DC: ASM, 2006.).

Viruses

- Hepatitis A and B virus
- * Macacine 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*

- Herpes B virus has an approximately 70% mortality rate in humans when not immediately treated.

- Herpes B virus may be present in materials from macaque monkeys, 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.

Macacine herpesvirus 1 (Herpes B Virus) is of specific concern when handling NHP material

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 must be worn depending on the splash risk.
- If there is a ***minimal splash risk***, such as when working inside a biosafety cabinet, closely fitting safety glasses should be worn.
- If there is a ***significant splash risk***, such as working on an open bench, goggles/safety glasses with a face mask must be worn.
- If you are working with ***live NHPs***, goggles/safety glasses worn under a face shield are required. In addition, all ULAR PPE requirements must be followed.
- Examples of eye protection:

  Penn logo safety glasses, UVEX Genesis part # 19 130 2060 (Fisher Scientific)

  Hybrid safety glasses/goggles, Peltor Maxim 2.2 Goggles part #125542 (Lab Safety Supply) Chin length face shield (wear with one of above goggles or glasses), Fisherbrand full length face shield part #S98127 (Fisher Scientific)

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), 5th 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 objects 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 materials must be in accordance with IATA regulations. ALL persons shipping hazardous or biological material must contact EHRS for training session information.

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. cut, puncture, needle-stick, etc.) occurs:

- Thoroughly wash the injured area with povidone-iodine, or chlorhexidine, or soap and water for 15 to 20 minutes.

IMMEDIATELY REPORT ALL EXPOSURES or possible exposures to:

- **Occupational Medicine**
  (Penn employees and non-university affiliates)
  Ravdin Building, 2nd Floor
  3400 Spruce St.
- **Student Health**
  (for Penn students)
  ProMed Building, Suite 100
  3535 Market St.

Hospital of the University of Pennsylvania
Emergency Department
(ALL after work hours)
Silverstein Pavilion
3400 Spruce St.

Green NHP Exposure Cards:

Wallet-sized "Green Cards," which describe procedures to follow in case of an exposure to NHP material, are available from EHRS. 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).

Progression of this disease leads to ascending meningoencephalitis and may result in cardiac or respiratory arrest in as little as 3 days after symptoms manifest. **This disease can be treated successfully if identified early. Universal precautions should be taken.** Additional information can be located at http://www.gsu.edu/bvirus

If the person with this card exhibits any of these symptoms, please contact Julia Hilliard, Ph.D. at the National B Virus Resource Center at 404-413-6550 or J. Scott Schmid, Ph.D. and colleagues at the **Centers for Disease Control** at 404-639-0066

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 1*) 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 Penn Tower Fourth Floor
• Students:	Student Health Services ProMed Building 3535 Market, Suite 100
• After hours, ALL report to:	Emergency Department Silverstein/HUP

Important Phone Numbers for Treating Physicians:

• Occupational Medicine	215-662-6110
• PAGER	215-524-8864
• Student Health Services	215-746-3535
• University Lab Animal Resources	215-898-6466

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 program is jointly administered by the Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and 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 CDC and/or USDA.

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

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 (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 **Appendix C** 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 amount under the control of a principal investigator *does not exceed, **at any time**, the amounts permissible by the select agent regulations.*

List of Excluded Microorganisms

For a current list of the exclusions, please visit the National Select Agent Registry (NASR) website.

List of Exempt Quantities of Toxins

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

HHS Toxins [§73.3(d)(3)]	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

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 these amounts 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)).*

THE BIOLOGICAL RESEARCH LABORATORY

There are several components that need to be in place for employees to safety work 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 on the EHRS website. All web-based trainings are accessible through Knowledge Link. For more information on Knowledge Link and how to use it, see the Knowledge Link Help pages or contact EHRS at traininghelp@ehrs.upenn.edu or 215-898-4453.

- Instructor led Laboratory Safety Training – 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.
- Annual Bloodborne Pathogen 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.
- Recombinant DNA training – required for any PENN employee who conducts research using recombinant DNA.
- Biosafety Cabinet Training - required for any PENN employee who conducts research in a Biosafety Cabinet.
- Instructor led Shipping & Packaging Infectious & Biological Substances training – required for any PENN employee who prepares and packages infectious and biological substances (classified as Dangerous Goods) for shipping. Shippers must be trained every two years to ensure compliance
- Instructor led Shipping & Safety Training for Personnel Who Collect & Ship Infectious Waste – required for any PENN employee who packages and/or signed off on shipping manifest for infectious waste.

Immunizations

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

Annual Lab Audits

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

Audit letters containing recommendations to improve safety in the lab are sent to the PI and it is the PI's responsibilities to take action on and review with the lab staff these recommendations. The PI must return the signed audit letter to the EHRS office indicated completion of the recommendations listed.

A Biosafety Officer performing the audit will address the following biohazard concerns:

- Hand washing
- Use of proper PPE
- No food or drink in the lab
- Properly labeled lab equipment
- Up to date room signs
- All personnel have completed training
- Chemical Hygiene Plan is current and posted
- If personnel shipping hazardous material are trained
- Completed Exposure Control Plan (Appendix C) when using primary human material

- Recombinant DNA material is registered with the IBC
- Proper decon procedures are used after manipulation of biohazardous material
- Infectious waste is properly segregated and disposed of
- Biosafety Cabinet is certified and not over crowded; air grilles are free of materials
- Bunsen burners are prohibited from use in the Biosafety Cabinet
- UV lights are properly used in the Biosafety Cabinet
- The vacuum trap in the Biosafety Cabinet has a HEPA filter on the tubing and is kept in a secondary container
- Infectious sharp materials (including razors, syringes with and without needles, pipettes, pipette tips, etc.) are disposed of in a hard sided sharps container.
- Sharps containers are used properly and disposed of when they are ¾ full.

Additional lab safety concerns may also be addressed. More information can be found on the EHRS website

Room Signs

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

Room sign information includes:

- Department, building name and room number
- Principal Investigator(s)
- Other researchers using this space
- Hazard Labels (Biological, Chemical, Radioactive, etc.)
- Name and contact number for after hour emergencies
- University of Pennsylvania main emergency numbers

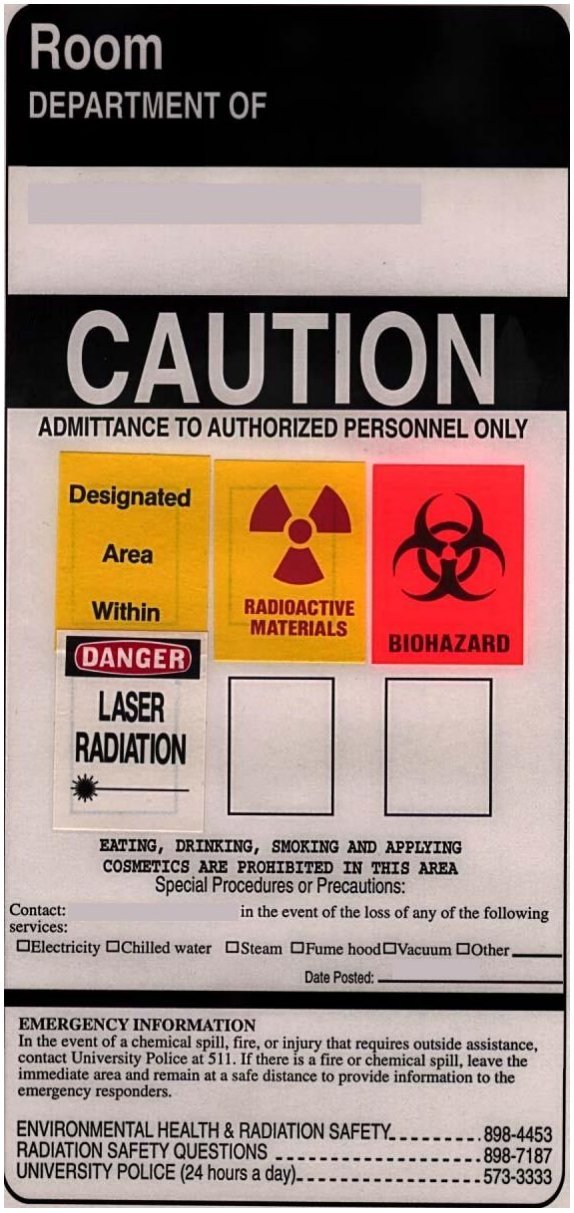


Figure 6-1: Laboratory Room Sign

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 (see below)

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 prevent 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 to protect hands from exposure to hazardous materials, including: organisms containing **recombinant** DNA, recombinant experimental animals, RG2 materials, BBP materials or surfaces and items contaminated with BBP materials.
- 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 and wash hands. See pages 49 and 50 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 coast made of 100% cotton are strongly recommended.
- **DO NOT WEAR LAB CLOTHING IN NONLAB AREAS** (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 by the facility. Personnel are not permitted to launder laboratory clothing at home.

E. Respiratory Protection

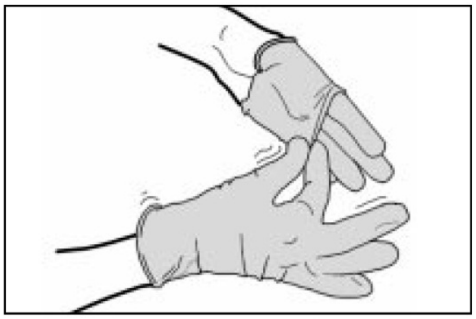
- The University of Pennsylvania has determined that certain employees are exposed to respiratory hazards during routine operations. Penn's respiratory protection program will ensure that all University of Pennsylvania employees and students are protected from exposure to these respiratory hazards.
- 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. Any 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

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

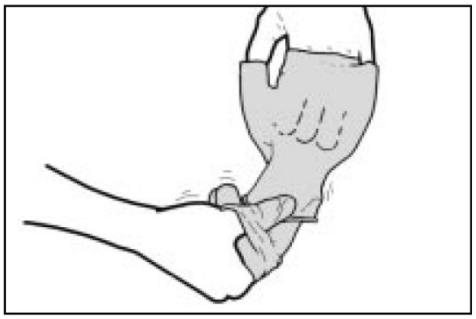
Removal of Gloves Technique

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

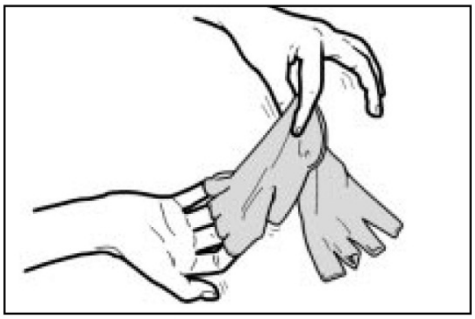


1. Grasp one glove at wrist and pull down to knuckles.

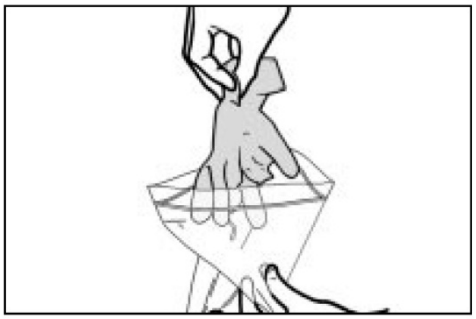
2. Grasp other glove at wrist and pull down to knuckles.



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.



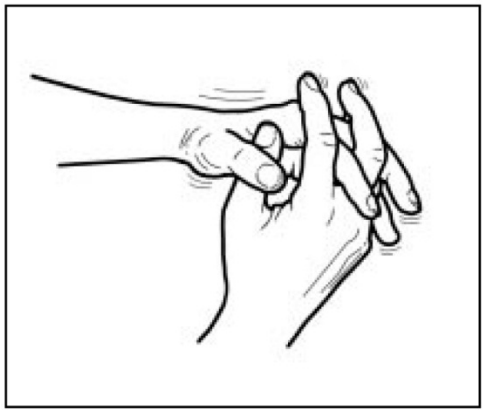
5. Dispose of gloves in an appropriate container.

6. Wash hands after removing and disposing of gloves.

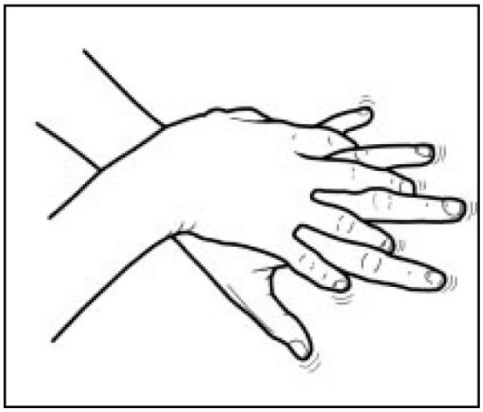
Hand Washing Technique

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

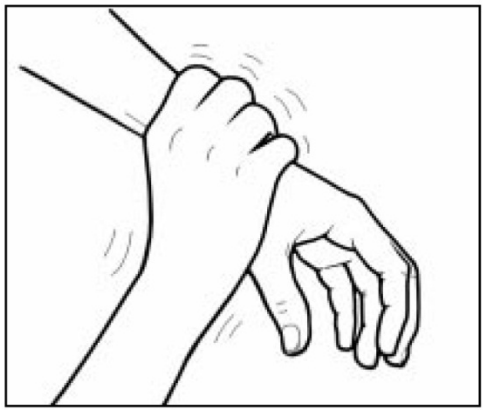
1. Wash palms.



2. Wash between fingers.



3. Wash back of hands.



4. Wash wrists.

DECONTAMINATION

Decontamination describes the process or treatment of medical devices, instruments, or environmental surfaces safe to handle. 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 biological indicators (*B. subtilis (globigii)* spore strips) to assure 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 EPA approved and must be shown to be effective against the target organism(s) to be deactivated.

Liquid disinfectants are divided into the following categories:

- Aldehydes
- Halogen-based biocides
- Quaternary Ammonium Compounds
- Phenolics
- Acids/Alkalis
- Heavy Metals
- Alcohols

See the tables that follow for examples, activity and information on the efficacy of each category of liquid disinfectant.

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 airhandling 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. If UV must be used, it should be used when areas are not occupied. 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 for more information.

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

Disinfectant Group	Examples	Activity	Efficacy
Aldehydes	Formaldhyde, Paraformaldehyde, Gluteraldehyde (Cidex-cold surface sterilization)	Biocidal activity Alkylation of carboxyl, hydroxyl and sulfhydryl group on proteins.	Surface and space decon as a gas and liquid
Halogen-based biocides	Iodine, Chlorine Wescodyne, Betadyne, Povidone-iodine and other iodophors are commercially available iodine-based disinfectants. Clidox® & Virex are chlorine based disinfectants.	Biocidal activity Bind to protein and modify sulfhydryl, amino, indole and phenolic groups. Acts as oxidizing agent.	Free organic matter, protein, will compete for chlorine ion reducing biocidal activity and making the disinfectant organic load dependent.
Quaternary Ammonium Compounds	Zephirin, CDQ, A-3	Cationic detergents, most effective against gram positive bacteria. Biostatic Generally ineffective against viruses, spores and *Mycobacterium tuberculosis* Action is causing membrane damage and leakage, followed by denaturation.	Activity is reduced in presence of heavy organic matter loads. Good for water baths, incubators, and applications where halide or phenolic residues are not desired.
Phenolics	O-phenophenoate-base compounds	Biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria.	Cresols, hexachlorophene, alkyl- and chloroderivatives and diphenyls are more active than phenol. Amphyl, O-syl, Tergisyl, Lysol, Vesphene, L-Phase and Expose
Acids/Alkalis	In general acids are better than alkalis.	Increase of H and OH species in solutions which interfere with certain microbial functions. Total effect is not only dependent on pH alone. Weak organic acids are more potent than inorganic acids.	Disruption of 2 and 3 conformation of enzymes and structural proteins.
Heavy Metals	Silver nitrate and mercuric chloride used in 1:1000 aqueous solutions	Attack on protein sulfhydryl groups and disruption of enzyme functions.	Organic matter can reverse the disinfectant properties of mercurials.
Alcohols	Ethanol and isopropanol in 70 to 80% aqueous solutions	Disruption of cellular membranes, stabilization of lipids and denaturation of proteins by acting directly on S-H functional groups. Active against lipid-containing viruses, broad spectrum of bacteria, ineffective against spore-formers. Evaporate quickly, no residue, but evaporation interferes with contact time. Immersion is best.	Absolute alcohol is not as effective. Water is required for disinfection process.

INFECTIOUS WASTE

Infectious waste refers to biological agents (viral and bacterial cultures, human cells lines, tissue samples, organs, blood and blood products, live and attenuated vaccines) used in research laboratories and waste created during the manipulation of these biological agents (media, serum, agar, consumables, gloves, etc).

Infectious waste must be disposed of through the Infectious Waste Stream. The Infectious Waste Stream refers to the segregation of different types of waste that are handled in specific manners.

Infectious Waste Streams

There are three categories of infectious waste: infectious solid waste, infectious liquid waste, and infectious sharps waste.

Infectious Sharps Waste - Any device/item having corners, edges, or projections capable of cutting or piercing the skin or waste bags. These include hypodermic needles, needles with attached tubing, suture needles, syringes, (with or without the attached needle), scalpels and razor blades, Pasteur pipettes, pipette tips, serological pipettes, plastic transfer pipettes, blood vials, slides, cover slips and other broken or unbroken glass or plasticware that have been used in the manipulation of infectious agents, animal procedures, and human patient care or treatment.

Figure 8-1: Infectious Sharps Containers

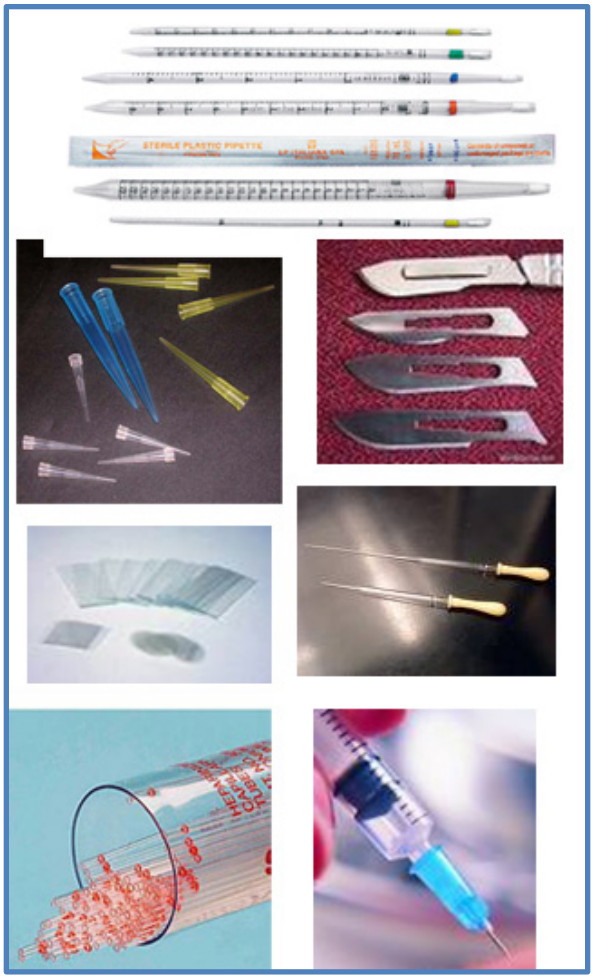


Figure 8-2: Examples of Sharps

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

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

Figure 8-4: Infectious Solid Waste Bag

Infectious Solid Waste – Other waste produced in the lab that may be potentially infected with biological agents, including non-sharp plastic consumables, agar gels, gloves, gowns, etc.

Infectious Waste Management

Sharps Waste

1. Sharps may be decontaminated at the end of use and placed in a lidded container for storage or disposed of directly into a sharps container.
2. Syringes and scalpels are not to be recapped prior to disposal.
3. DO NOT discard infectious sharps through the regular waste stream.
4. DO NOT discard sharps in any bag.
5. DO NOT autoclave chemically contaminated sharps. Instead, label with "**Chemical Contaminated Sharps DO NOT AUTOCLAVE**", close when container is ¾ full, and dispose of through the infectious waste stream.

Liquid Waste

Liquids must be decontaminated prior to disposal by either an appropriate disinfectant or autoclaving.

Solid Waste

1. Biological material should be inactivated or decontaminated at the end of its use.
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 biohazard symbol, whether used or not, must be disposed of as infectious waste.
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 then that for which it is intended.

Procedures

1. All personnel handling infectious waste must complete **Management of Laboratory Waste**, found in the Knowledge Link Catalog**.**
2. Each generator is responsible for segregating and autoclaving their infectious waste. See the Lab Waste Guide fact sheet for more details.
3. Dispose of all material with the Universal Biohazard Warning Sign through the infectious waste stream.
4. See the Waste Matrix specific to your school or building for more details on collection and disposal of all laboratory waste.
5. Infectious waste needs to be properly packaged for removal and incineration by the University's infectious waste hauler.
6. 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**

Figure 8-5: Biohazard Bags

the Infectious Waste Manifest. Contact EHRS to be trained.

Sharps Waste

1. Sharps containers must be used with their lids in place.
2. Sharps containers must be closed and discarded when they are ¾ full.
3. A designated person autoclaves the sharps containers (except for those marked "Chemical Contaminated Sharps DO NOT AUTOCLAVE") and places them into the collection bin for the infectious waste stream.
4. Sharps containers marked with "Chemical Contaminated Sharps DO NOT AUTOCLAVE" are placed into the collection bin for the infectious waste stream.
5. Sharps containers marked with RADIOACTIVE **MUST NOT** be put into the infectious waste stream. Contact Radiation Safety for more information

Liquid Waste

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

Solid Waste

Figure 8-6: Sharps container for chemical contaminated sharps.

1. See the Waste Matrix specific to your school or building 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 autoclaved and disposed of on a regular basis.

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

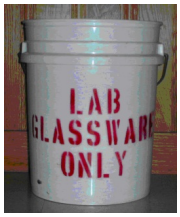
KNOW WHERE TO THROW!

RED BAG WASTE

INFECTIOUS WASTE

SHARPS CONTANERS

ARE NOT DISPOSED OF IN:



YOU MUST OBEY THE LAW!

INFECTIOUS WASTE SHARPS

Sharps include any object the 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"

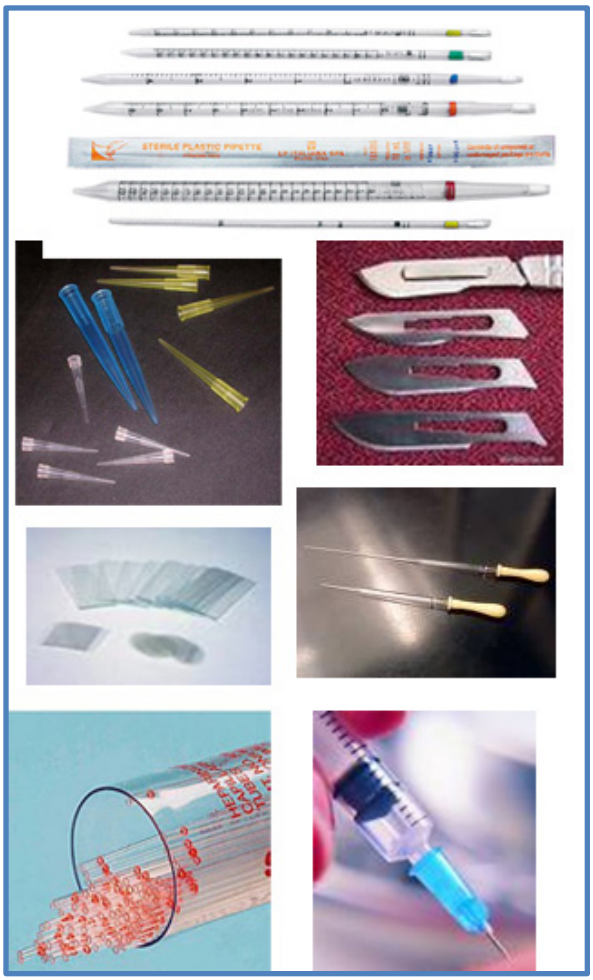


Figure 8-7: Examples of Sharps

Infectious Waste Management

1. Sharps may be decontaminated at the end of use or disposed of directly into a sharps container.

Figure 8-8: Infectious Sharps Containers

2. Contaminated sharps must not be reused.
3. Syringes are not to be recapped prior to disposal.
4. DO NOT discard sharps through the regular waste stream.
5. DO NOT discard sharps in any bag.
6. DO NOT autoclave chemically contaminated sharps. Instead, label with "Chemical Contaminated Sharps DO NOT AUTOCLAVE", close when container is ¾ full, and dispose of through the infectious waste stream

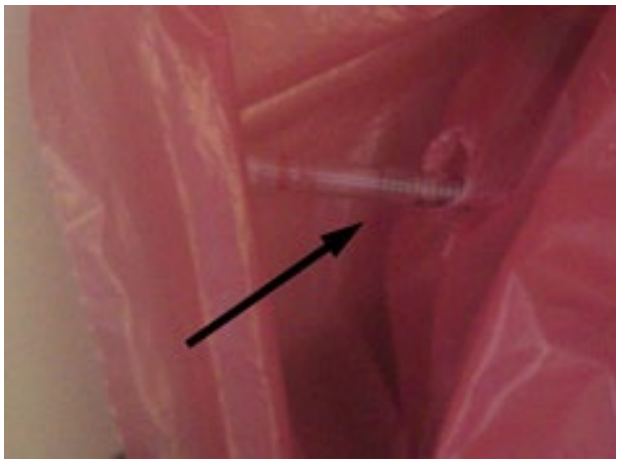
Figure 8-9: Sharps container for chemical contaminated sharps.

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

Procedures

1. Each generator is responsible for segregating and autoclaving their infectious waste. See the Know Where to Throw fact sheet for more detail or consult a Biosafety Officer.
2. See the Waste Matrix specific to your school or building 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. A designated person autoclaves the sharps containers (except for those marked "Chemical Contaminated Sharps DO NOT AUTOCLAVE") and places them 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.
7. Sharps containers marked as RADIOACTIVE **MUST NOT** be put into the infectious waste stream. Contact Radiation Safety for more information.

DO NOT place sharps in any bag!



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

16. DO NOT autoclave infectious waste that may be contaminated with either radioactive material or chemicals.
17. 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.**
18. DO NOT put radioactive material in the infectious waste stream!
19. 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 EHRS, 215-898-4453, 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.

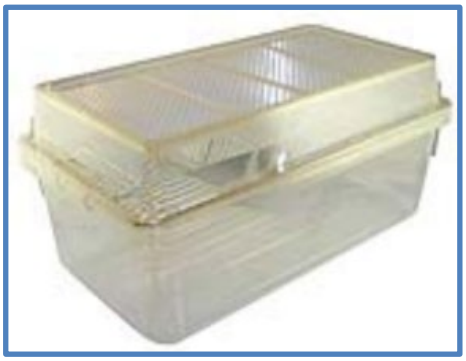
Chemical Contaminated Waste

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.

Contact EHRS for disposal guidance **PRIOR** to creating mixed waste or chemical contaminated infectious waste.

RESEARCH ANIMAL INFECTIOUS WASTE

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 must be treated as infectious waste and disposed of through the infectious waste stream.



Infectious Waste Management

6. 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.
7. Radioactive animal carcasses are collected by EHRS for decay and then disposed of through the infectious waste stream.
8. 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 surgucal mask should be worn when disposing of animal carcasses and bedding.

Animal waste **MUST NOT** be discarded in the regular trash!

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.

> Training is needed **BEFORE** operating an autoclave!

Infectious Waste Management

1. Autoclave all cultures and stocks of infectious agents, consumables used in the manipulation of said cultures, recombinant DNA (rDNA), human and infectious animal tissue waste, cages and bedding of potentially infected animals, and reusable lab ware.
2. DO NOT AUTOCLAVE items contaminated with solvents, volatile or corrosive chemicals, or items containing carcinogens, mutagens or teratogens.

- Contact EHRS at 215-898-4453 for more information 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

- Autoclave tape gives you a visual indication that the item has passed thru steam sterilization. DO NOT use as the only indicator of sterilization and decontamination!
- 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. This should be used with each autoclave run.
- 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. These should be used monthly to assure proper function of the autoclave. Results should be recorded in a Quality Control log.
- EHRS recommends that a service contract be established for each autoclave and maintenance be performed annually and when needed.

Figure 8-10: Autoclaves

Figure 8-11: Devices used in the validation of autoclaves.

Procedures

The following are guidelines on proper use of an autoclave and do not replace one-on-one training.

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.

Figure 8-12: Proper PPE to be worn when working at an autoclave.

- Use autoclavable polypropylene / polyethylene biohazard bags ONLY.
- Use a heat resistant secondary container to retain any leakage that may occur.
- DO NOT overfill bags or autoclave chamber as this decreases its effectiveness.

Figure 8-13: Autoclavable Infectious Waste Bags

- Leave 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.

Figure 8-14: Biohazard bags placed in autoclavable secondary containers prior to autoclaving.

DURING Autoclaving

- Use appropriate cycle times for the items you will be autoclaving:

  Sterilizing Clean Materials: 30 min. at 121°C and 15 psi

  Decontaminating Waste: 60 min. at 121°C and 15 psi

  Dense Loads: lengthen running time

  Liquids: use slow exhaust

  Glassware: use fast exhaust

- Segregate autoclave loads (infectious waste, liquid, or labware).
- **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.

Dispose of ALL autoclaved biohazard waste through the Infectious Waste Stream

TRANSPORT AND SHIPPING OF BIOHAZARDOUS MATERIAL

For a detailed discussion of shipping 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 shipping requirements. 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 2 years 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, import and export of biological materials and 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: known or suspected of being infected with any disease transmissible to man. 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.
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 man.
3. Insects: Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to man. 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 (202) 358-2095

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 CDC because the materials have been judged to be noninfectious, but may be construed to be infectious by U. S. Customs inspection personnel, a formal letter should be composed describing the material by the shipper to accompany the shipment. 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.

Application to CDC for the importation permit should be made 10 working days in advance of the shipment date to allow time for processing, issuance and delivery of the permit and shipping labels to the permittee. Go to http://www.cdc.gov/od/eaipp/importApplication/ for on line application.

Animal, Plant and Other Permits:

U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required to import or transport infectious agents of livestock and biological materials containing animal, particularly livestock, material. 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 online or from EHRS (215) 898-4453. Further information may be obtained by calling the USDA/APHIS at (301) 734-7834.

Export 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 in the country where the package is being shipped. If your shipment requires an export permit, it must be completed and approved by the appropriated government agency **prior** to shipment. If you need an import permit or have questions, please contact Elizabeth Peloso, Director Export Compliance epeloso@upenn.edu or 215-746-0234

Export Administration Regulations Database:

Please consult **Resources** page for links.

Training is required PRIOR to shipping any biohazardous material, chemicals, or dry ice.

RISK GROUPS: Bacteria

BL – Biosafety Level; RG – Risk Group; V – Vaccine available; T – Toxin produced; HP – Human Pathogen; AP – Animal Pathogen; SA – Select Agent

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Acinetobacter	lwoffi	formerly Mima polymorpha		2	+		
Actinobacillus	actinomycetemcomitans			2	+		
Actinobacillus	spp			2	+		
Actinomadura	madurae				+		
Actinomadura	pelletieri				+		
Actinomyces	bovis					+	
Actinomyces	gerencseriae				+		
Actinomyces	israelii				+		
Actinomyces	naeslundii						
Actinomyces	pyogenes			2	+	+	
Actinomyces	spp				+	+	
Aerococcus	spp	was A.viridans, A.urinae, A. christensenii					
Aeromonas	hydrophila			2	+	+	
Aeromonas	punctata						
Aeromonas	spp.				+		
Afpia spp							
Amycolata	autotrophica			2	+		
Arachnia	propionica	was Propionibacterium propionicum			+		
Arcanobacterium	haemolyticum	was Corynebacterium		2	+		
Archanobacterium	equi	was Corynebacterium all serotypes			+		
Arizona	hinshawii			2	+		
Bacillus	anthracis		2/3	2	+	+	+
Bacillus	cereus				+	+	
Bacillus	thuringiensis				+	+	
Bacteroides	fragilis				+	+	
Bacteroides	spp				+	+	
Bartonella	bacilliformis				+		
Bartonella	elizabethae				+		
Bartonella	spp.	see species listed in NIH Guidelines			+		
Bartonella	henselae			2	+		
Bartonella	quintana		2	2	+		
Bartonella	vinsonii		2	2	+		
Bordetella	all spp			2	+		
Bordetella	bronchiseptica			2	+	+	
Bordetella	parapertussis			2	+		
Bordetella	pertussis		2	2	+		
Borrelia	burgdorferi			2	+	+	
Borrelia	duttoni				+		
Borrelia	recurrentis			2	+		
Borrelia	spp	see spp. for RG2			+	+	
Borrelia	vincenti						
Botulinum neurotoxin		protein toxin					+
Branhamella	catarrhalis						
Brucella	abortus		3	3	+	+	+
Brucella	canis		3	3	+	+	
Brucella	melitensis	(USDA restricted)	3	3	+	+	+

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Brucella	ovis			3	+	+	
Brucella	spp	except B. ovis	3	3	+	+	
Brucella	suis		3	3	+	+	+
Burkholderia	spp		see spp.	2, unless listed at 3	+	+	
Burkholderia	mallei			3	+	+	+
Burkholderia	pseudomallei	was Pseudomonas	2/3	3	+	+	+
Calymmatobacterium	granulomatis						
Campylobacter	coli		2	2	+	+	
Campylobacter	fetus		2 ssp fetus	2	+		
Campylobacter	jejuni		2	2	+	+	
Campylobacter	spp		2 implied	see spp	+	+	
Campylobacter	sputorum				+		
Capnocytophaga	spp				+		
Cardiobacterum	hominis				+		
Chlamydia	pneumoniae			2	+		
Chlamydia	psittaci	avian strains, 3	2	2	+	+	
Chlamydia	spp		see 2 spp	see 3 spp	+		
Chlamydia	trachomatis	lymphogranuloma venereum	2	2	+	+	
Citrobacter	spp				+		
Clostridium	botulinum	V, T	2 /3	2	+	+	+
Clostridium	chauvoei			2		+	
Clostridium	difficile				+		
Clostridium	equi					+	
Clostridium	haemolyticum			2		+	
Clostridium	histolyticum			2	+		
Clostridium	novyi			2		+	
Clostridium	perfringens				+	+	
Clostridium	septicum			2		+	
Clostridium	sordelli					+	
Clostridium	spp			2			
Clostridium	tetani	T, V	2	2	+	+	
Corynebacterium	bovis					+	
Corynebacterium	diphtheriae		2	2	+		
Corynebacterium	matruchotii						
Corynebacterium	minutissimum				+		
Corynebacterium	pseudotuberculosis			2	+	+	
Corynebacterium	renale			2		+	
Corynebacterium	spp			see spp.	+		
Corynebacterium	ulcerans						
Cowdria	ruminantium						+
Coxiella	burnetii		3	3			+
Dermatophilus	congolensis			2	+	+	
Edwardsiella	tarda			2	+	+	
Eikenella	corrodens				+		
Enterobacter	aerogenes/cloacae				+		
Enterobacter	spp.				+		
Enterococcus	spp	Streptococcus faecalis			+	+	
Erlichia	sennetsu	Rickettsia					
Erlichia	spp						
Erysipelothrix	rhusiopathiae	was spp insidiosa		2	+	+	
Erysipelothrix	spp.						

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Escherichia	coli,enterohemorrhagic coli, enteroinvasive coli, enteropathogenic coli, enterotoxigenic	pathogenic strains		2			
Escherichia	coli, K12	genetically crippled		1			
Flavobacterium	meningosepticum				+		
Flavobacterium	spp						
Fluoribacter	bozemanae	was Legionella			+		
Francisella	novocida			2	+		
Francisella	tularensis, Type A V	V	3	3	+	+	+
Francisella	Tularensis, Type B V	V	2		+	+	+
Fusobacterium	necrophorum			2	+	+	
Fusobacterium	spp				+		
Gardnerella	vaginalis				+		
Haemophilus	ducreyi			2	+		
Haemophilus	influenzae			2	+		
Haemophilus	spp			see spp	+	+	
Hartmanella	spp				+		
Helicobacter	pylori			2	+		
Herellea	vaginicola	see Actinetobacter baumannii					
Kingella	kingae				+		
Klebsiella	oxytoca			1	+		
Klebsiella	pneumoniae			2	+		
Klebsiella	spp			2	+		
Lactobacillus	spp			1			
Legionella	pneumophila		2	2	+	+	
Legionella	spp		2	2	+		
Legionella-like organisms			2				
Leptospira	interrogans		2	2	+	+	
Liberobacter	africanus						+
Liberobacter	asiaticus						+
Listeria	ivanovii			2	+	+	
Listeria	monocytogenes			2	+	+	
Listeria	spp			2			
Mima	polymorpha						
Moraxella	spp			2	+	+	
Morganella	morganii				+	+	
Mycobacterium	africanum			2 implied	+	+	
Mycobacterium	asiaticum		2	2	+	+	
Mycobacterium	avium-intracelluare	(avium in Canada)	2	2	+	+	
Mycobacterium	bovis	BCG is 2	3	3	+	+	
Mycobacterium	chelonei		2	2	+	+	
Mycobacterium	fortuitum		2	2	+	+	
Mycobacterium	kansasii		2	2	+		
Mycobacterium	leprae		2	2	+		
Mycobacterium	malmoense		2	2	+		
Mycobacterium	marinum		2	2	+	+	
Mycobacterium	microti			2	+		
Mycobacterium	paratuberculosis	Mycobacterium avium subsp. paratuberculosis	2	2	+	+	
Mycobacterium	scrofulaceum		2	2	+		
Mycobacterium	simiae		2	2	+	+	
Mycobacterium	spp	except. MDR M.tb complex	see spp	2 Unless listed as 3			

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Mycobacterium	szulgai		2	2	+		
Mycobacterium	tuberculosis	3, V,T for multiply resistant	3	3			
Mycobacterium	ulcerans		2	2	+	+	
Mycobacterium	xenopi		2	2	+	+	
Mycoplasma	capriolum/M.F38/M./M. mycoide s capri (contagious caprine pleuropneumonia agent)						+
Mycoplasma	hominis		2	2	+		
Mycoplasma	mycoides	Restricted				+	+
Mycoplasma	pneumoniae			2	+		
Mycoplasma	agalactiae	Restricted				+	
Mycoplasma	spp	except M. mycoides, M agalactiae. M. capricolum		2	+		
Neisseria	gonorrhoeae		2	2	+		
Neisseria	meningitidis	V	2	2	+		
Neisseria	spp		see spp.	see spp.	+	+	
Nocardia	asteroids			2	+	+	
Nocardia	brasiliensis			2	+	+	
Nocardia	caviae						
Nocardia	farcinica				+	+	
Nocardia	nova				+		
Nocardia	spp			see spp.	+		
Nocardia	transvalensis			2			
Nocardia	otitidis-caviarum			2	+		
Pasteurella	haemolytica			2		+	
Pasteurella	multocida			3	+	+	
Pasteurella	pneumotropica						
Pasteurella	spp	type B, "buffalo", other virulent strains		2, unless listed as 3	+	+	
Peptostreptococcus	anaerobius				+		
Plesiomonas	shigelloides				+	+	
Porphyromonas	spp				+	+	
Prevotella	spp				+		
Proteus	mirabilis				+		
Proteus	penneri				+		
Proteus	spp.				+		
Proteus	vulgaris				+		
Providencia	alcalifaciens				+		
Providencia	rettgeri				+		
Providencia	spp				+		
Pseudomonas	aeruginosa			2	+		
Pseudomonas	spp	except B. mallei, B. pseudomalleii			+		
Rhodococcus	equi			2	+	+	
Rickettsia	(vole)			2			
Rickettsia	akari		3	3	+	+	
Rickettsia	australis		3	3	+		
Rickettsia	canadensis		3	3	+		
Rickettsia	conorii		3	3	+	+	
Rickettsia	montana				+		

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Rickettsia	mooseri						
Rickettsia	parkeri						
Rickettsia	prowazekii		3	3	+		+
Rickettsia	rhipicephali				+		
Rickettsia	rickettsii		3	3	+	+	+
Rickettsia	sennetsu						
Rickettsia	sibirica		3	3			
Rickettsia	spp.			3 (except vole, 2)	+	+	
Rickettsia	tsutsugamushi		3	3	+	+	
Rickettsia	typhi (mooseri)			3	+	+	
Ralstonia	solanacearum						+
Salmonella	arizonae			2	+	+	
Salmonella	cholerasuis			2	+	+	
Salmonella	enteritidis			2	+	+	
Salmonella	gallinarum-pullorum			2	+	+	
Salmonella	meleagridis			2			
Salmonella	paratyphi,A,B,C			2	+		
Salmonella	spp	all spp and serotypes/ serovars	see spp.	2	+	+	
Salmonella	typhi		2, 3	2	+		
Salmonella	typhimurium			2	+	+	
Serpulina	spp				+	+	
Serratia	marcescens				+		
Serratia	liquefaciens						
Shigella	boydii		2	2	+		
Shigella	dysenteriae		2	2	+		
Shigella	flexneri		2	2	+		
Shigella	sonnei		2	2	+		
Shigella	spp.		2	2			
Sphaerophorus	necrophorus			2			
Staphylococcus	aureus			2	+	+	
Staphylococcus	epidermidis					+	
Streptobacillus	moniliformis			2	+	+	
Streptobacillus	spp			see spp.	+		
Streptococcus	agalactiae			2	+	+	
Streptococcus	pneumoniae			2	+		
Streptococcus	pyogenes			2	+		
Streptococcus	spp.			2	+	+	
Streptococcus	suis				+	+	
Treponema	carateum			2	+		
Treponema	pallidum		2	2	+		
Treponema	pertenue				+		
Treponema	spp			see spp.	+	+	
Treponema	vincentii				+		
Ureaplasma	urealyticum				+	+	
Vibrio	cholerae		2	2	+		
Vibrio	parahaemolyticus		2	2	+		
Vibrio	spp		see spp.	see spp.	+	+	
Vibrio	vulnificus			2	+	+	
Xanthomonas	oryzae						+
Xylella	fastidiosa						+
Yersinia	enterocolitica			2	+	+	
Yersinia	pestis		2; 3 for aerosols & antibiotic resistant strains	3	+	+	+

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Yersinia	pseudotuberculosis				+	+	
Yersinia	spp		see spp.	see spp.	+		

RISK GROUPS: Fungi

BL – Biosafety Level; RG – Risk Group; V – Vaccine available; T – Toxin produced; HP – Human Pathogen; AP – Animal Pathogen; SA – Select Agent

Absidia	corymbifera	A. ramose					
Aspergillus	flavus				+	+	
Aspergillus	fumigatus				+	+	
Aspergillus	spp						
Blastomyces	dermatitidis	Ajellomyces dermatitidis, Zymonema dermatitidis	2	2	+	+	
Candida	albicans				+	+	
Candida	spp						
Cladosporium	bantianum		2	2	+		
Cladosporium	carrionii				+		
Cladosporium	trichoides	(Xylohypha – RG2)	2	2			
Coccidioides	immitis	RG3 for soil and sporulating culture; use BL3 for arthroconidia and contaminated soil	2, 3	3	+		+
Coccidioides	posadasii						+
Cryptococcus	neoformans	var. neoformans, Filobasidiella neoformans	2	2	+	+	
Cryptococcus	neoformans	var. gaitii, Filobasidiella bacillispora	2	2	+	+	
Dactylaria	gallopava	(Ochroconis gallopavum – RG2)	2	2	+	+	
Dermatophilus	congolensis					+	
Emmonsia	parva	var. parva and var. crescens			+	+	
Epidermophyton	floccosum		2	2	+		
Epidermophyton	spp	pathogenic members	2	2			
Exophiala	dermatitidis	(Wangiella)	2				
Fonsecaea	compacta	Phialophora compacta, Rhinocladiella compacta			+		
Fonsecaea	pedrosoi	Phialophora pedrosoi, Rhinocladiella pedrosoi	2		+		
Geotrichum	spp						
Histoplasma	capsulatum	var. capsulatum (Ajellomyces capsulatus), Canada	3	3	+	+	
Histoplasma	capsulatum	var duboisii, NIH, Canada		3	+		
Histoplasma	farcinimosum				+	+	
Histoplasma	spp.						

GENUS	SPECIES	COMMENTS	BSL (CDC)	RG (NIH)	HP	AP	SA
Loboa	lobai						
Madurella	grisea				+		
Madurella	mycetomatis				+		
Microsporum	spp		2	2	+		
Mucor	spp						
Neotestudina	rosatii				+		
Paracoccidioides	brasiliensis			2	+		
Penicillium	marneffei		2	2	+	+	
Peronosclerospora	philippinensis						+
Phakopsora	pachyrhizi						+
Rhizopus	cohnii					+	
Rhizopus	microspous					+	
Sclerophthora	rayssiae	var. zeae = select agent					+
Synchytrium	endobioticum						+
Sporothrix	schenckii		2	2			
Trichophyton	rubrum		2	2			
Trichophyton	spp		2	2	+		
Trichosporon	spp						
Xylohypha	bantania						

RISK GROUPS: Viruses

BL – Biosafety Level; RG – Risk Group; V – Vaccine available; AT – arbovirus table; T – Toxin produced; HP – Human Pathogen; AP – Animal Pathogen; SA – Select Agent

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Absettarov, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4	+		
Acute haemorrhagic conjunctivitis virus (AHC)	Picornaviridae				+		
Adenovirus, human, all types Types 1, 2, 3, 4, 5, 7 Types 40, 41	Adenoviridae			2	+		
African horse sickness disease							+
African swine fever virus							+
Aino	X-Arboviruses		4				
Akabane	X-Arboviruses		4				+
Alastrim	Poxviridae	Restricted					
Aphthovirus	Picornaviridae					+	
Araguari	X-Arboviruses		3				
Astroviridae	Astroviridae	(children and lambs feces)			+	+	
Avian influenza virus		(highly pathogenic)					+
Avian leukosis virus (ALV)	Viral vector/Animal retrovirus			1		+	
Avian sarcoma virus	Viral vector/Animal retrovirus			1		+	
Baculovirus	Viral vector/Animal virus			1		+	
Barmah Forest	Togaviridae/ Alphavirus (Grp A Arbovirus)		2				
Batama	X-Arboviruses		2				
Batken	X-Arboviruses						

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Bebaru virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2	2	+		
Bhanja	X-Arboviruses		4				
Bimbo	X-Arboviruses		3				
Bloodborne hepatitis viruses not yet identified	Unclassified viruses		2	2	+		
Bluetongue	X-Arboviruses	exotic viruses - SA	2				+
Bobaya	X-Arboviruses		3				
Bobia	X-Arboviruses		3				
Bovine immunodeficiency virus (BIV)	Viral vector/Animal retrovirus					+	
Bovine leukemia virus (BLV)	Viral vector/Animal retrovirus			1		+	
Bovine papilloma virus	Papovavirus/ Animal virus vector			1		+	
Bovine spongiform encephalopathy (BSE)	Unconventional agents, prions					+	+
Buenaventura	X-Arboviruses		3				
Buffalopox virus: 2 viruses (1a vaccinia variant)	Poxviridae		2	2	+	+	
Bunyamwera virus	Bunyaviridae/ Bunyavirus Group		2	2	+	+	
Bunyavirus	Bunyaviridae/ Bunyavirus Group						
Cabassou	X-Arboviruses		3				
Cache valley	X-Arboviruses		2	2 AT			
California encephalitis virus	Bunyaviridae/ Bunyavirus Group		2	2 AT	+	+	
Camel pox virus	Poxviridae		2	2		+	+
Cardiovirus	Picornaviridae					+	
Central European Tick-borne encephalitis virus, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4			+
Cercopithecine herpes virus Herpesvirus simiae (B virus, herpes virus)	Herpesviridae/ Alpha-herpesviridae		3/4	4			+
Chick embryo lethal orphan CELO	Viral vector/Animal virus						
Chikungunya virus	Togaviridae/ Alphavirus (Grp A Arbovirus)	2, high passage strains; vaccine strain131/25	3 arbovirus table	3 T	+		
Chim	X-Arboviruses		3				
Classical swine fever virus							+
Cocal	X-Arboviruses		3				
Coltiviruses	Reoviridae	RG2 – includes Colorado Tick Fever		2	+	+	
Congo Crimean haemorrhagic fever TBE	Bunyaviridae/ Nairoviruses	Crimean-Congo – RG4	4	4	+		+

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Coronavirus	Coronaviridae		3 SARS	2	+	+	+
Cowpox virus	Poxviridae		2 V	2	+	+	
Coxsackie	Picornoviridae/ Enterovirus	Types A and B – RG2		2	+		
Creutzfeldt-Jacob disese	Unconventional agents/prion		2	3	+		
Cytomegalovirus (CMV) Genus Lymphocryptovirus	Herpesviridae/ Betaherpesviridae		2	2	+		
Dengue virus	Flaviviridae/ Flavivirus (Grp B Arbovirus)	Type 1-4 - RG2	2	2 / 3	+		
Dhori	X-Arboviruses		3 AT	3 AT			
Dog sarcoma	Viral vector/ Animal virus						
Dugbe	X-Arboviruses		3				
Eastern equine encephalomyelitis (EEE)	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 V	2 V	+	+	+
Ebola virus	Filoviridae		4	4	+	+	+
Echoviruses	Picornoviridae/ Enterovirus			2	+		
Ectromelia (mousepox)	Orthopoxvirus						
Elephantpox virus (variant of cowpox)	Poxviridae			2	+	+	
Enterovirus	Picornoviridae/ Enterovirus						
Epstein-Barr virus (EBV)	Herpesviridae/ Gamma-herpesviridae		2	2	+		
Everglade virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3	3	+		
Far Eastern Tick-borne encephalitis	flavivirus						+
Feline leukemia virus, FeLV	Viral vector/Animal retrovirus			1		+	
Feline sarcoma virus, FeSV	Viral vector/Animal retrovirus			1		+	
Flanders-Hart Park virus	Rhabdoviridae	mosquitoes and birds, humans	2 AT	2 AT			
Flexal (South American hemorrhagic fever virus)	Arbovirus		3		+		+
Foot and Mouth disease virus							+
Gammaherpes	Herpesviridae / Gamma- herpesvirinae						
Ganjam	X-Arboviruses	USDA permit required					
Garba	X-Arboviruses		3 AT	3 AT			
Germiston	X-Arboviruses		3	3 AT			
Gerstmann-Straussler- Scheinker syndrome	Unconventional agents, prions		2	3	+		
Getah	X-Arboviruses		3 AT	3 AT			
Gibbon leukemia virus (GaLV)	Viral vector/Animal retrovirus			1			
Goat pox virus							+

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Gordil	X-Arboviruses		3 AT	3 A			
Guanarito (South American hemorrhagic fever virus)	Arenaviruses		4	4	+		+
Guaratuba	X-Arboviruses		2 arbovirus	2 arbovirus			
Guaratuba							
Guinea pig herpes	Viral vector/Animal virus			1			
Hamster leukemia	Viral vector/Animal virus			1			
Hantaan (Korean haemorrhagic fever)	Bunyaviridae/ Hantaviruses	see also sin nombre virus	3	3	+		
Hanzalova,TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4	+		
Hart Park virus	Rhabdoviridae		2 AT	2 AT			
Hazara virus	Bunyaviridae/ Nairoviruses		2 AT	2 AT	+		
Hepatitis A virus, human enterovirus type 72	Picornoviridae/ Hepatovirus		2 V	2	+		
Hepatitis B virus	Hepadnaviridae		2 V	2	+		
Hepatitis C virus	Togaviridae/ Pestivirus (Canada)	(hepatitis non-A,non-B)	2	2	+		
Hepatitis D (Delta) virus (b)	Hepadnaviridae	Delta, only pathogenic with HBV inf.	2	2	+		
Hepatitis E virus	Calciviridae		2	2	+		
Herpes saimiri	Herpesviridae/ Rhadinovirus					+	
Herpes simplex viruses	Herpesviridae/ Alpha-herpesviridae	Type 1 and 2	2	2 (types 1 and 2)	+		
Herpesvirus ateles	Herpesviridae/ Rhadinovirus			1			
Herpesvirus saimiri, Genus Rhadinovirus	Herpesviridae/ Animal virus vector			1			
Herpesvirus zoster (Varicella)	Herpesviridae/ Alpha-herpesviridae		2	2	+		
Human B lympho-tropic virus	Herpesviridae	Herpes types 6 and 7 – RG2		2	+		
Human Immunodeficiency virus (HIV) Types 1 & 2 Oncornavirus C	Retroviridae/ Lentiviridae	1, 2 (AIDS causing) – RG3	2/2+/3	3	+		
Human T-cell lymphotropic viruses (HTLV)	Retroviridae/ Oncovirinae/ Genus Oncornavirus C	Types 1-3 (EC 1 and 2); Types 1 and 2 – RG3	2/3	3	+		
Hypr,TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4	+		
Ibaraki	X-Arboviruses		3 AT	3 AT			
Influenza virus (vaccine strain)	Orthomyxoviridae	vaccine strains: A/PR8/34, A/WS/33	1				
Influenza virus, Types A-C	Orthomyxoviridae	Types A,B, and C (EC)	2	2 (types A,B, C)	+	+	

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Inhangapi	X-Arboviruses		3 AT	3 AT			
Inini	X-Arboviruses		3 AT	3 AT			
Israel Turkey Mening.	X-Arboviruses		3 AT	3 AT			
Issyk-Kul	X-Arboviruses		3 AT	3 AT			
Itaituba	X-Arboviruses		3 AT	3 AT			
Japanese B encephalitis	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+		+
Japanese encephalitis, Nakayama	Flaviviridae/ Flavivirus (Grp B Arbovirus)				+		+
Junin virus (South American hemorrhagic fever virus)	Arenaviruses		3V/4	4, 3 V	+		+
Kairi(x)	X-Arboviruses		3 AT	3 AT			
Khasan, Koutango	X-Arboviruses		3 AT	3 AT			
Kokobera	Flaviviridae/ Flavivirus (Grp B Arbovirus)						
Kumlinge,TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4	+		
Kunjin	Flaviviridae/ Flavivirus (Grp B Arbovirus)		2 AT	2 AT			
Kuru	Unconventional agents/prion		2	3	+		
Kyasanur Forest, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4	+	+	+
Kyzylagach	X-Arboviruses		3 AT	3 AT			
LaCrosse virus	X-Arboviruses		2 AT	2 AT			
Langat virus	X-Arboviruses		2 AT	2 AT			
Lassa fever virus	Arenaviruses		4	4	+		+
Lentiviridae , except HIV-1 and HI	Retroviridae						
Looping ill , TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+	+	
Lucke (frog) virus	Viral vector/ Animal virus						
Lumpy skin disease virus							+
Lymphocytic choriomeningitis (neurotropic) virus	Arenaviruses	neurotropic strains	2/3	3	+	+	
Lymphocytic choriomeningitis virus	Arenaviruses	other viscerotrophic (Canada:lab adapted)	2	2	+	+	
Machupo virus (South American hemorrhagic fever virus)	Arenaviruses		4	4	+		+
Malignant catarrhal fever							+
Marburg virus	Filoviridae		4	4	+	+	+
Marek's disease virus	Herpesviridae/ Animal virus vector			1 vector		+	
Mason-Pfizer monkey virus	Viral vector/ Animal retrovirus			1			
Mayaro virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 AT	3 AT	+		
Measles virus	Paramyxoviridae/ Morbillivirus			2	+		
Menangle virus	Paramyxoviridae		3+				+

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Middelburg	X-Arboviruses		3 AT	3 AT			
Milker's node virus	Poxviridae	also Pseudocowpox virus	2	2	+	+	
Molluscum contagiosum virus	Poxviridae		2	2	+		
Monkeypox virus	Poxviridae/ Orthopoxvirus		2 V	3	+	+	+
Mopeia virus (other Tacaribe viruses)	Arenaviruses		3 AT	3 AT	+		
Morbillivirus,except Rinderpest	Paramyxoviridae/ Morbillivirus				+	+	
Mouse mammary tumor virus	Viral vector/Animal retrovirus			1			
Mucambo virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 AT	3 AT	+		
Mumps virus	Paramyxoviridae/ Paramyxovirus			2	+		
Murine cytomegalovirus	Herpesviridae/ Animal virus vector			1			
Murine leukemia virus	Viral vector/ Animal retrovirus			1		+	
Murine sarcoma virus	Viral vector/ Animal retrovirus			1		+	
Murray Valley encephalitis (Australia encephalitis)	Flaviviridae/ Flavivirus (Grp B Arbovirus)	(Australian encephalitis)	3 AT	3 AT	+		
Nairobi Sheep Disease	Bunyaviridae/ Nairovirus	USDA Restricted	3 AT	Restric ted	+	+	
Nariva, Negishi	X-Arboviruses		3 AT	3 AT			
Ndumu	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 AT	3 AT	+	+	
New Minto, Nodamura, Northway	X-Arboviruses		3 AT	3 AT			
Newcastle Disease virus	Paramyxoviridae/ Paramyxovirus			2		+	+
Nipah and Hendra complex viruses	Equine morbillivirus		3+				+
Norwalk virus	Calciviridae			2	+		
O'Nyong-Nyong virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 AT	2 AT	+		
Omsk (hemorrhagic fever) TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)	hemorrhagic fever	4	4	+		+
Oncornavirus B	Retroviridae/ Oncovirinae						
Oncornavirus C, except HTLV I & II	Retroviridae/ Oncovirinae						
Orbiviruses	Reoviridae			2			
Orf virus	Poxviridae/ Parapoxvirus		2	2	+	+	
Oropouche virus	Bunyaviridae/ Bunyavirus Group		3 AT	3 AT	+		
Other bunyaviridae known to be pathogenic	Bunyaviridae						

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Other calciviridae	Calciviridae			2			
Other flaviviruses known to be pathogenic	Flavivirus						
Other hantaviruses	Bunyaviridae/ Hantaviruses	epidemic nephrosis virus		3			
Other known alphaviruses	Togaviridae/ Alphavirus (Grp A Arbovirus)						
Other pathogenic orthopoxviruses not in RG 2 or 4	Poxviridae/ Orthopoxvirus						
Ouango, Oubangui	X-Arboviruses		3				
Papillomaviruses (human)	Papovaviridae			2	+		
Parainfluenza virus Type 3, SF4 strain	Paramyxoviridae/ Paramyxovirus						
Parainfluenza viruses	Paramyxoviridae/ Paramyxovirus	Types 1 to 4 – RG2		2	+	+	
Paramushir, Piry	X-Arboviruses						
Paravaccinia virus	Poxviridae		2	2			
Parvovirus (human)	Parvoviridae	B19		2 (B19)	+		
Peste des petits ruminants							+
Plum pox potyvirus							+
Polioviruses	Picornoviridae/ Enterovirus	all types, wild and attenuated – RG2	2 V	2	+		
Polyomavirus, BK and JC viruses	Papovaviridae				+		
Powassan	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+	+	
Prospect Hill virus	Bunyaviridae/ Hantaviruses		2 AT	2 AT	+		
Pseudorabies virus	Herpesviridae/ Alphaherpesviridae					+	
Puumala virus	Bunyaviridae/ Hantaviruses		3 AT	3 AT	+		
Rabbitpox virus (vaccinia variant)	Poxviridae		2	2	+	+	
Rabies virus	Rhabdoviridae/ Lyssavirus	fixed virus is 2, street virus, 3	2 V /3	2/3	+	+	
Rat leukemia virus	Viral vector/ Animal retrovirus			1			
Razdan	X-Arboviruses		3 AT	3 AT			
Reoviruses	Reoviridae			2			
Respiratory syncytial virus	Paramyxoviridae/ Pneumovirus			2	+	+	
Rhadinovirus, except H. ateles and H. saimiri	Herpesviridae/ Rhadinovirus						
Rhadinovirus, except H.ateles,H. saimiri	Herpesviridae						
Rhinovirus	Picornaviridae/ Rhinoviruses	Bovine, Equine, Human		2 (all types)	+	+	
Rift Valley Fever, (Zinga virus)	Bunyaviridae/ Phleboviruses	RG2 - vaccine strain MP-12; Grp C Bunyaviridae	3 Zinga	3	+	+	+
Rinderpest virus							+
Rochambeau	X-Arboviruses		3 AT	3 AT			
Rocio	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+		

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Ross River virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 AT	2 AT	+		
Rotavirus (human)	Reoviridae			2f	+	+	
Rubivirus (Rubella)	Togaviridae/ Rubivirus			2	+		
Russian spring-summer encephalitis, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4	+		+
Sabia (South American hemorrhagic fever virus)	Arenaviruses		4				+
Sagiyama	X-Arboviruses		3 AT	3 AT			
Salanga, Santa Rosa, Saumarex Reef	X-Arboviruses		3 AT	3 AT			
Sammarez Reef	Flaviviridae/ Flavivirus (Grp B Arbovirus)						
Sandfly fever virus	Bunyaviridae/ Phleboviruses		2 AT	2 AT	+		
Scrapie	Unconventional agents prions:					+	
Semliki Forest virus	Togaviridae/ Alphavirus (Grp A Arbovirus)	most recombinant activities BL2	3 AT	3 AT	+		
Sendai virus (murine parainfluenza virus type 1)	Paramyxoviruses/ Parainfluenza viruses						
Seoul virus	Bunyaviridae/ Hantaviruses		3 AT	3 AT	+		
Sepik, Slovakia, Spondweni	X-Arboviruses		3 AT	3 AT			
Sheep pox virus							+
Shope papilloma virus	Papovavirus/ Animal virus vector			1		+	
Simian immunodeficiency virus	Retroviridae/ Lentiviridae		2; 2+; 3	3		+	
Simian sarcoma virus, SSV-1	Retroviridae					+	
Simian virus 40 (SV40)	Papovaviridae/ Animal virus vector			1		+	
Sin nombre virus	Bunyaviridae/ Hantaviruses	hantavirus pulmonary syndrome	3		+		
Sindbis virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 AT	2 AT	+	+	
St. Louis encephalitis	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+	+	
Subsclerosing pancencephalitis	Paramyxoviridae						
Swine vesicular disease virus							+
Tacaribe complex	Arenaviruses		2	2	+		
Tamdy, Telok Forest, Tiacotalpan	X-Arboviruses		3 AT	3 AT			
Tanapox	Poxviridae	see Yabapox	2	2			
Tensaw virus	Bunyaviridae/ Bunyavirus Group		2 AT	2 AT			
Thetalymphocrypto virus	Herpesviridae/ Animal virus vector						
Tick-borne encephalitis complex	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4				+

NAME	VIRAL GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP	SA
Tick-borne orthomyxoviridae,TBE	Orthomyxoviridae	RG4 - Dhori & Thogoto (EC)	4	4	+		
Tocio	X-Arboviruses		3 AT	3 AT			
Tonate virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 AT	3 AT	+		
Toroviridae	Toroviridae						
Toscana virus	Bunyaviridae/ Phleboviruses		2 AT	2 AT	+		
Turlock virus	X-Arboviruses		2	2 AT			
unassigned herpesviruses HHV 7, HHV8	Herpesviridae						
Vaccinia virus	Poxviridae/ Orthopoxvirus		2 V	2	+	+	
Variola (major and minor) virus	Poxviridae		Restric ted	Restric ted			+
Venezuelan equine encephalomyelitis	Togaviridae/ Alphavirus (Grp A Arbovirus)	RG2 vaccine strain, TC-83	3	3	+	+	+
Vesicular stomatitis virus	Rhabdoviridae	RG2 - Lab adapted stains:Indiana, San Juan, Glascow, Alagoas	3 (exotic, Piry)	3 / 2 lab strains	+	+	+ exo tic
Wesselsbron virus	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+	+	
West Nile fever virus	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 AT	3 AT	+	+	
Western equine encephalomyelitis	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 ,V	2,V	+	+	
Whitepox (Variola)	Poxviridae		Restric ted	Restric ted	+		
Yabapox virus (Tana and Yaba)	Poxviridae		2	2	+	+	
Yellow fever virus (vaccine strain 17D)	Flaviviridae/ Flavivirus (Grp B Arbovirus)	2 see wild type	2	2	+		
Yellow fever virus, wild type	Flaviviridae/ Flavivirus (Grp B Arbovirus)	3 V see vaccine strain 17D	3 HEPA-exhaus t all air	3	+		
Zinga (See Rift Valley Fever)	Bunyaviridae/ Phleboviruses	see Rift Valley Fever					

b Hepatitis D virus is pathogenic in workers only in the presence of simultaneous or secondary infection caused by hepatitis B virus.
Vaccination against hepatitis B virus will therefore protect workers who are not affected by hepatitis B virus against hepatitis D virus (Delta).

c Recommended for work involving direct contact with these agents.

RISK GROUPS: Parasites

BL – Biosafety Level; RG – Risk Group; V – Vaccine available; T – Toxin produced; HP – Human Pathogen; AP – Animal Pathogen; SA – Select Agent

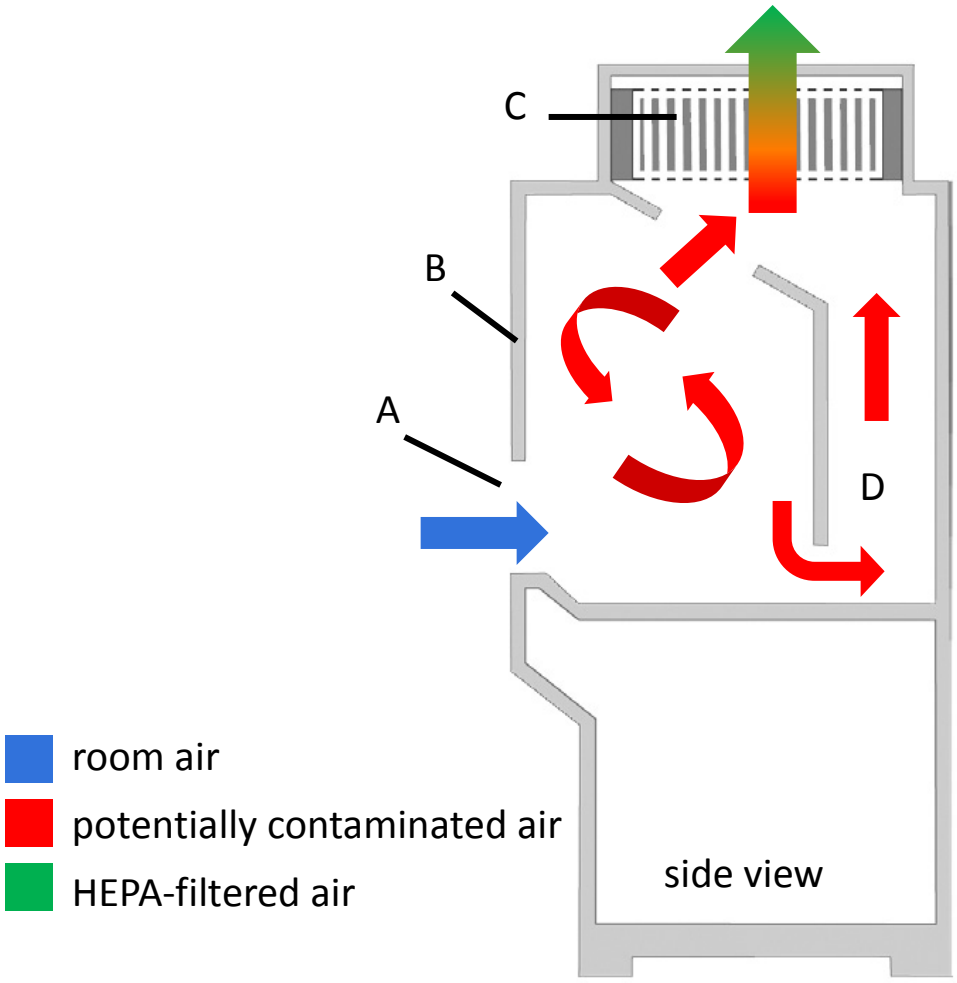
GENUS	SPECIES	GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP
Acanthamoeba	castellani	Protozoa				+	
Acanthamoeba	spp	Protozoa					
Acanthocheilonema	spp	Helminth, Nematode					
Ancylostoma	duodenale	Helminth, Nematode	hookworm	2	2	+	+
Ancylostoma	spp	Helminth, Nematode	hookworm	2	2		
Ancylstoma	ceylanicum	Helminth, Nematode	larval migrans, cat hookworm	2	2		
Angiostrongylus	cantonensis	Helminth, Nematode				+	
Angiostrongylus	costaricensis	Helminth, Nematode				+	
Angiostrongylus	spp	Helminth, Nematode					
Ascaris	lumbricoides	Helminth, Nematode		2	2	+	
Ascaris	spp	Helminth, Nematode		2	2	+	
Ascaris	suum	Helminth, Nematode				+	+
Babesia	divergens	Protozoa		2	2	+	+
Babesia	microti	Protozoa		2	2	+	
Babesia	spp	Protozoa		2	2		
Balantidium	coli	Protozoa					
Balantidium	spp	Protozoa				+	
Brugia	malayi	Helminth, Nematode		2	2	+	
Brugia	pahangi	Helminth, Nematode		2	2	+	
Brugia	spp	Helminth, Nematode	filaria worm	2	2		
Brugia	timori	Helminth, Nematode			2		
Capillaria	philippinensis	Helminth, Nematode				+	
Capillaria	spp	Helminth, Nematode				+	
Clonorchis	sinensis	Helminth, Trematode				+	
Clonorchis	spp	Helminth, Trematode					
Clonorchis	viverrini	Helminth, Trematode				+	
Coccidia	spp	Protozoa		2	2		
Cyclospora	cayetanensis					+	
Cryptosporidium	parvum	Protozoa		2	2	+	
Cryptosporidium	spp	Protozoa		2	2	+	
Cysticercus	cellulosae	Helminth, Cestode larva	larval T. solium	2	2		
Cysticercus	spp	Helminth, Cestode		2	2		
Dicrocoelium	spp	Helminths, Trematode					
Dipetalonema	perstans	Helminth, Nematode					
Dipetalonema	spp	Helminth, Nematode					
Dipetalonema	streptocerca	Helminth, Nematode				+	
Diphyllobothrium	latum	Helminth, Cestode				+	
Diphyllobothrium	spp	Helminth, Cestode					
Dipylidium	spp	Helminth, Cestoda					
Dracunculus	medinensis	Helminth, Nematode	guinea worm			+	
Dracunculus	spp	Helminth, Nematode					
Echinococcus	granulosis	Helminth, Cestode		2	2	+	+
Echinococcus	multilocularis	Helminth, Cestode		2	2	+	+
Echinococcus	spp	Helminth, Cestode		2	2		
Echinococcus	vogeli	Helminth, Cestode		2	2	+	
Entamoeba	histolytica	Protozoa		2	2	+	+
Enterobius	spp	Helminth, Nematode		2	2		
Fasciola	gigantica	Helminth, Trematode		2	2	+	+

GENUS	SPECIES	GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP
Fasciola	hepatica	Helminth, Trematode		2	2	+	+
Fasciola	spp	Helminth, Trematode	BL2 for metacercaria e	2	2		
Fasciolopsis	buski	Helminth,				+	+
	Trematode						
Fasciolopsis	spp	Helminth,					
	Trematode						
Giardia	lamblia	Protozoa	(intestinalis) EU	2	2	+	
Giardia	spp	Protozoa		2	2	+	
Hartmanella	spp	Protozoa					
Heterophyes	spp	Helminth,		2	2		
	Trematode						
Hymenolepis	diminuta	Helminth, Cestode			2	+	
Hymenolepis	nana	Helminth, Cestode	Human origin	2	2	+	
Hymenolepis	spp	Helminth, Cestode		2	2		
Isospora	spp	Protozoa	BL2 fo Coccidia	2	2	+	+
Leishmania	braziliensis	Protozoa		2	2	+	+
Leishmania	donovani	Protozoa		2	2	+	+
Leishmania	ethiopica	Protozoa	Cethiopica (EU)	2	2	+	
Leishmania	major	Protozoa		2	2	+	
Leishmania	mexicana	Protozoa		2	2	+	+
Leishmania	peruviania	Protozoa		2	2	+	
Leishmania	spp.	Protozoa	Australia, mammalian species	2	2	+	
Leishmania	tropica	Protozoa		2	2	+	+
Linguatula	spp	Arthropod					
Loa	loa	Helminth, Nematode		2	2	+	
Loa	spp	Helminth, Nematode	filaria	2	2		
Macracanthorhynchu sspp	Acanthocephala						
Mansonella	ozzardi	Helminth, Nematode				+	
Mansonella	perstans	Helminth, Nematode				+	
Microsporidium	spp.	Protozoa		2	2		
Naegleria	fowleri	Protozoa		2	2	+	
Naegleria	gruberi	Protozoa		1	1		
Naegleria	spp	Protozoa		see 2 sp	1 or 2		
Necator	americanus	Helminth, Nematode		2	2	+	
Necator	spp	Helminth, Nematode	hookworm	2	2		
Onchocerca	spp	Helminth, Nematode	filaria worm	2	2		
Onchocerca	volvulus	Helminth, Nematode	filaria worm	2	2	+	
Opisthorchis	felineus	Helminth, Trematode				+	
Opisthorchis	spp	Helminth, Trematode				+	
Paragonimus	spp	Helminth, Trematode					
Paragonimus	westermanii	Helminth, Trematode				+	+
Piroplasma	spp	Protozoa					
Plasmodium	cynomologi	Protozoa		2	2		
Plasmodium	falciparum	Protozoa		2	2	+	
Plasmodium	malariae	Protozoa		2	2		

GENUS	SPECIES	GROUP	COMMENTS	BL (CDC)	RG (NIH)	HP	AP
Plasmodium	simian parasites	Protozoa		2	2		
Plasmodium	spp	Protozoa	human &simian	2	2		
Plasmodium	vivax	Protozoa		2	2		
Pneumocystis	carinii	Protozoa				+	
Sarcocystis	spp	Protozoa		2	2		
Sarcocystis	suihominis	Helminth, Cestode larva	ssp hominis	2	2	+	+
Schistosoma	haematobium	Helminth, Trematode		2	2	+	
Schistosoma	intercalatum	Helminth, Trematode		2	2	+	
Schistosoma	japonicum	Helminth, Trematode		2	2	+	
Schistosoma	mansoni	Helminth, Trematode		2	2	+	
Schistosoma	mekongi	Helminth, Trematode		2	2	+	
Schistosoma	spp	Helminth, Trematode		2	2		
Strongyloides	spp	Helminth, Nematode	hominis	2	2	+	
Strongyloides	stercoralis	Helminth, Nematode		2	2		
Taenia	saginata	Helminth, Cestode				+	+
Taenia	solium	Helminth, Cestode	see Cysticercus	2	2	+	+
Taenia	spp	Helminth, Cestode			2		
Toxascaris	spp	Helminth, Nematode					
Toxocara	canis	Helminth, Nematode			2	+	+
Toxocara	spp	Helminth, Nematode			2		
Toxoplasma	gondii	Protozoa		2	2	+	+
Toxoplasma	spp	Protozoa		2	2		
Trichinella	spiralis	Helminth, Nematode			2	+	+
Trichinella	spp	Helminth, Nematode	RG1 for species listed		1	+	+
Trichomonas	vaginalis	Protozoa				+	
Trichostrongylus	spp	Helminth, Nematode				+	
Trichuris	trichiura	Helminth, Nematode				+	
Trypanosoma	brucei	Protozoa	ssp brucei	2	2	+	+
Trypanosoma	brucei	Protozoa	ssp.gambiense	2	2	+	
Trypanosoma	brucei	Protozoa	ssp rhodesiense	2	2	+	+
Trypanosoma	cruzi	Protozoa		2	2	+	
Trypanosoma	spp	Protozoa		2	2		
Wuchereria	bancroftii	Helminth, Nematode		2	2	+	
Wuchereria	spp	Helminth, Nematode	filaria worm	2	2		

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.



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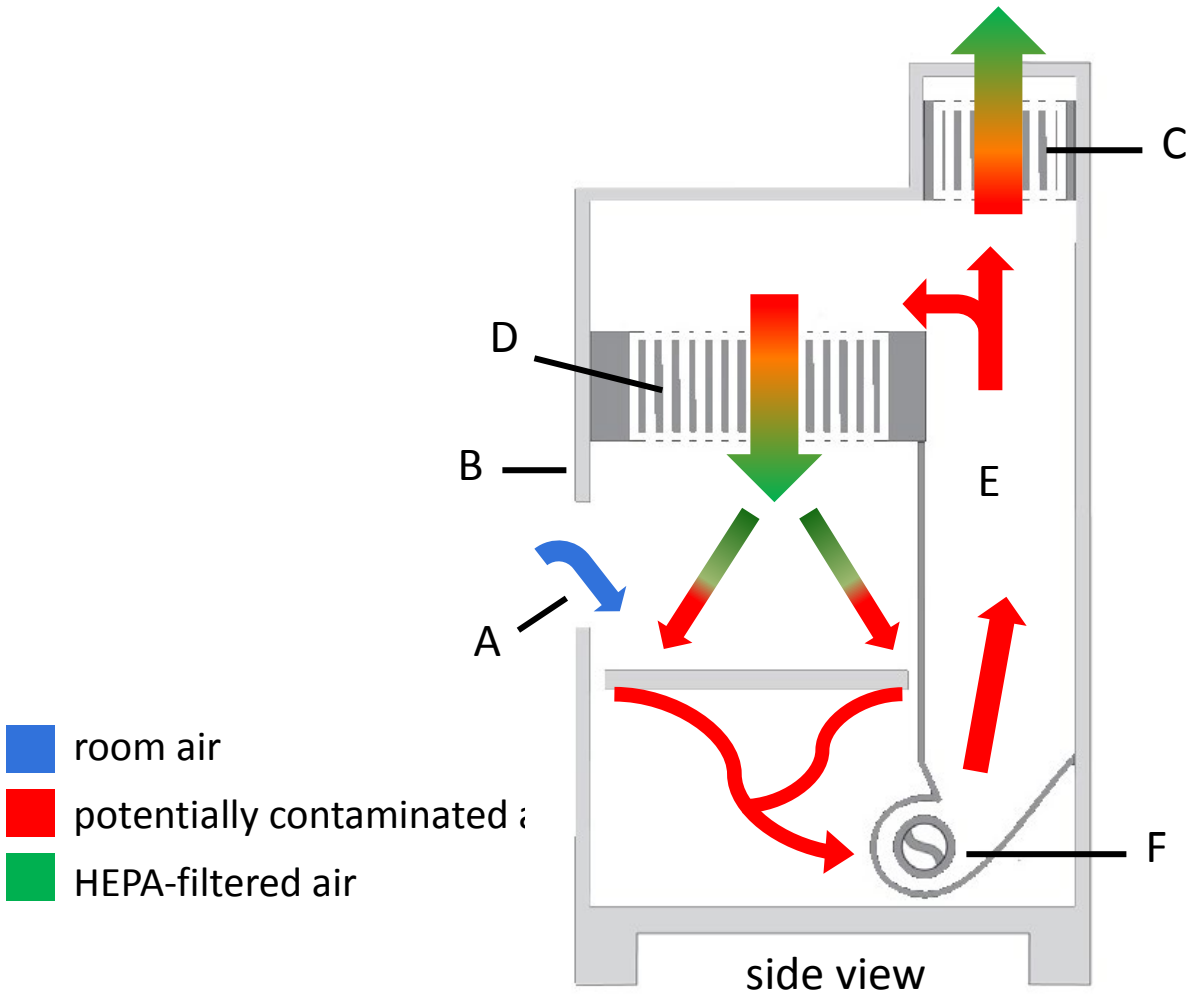
Class II A1

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



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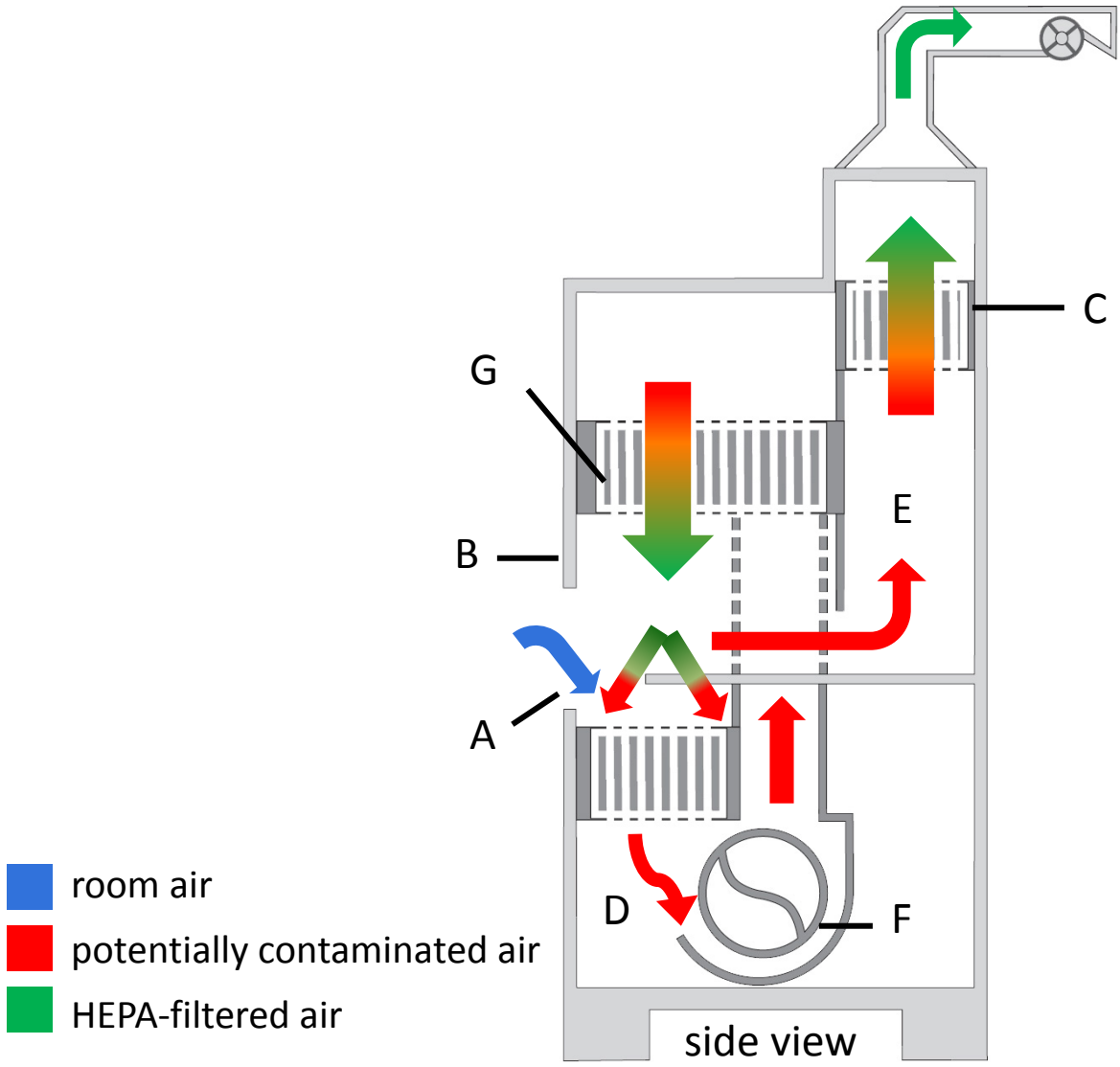
Class II B1

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.



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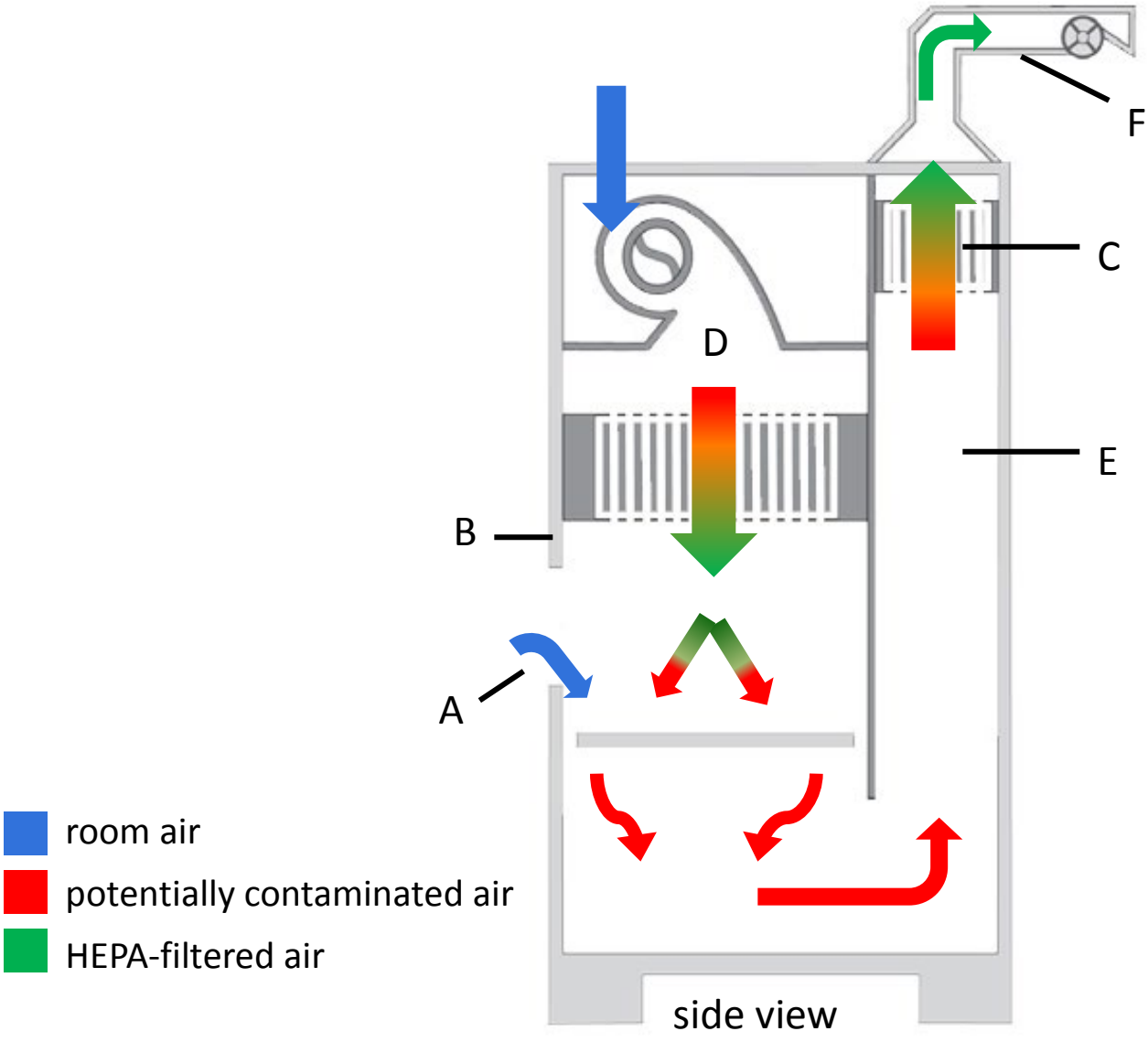
Class II B2

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.



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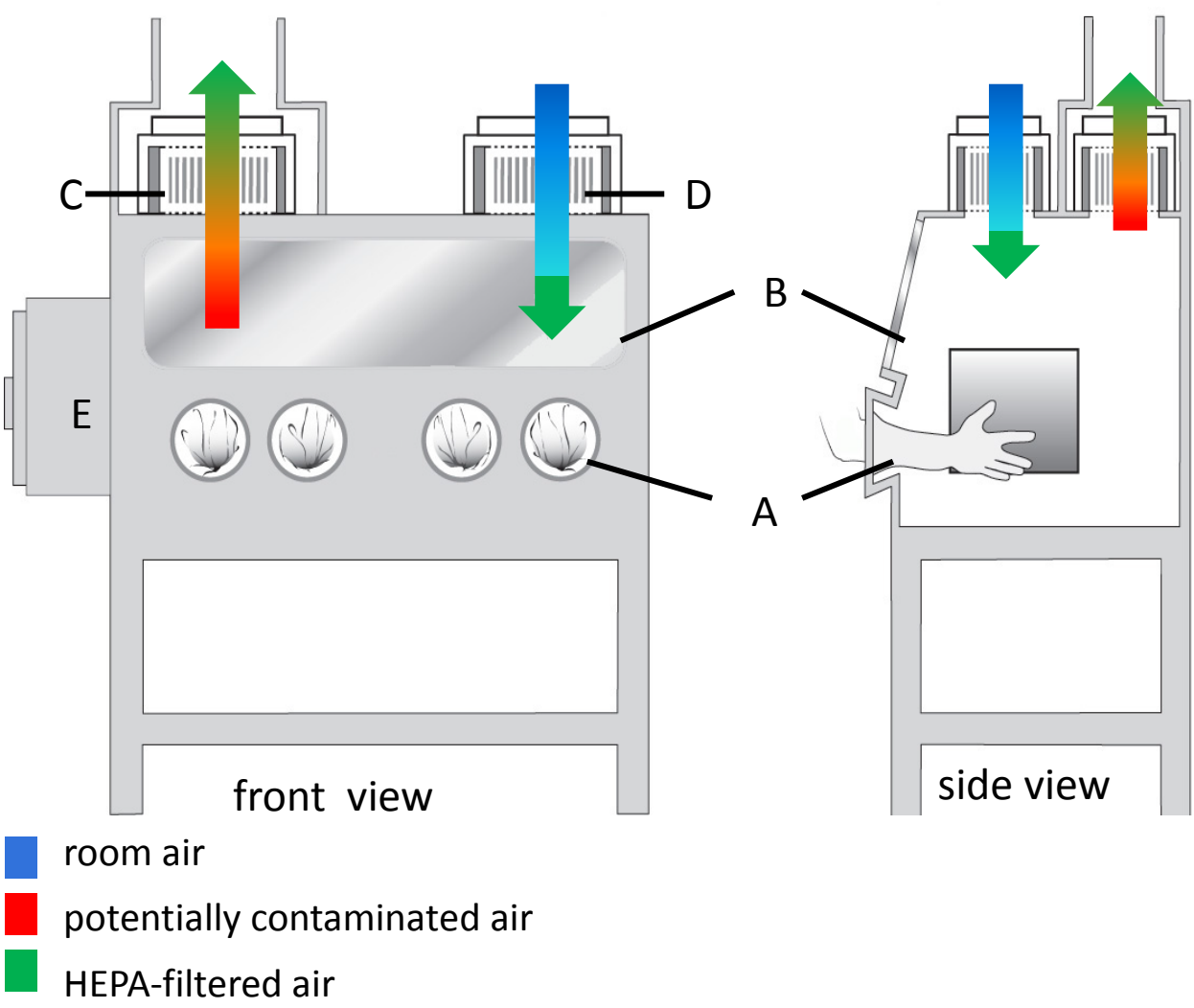
Class III

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 th ework surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to the building exhaust system.



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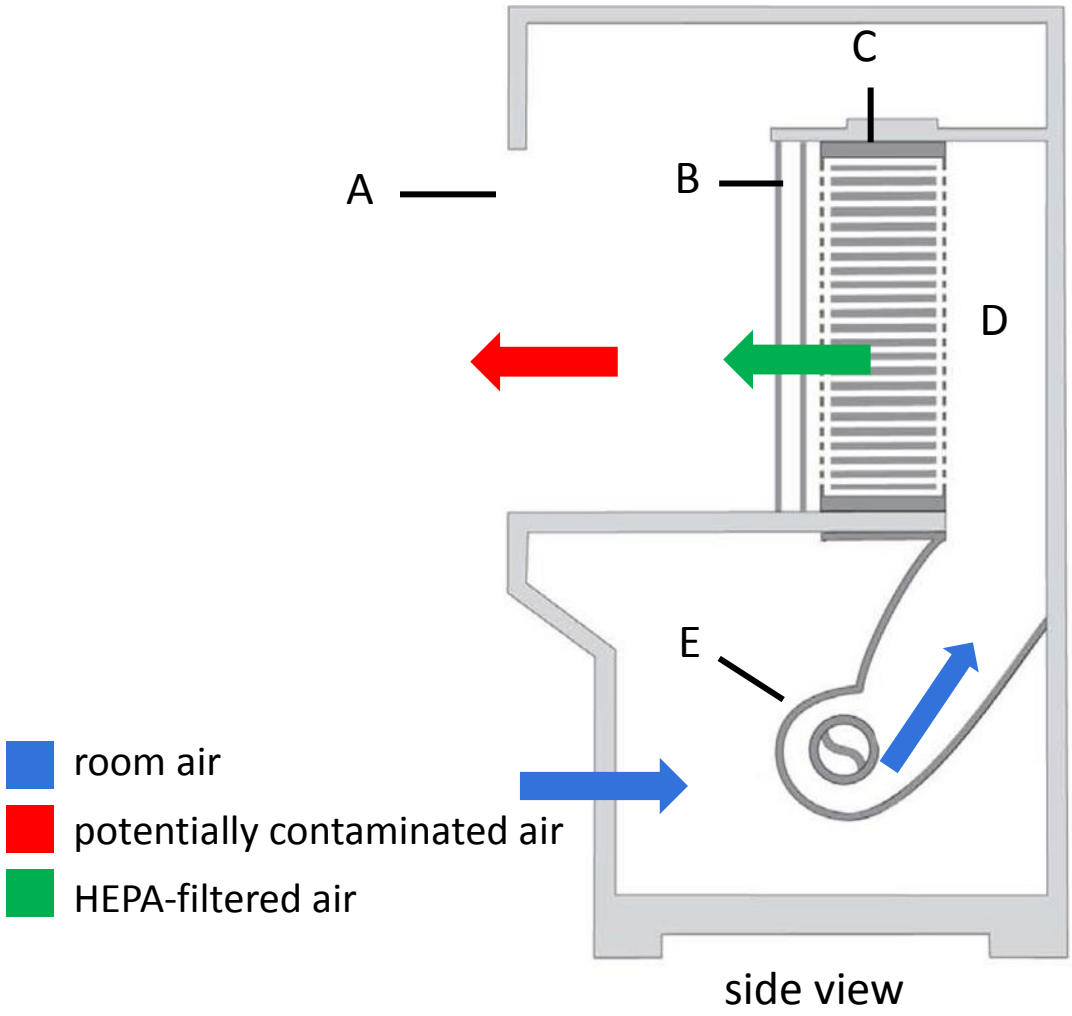
Horizontal Laminar Flow „Clean Bench"

Horizontal Laminar Flow „Clean Bench" (A) front opening; (B) supply grille; (C) supply HEPA filter; (D) supply plenum; (E) blower.



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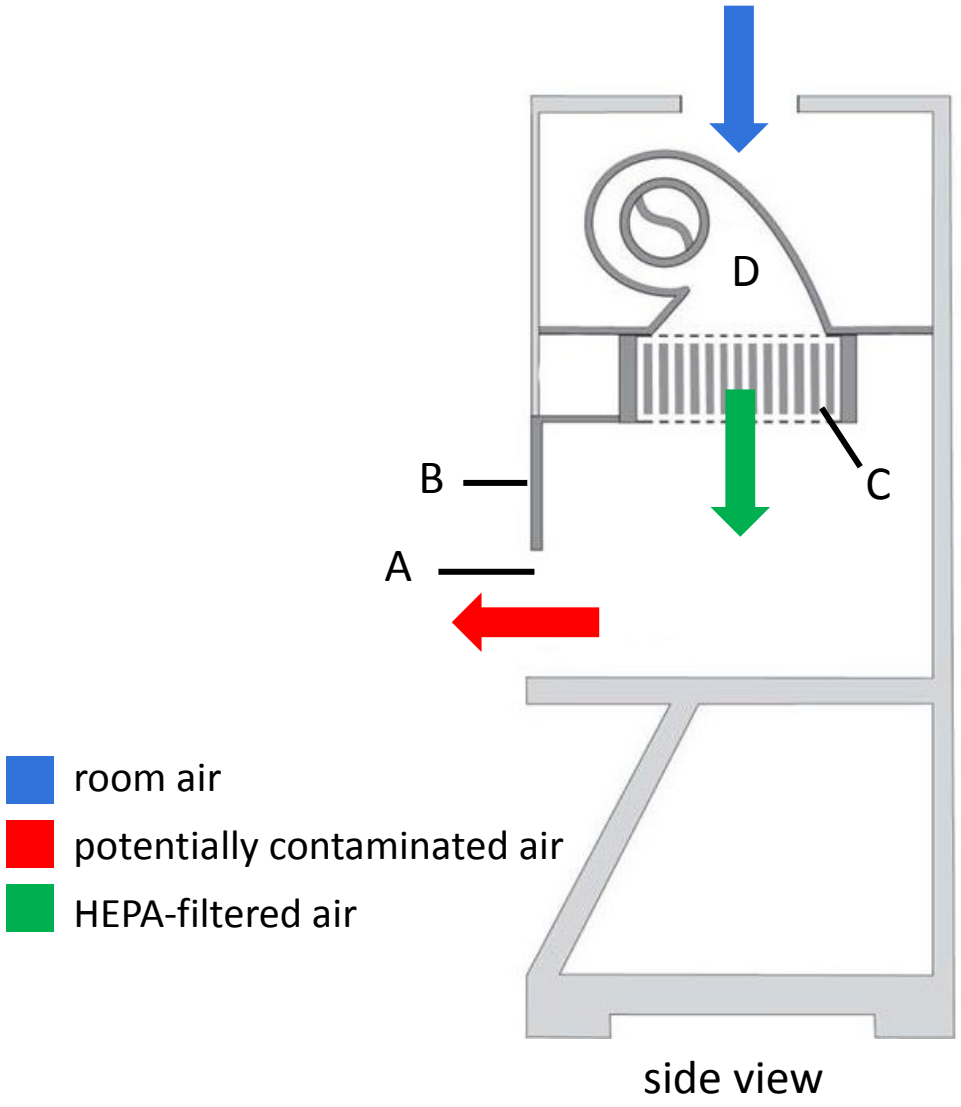
Vertical Laminar Flow „Clean Bench"

Vertical Laminar Flow „Clean Bench" (A) front opening; (B) sash; (C) supply HEPA filter; (D) blower. Note: Some vertical flow clean benches have recirculated air through the front and/or rear perforated grilles.



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Select Agents & Toxins List

(HHS and USDA Select Agents and Toxins 7CFR Part 331, 9 CFR 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 $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)[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 the 1918 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 AND TOXINS

61. *Coniothyrium glycines* (formerly *Phoma glycinicola* 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**

University Offices

University of Pennsylvania

Center for Technology Transfer (CTT)

Institutional Animal Care and Use Committee (IACUC)

Institutional Biosafety Committee (IBC)

Institutional Review Board (IRB)

Laboratory Animal Resources (ULAR)

Office of Biotechnology Activities (OBA)

Office of Environmental Health and Radiation Safety (EHRS)

Office of Facilities, Real Estate Services (FRES)

Office of Human Research (OHR)

Office of Regulatory Affairs (ORA)

Office of Research Services (ORS)

School of Medicine Space Planning and Operations (SPO)

Office of the Vice Provost for Research

Training Resources

Penn Profiler

Knowledge Link

Knowledge Link Help

Training Help Email

Biosafety Resources

American Biological Safety Association: Risk Groups Classification for Infectious Agents

Audit Program EHRS

Biological Agent Registration (BAR)

Biological Safety Manual of UPenn

Biosafety Cabinet information EHRS

Environmental Protection Policy Statement of UPenn (October 7, 2008).

EPA Approved Disinfectants

Exposure Control Plan for Bloodborne Pathogens of UPenn

Hazard Labels Request

MicroClean – Biosafety Cabinet (BSC) certification & repair vendor 800-523-9852

Occupational Medicine HUP – 215-662-2354

Personal Protective Equipment

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

Use of UV Lights in BSCs Burgener, 2006, Applied Biosafety, 11:4, pp228-230

Radiation Safety Researcher's User Guide

Respiratory Program EHRS

Room Sign Request Form

Room Sign Request Form for Perelman School of Medicine Laboratories

Shipping Manual for Infectious Substances and Biological Materials

Waste Management Matrixes

Know Where to Throw fact sheet

Regulatory Resources

Biosafety in Microbiological & Biomedical Laboratories, 5th ed. (CDC/NIH)

Canadian Material Safety Data Sheets for Infectious Substances

CDC's *Biosafety in Microbiological and Biomedical Laboratory (BMBL), 5th Edition*

CDC Etiologic Agent Import Permit Program http://www.cdc.gov/od/eaipp/

CDC import permit application http://www.cdc.gov/od/eaipp/importApplication/

National Select Agent Registry

Exclusions to the National Select Agent Registry

NIH Guidelines for Research Involving Recombinant DNA Molecules

Occupational Safety and Health Administration (OSHA)

OSHA Bloodborne Pathogen Standard

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

WHO Biosafety Manual

UPENN research services http://www.upenn.edu/researchservices/exportcontrols.html

USDA/APHIS Import permits http://www.aphis.usda.gov/permits/

US Department of commerce: Export Administration Regulation Downloadable FIleshttp://www.access.gpo.gov/bis/ear/ear_data.html