Hackensack Meridian Health Center for Discovery and Innovation Biosafety Manual

Center for Discovery & Innovation
Member of Hackensack Meridian *Health*

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Biological Risk Assessment

Risk assessment is the process of evaluating biological research projects for the potential hazards and characterizing those hazards into classes with proper containment and work practices by which that research can be performed safely and effectively. Biological Risk assessment is an iterative process continually carried out in biological research. Biological research projects go through a review process carried out on multiple levels of the research administration. Research involving recombinant DNA and synthetic nucleic acids will be reviewed by the Institutional Biosafety Committee. Research involving animals will be reviewed by the Animal Care and Use Committee. Research involving human subjects would be reviewed by the Institutional Review Board. Within these committees, numerous research support personnel including biological safety officers, environmental safety officers, veterinarians, faculty and staff will take part in this process to ensure a safe and well-reviewed research project.

Classification of a Hazardous Biological Agent:

Hazardous, biological research agents can be classified into four categories based on their assigned risk group. The risk groups are defined by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). These risk groups break down agents based on their potential to cause harm to a healthy human adult.

Classification of Infectious Microorganisms by Risk Group:

Risk Group Classification	**CDC/NIH/WHO**
Risk Group 1	Agents not associated with disease in healthy adult humans. (No or low individual and community risk)
Risk Group 2	Agents associated with human disease that are rarely serious and for which preventive or therapeutic interventions are often available. (Moderate individual risk but low community risk)
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).
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).

Standard Microbiological Practices

The following provide a basis of proper work practices which must be used to protect you and others from exposure to hazardous materials. These basic work practices also aid in the reduction of cross-contamination and improve the quality of the work performed.

- Use good aseptic technique for all procedures whether they involve infectious material use or not.
- Personnel must wash their hands after working with materials and before leaving the laboratory.
- Gloves, laboratory coats, eye protection and other relevant personal protective equipment must be worn and dedicated for research use.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
- Precautions must always be taken with sharp items. These include:
    - Careful management, transportation and use of needles and other sharps are of primary importance.
    - Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    - Non-disposable sharps must be placed in a rigid container for transport to a processing area for decontamination, preferably by autoclaving.
    - Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
    - Plastic should be substituted for glassware whenever possible.

- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash using an appropriate disinfectant for the material.
- Decontaminate all cultures, stocks, and other liquid cultures before disposal.
- Transportation of any viable cultures, stocks and other liquid cultures should be carried in a durable, leak proof container, where the outer surface is easily decontaminated and secured for transport.
- Laboratory personnel must have proper training in procedures and methods being performed in the laboratory by a qualified and experienced person. a. Personnel must receive annual updates or additional training when procedural or policy changes occur.
- Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. All laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Responsibilities of the Principal Investigator

The responsibilities summarized here based on the requirements provided by the NIH rDNA Guidelines, OSHA Laboratory Standard, and CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition, as well as other applicable regulatory agencies.

Principal Investigators (PIs) or the Laboratory Director are responsible for the health and safety of all personnel and compliance with all applicable regulations and the criteria established in this manual in their laboratories.

Before beginning any research with recombinant/synthetic nucleic acids or any genetic manipulations of biological organisms, please contact the institutional biosafety officer at sean.fitzgerald@hmhn.org to begin an assessment of your work, assistance in setting up the appropriate safety controls, and the IBC research project registration.

Responsibilities of the PI include:

- Ensuring that specific laboratory hazards are effectively communicated to laboratory personnel.
- Ensuring that personnel have received appropriate training and are competent to perform procedures used in the laboratory.
- Developing laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory.
- Ensuring that engineering controls are available, in good working order, and are used appropriately to minimize exposure to biohazardous agents.
- Ensuring that appropriate personal protective equipment is available and used by laboratory personnel.
- Ensuring that laboratory workers are provided immunizations and medical surveillance prior to, and in the event of, exposure to biohazardous agents as appropriate (based on current CDC and IBC recommendations).
- Notifying the Institutional Biosafety Officer at (201) 250-1024 of any spills, incidents, involving biological agents that may result in exposure to laboratory personnel, the public, or release to the environment.
- Ensuring that biological agents are disposed of according to regulations, as outlined in this manual.
- Ensuring that any transportation or shipping of biohazardous materials are packaged and documented in accordance with regulations.
- Ensuring that periodic inspections of the laboratory are conducted with Biological Safety and a laboratory representative.

Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is a institution wide committee responsible for reviewing research activities utilizing recombinant DNA (rDNA) and/or infectious agents, assuring the safe conduct of research, assessing decontamination and biocontainment levels, and ensuring research is done in compliance with government and institution regulations. Federal regulations require that all procedures involving the use of rDNA, which according to the "*NIH Guidelines for Use of Recombinant or Synthetic Nucleic Acid Molecules*" (http://osp.od.nih.gov/sites/default/files/resources/NIH_Guidelines.pdf) are Non-Exempt, undergo review by the IBC.

- All NIH-funded projects involving recombinant or synthetic nucleic acid molecules must comply with the NIH Guidelines.
- All non-NIH funded projects involving recombinant or synthetic nucleic acid molecules conducted at or sponsored by an Institution that receive NIH funding for research involving recombinant or synthetic nucleic acid molecules must comply with the NIH Guidelines.

IBC Policy also requires that work with organisms that require BSL3 or ABSL3 containment undergo review by the IBC.

IBC Registration

Experiments in Sections III-A, III-B, and III-D of the NIH Guidelines require Institutional Biosafety Committee (IBC) approval **prior to initiation**. To register or amend research email sean.fitzgerald@hmhn.org.

Upon receipt, the protocol is assigned a unique identification number and the PI will be notified of its receipt. The PI may be contacted by Biosafety Officers or Reviewers prior to the scheduled IBC meeting for additional information. The PI will be notified of the results of the committee's review and containment requirements after the meeting.

Before initiating research subject to the NIH Guidelines, the PI must:

- Determine whether the research is subject to Section III-A, III-B, III-C, III-D, or III-E of the NIH Guidelines.
- Propose physical and biological containment levels in accordance with the NIH Guidelines when registering research with the IBC.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.
- Submit a research protocol to the IBC for review and approval.
- Seek NIH OBA's determination regarding containment for experiments that require case-by-case review.
- Petition NIH OBA, with notice to the IBC, for proposed exemptions from the NIH Guidelines.
- Obtain IBC approval before initiating research subject to the NIH Guidelines.
- Seek NIH approval, in addition to IBC approval, to conduct experiments specified in Sections III-A and III-B of the NIH Guidelines.

While conducting research subject to the NIH Guidelines, the PI must:

- Determine the need for IBC review before modifying recombinant or synthetic nucleic acid research already approved by the IBC.
- Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval.
- Remain in communication with the IBC throughout the duration of the project.
- Report any significant problems pertaining to the operation and implementation of containment practices and procedures, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the IBC, NIH OBA, and, as applicable, the Biological Safety Officer, Animal Facility Director, and other appropriate authorities.

Personal Protective Equipment

Personal protective equipment (PPE) is used to directly protect personnel from contact with infectious agents. Supervisors are responsible for conducting laboratory PPE assessments, providing PPE, and training personnel in the proper use. PPE must not be taken home or worn outside the laboratory.

Appropriate Laboratory PPE should be determined following a thorough risk assessment of the work being performed. For assistance in selecting PPE for work with biological materials, contact sean.fitzgerald@hmhn.org.

Recommended Laboratory PPE

- Laboratory outer garments
    - Dedicated long sleeved outer garments, such as lab coats, are used to prevent contamination of the skin and street clothes.
- Gloves
    - Gloves must be worn when working with infectious and potentially infectious materials. Gloves are recommended for all manipulations of biological materials to avoid cross contamination of experiments.
    - If personnel develop or have latex allergies, then a suitable alternative should be available for use.
    - Double-gloving adds further protection when applied appropriately.
- Face protection
    - Safety glasses are recommended for all laboratory procedures.
    - Goggles, face shields, or other splatter guards should be used for anticipated splashes or sprays of infectious material when procedures are conducted outside of containment.
- Respiratory protection
    - May be necessary in some cases depending on the materials being used or procedures performed.
    - Personnel who are required to wear respiratory protection must be evaluated by a physician and trained in respirator selection and usage.

PPE should be provided for visitors, maintenance and custodial personnel if needed.

Disinfection and Sterilization

Sterilization is the process to render an item, device, or solution completely free of all living microorganisms and viruses.

Disinfection is the targeted process to eliminate nearly all recognized pathogenic microorganisms but not necessarily all microbial forms of life.

- Disinfection is aimed at the organism of interest. This process utilizes specific materials and processes to the organism that you are aiming to eliminate. Disinfection is less intrusive, costly, and allows a level of control that sterilization does not allow.
- Disinfection is dependent on concentration of the disinfectant of choice and the contact time.

There are three levels of disinfection:

- High-Level Disinfection
    - This destroys vegetative microorganisms and inactivates viruses, but not necessarily high levels of spores.
    - These are typically fast acting and have a short contact time.
- Intermediate-Level Disinfection
    - This destroys vegetative microorganisms and inactivates most viruses.
- Low-Level Disinfection
    - This destroys vegetative microorganisms, but it is not considered tuberculocidal.

Descending Order of Resistance to Disinfection

Bacterial Spores (*Bacillus* spp., *Clostridium* spp.)
↓
Mycobacteria (*Mycobacterium tuberculosis*)
↓
Non-lipid or Small Viruses (Poliovirus, Rhinovirus)
↓
Fungi (*Candida* spp., *Cryptococcus* spp.)
↓
Vegetative Bacteria (*Staphylococcus* spp., *Salmonella* spp.)
↓
Lipid or Medium-Size Viruses (HCV, CMV, Herpes Simplex Virus, HIV, SARS-CoV-2)

Common Disinfectant Examples:

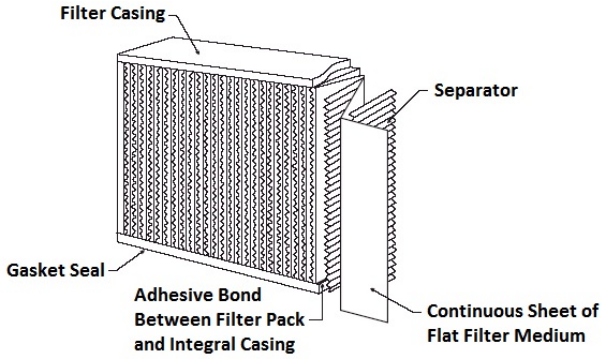
Classes of Chemical	**Alcohols**	**Chlorine Compounds**	**Oxidizing agents**
Disinfectant Examples	70% Ethanol	Bleach	Hydrogen Peroxide
Active Concentration	70%	10% (v/v for liquids)	3-6%
Activity Level	Intermediate	Intermediate	High to Intermediate

Biological Safety Cabinets

Biological safety cabinets (BSC) are the primary means of containment for working safely with infectious materials. Biosafety cabinets operate by controlling airborne contaminants during work using laminar airflow and high efficiency particulate air (HEPA) filtration. Three types of BSCs, designated as Class I, II, and III, have been developed to meet varying research and clinical needs.

High Efficiency Particulate Air (HEPA) Filters:

Control of airborne particulate materials is achieved with high-efficiency particulate filters that efficiently remove microscopic contaminants from the air. The HEPA filter removes particles equal to and greater than 0.3 µm (which essentially includes all bacteria, spores, and viruses) with an efficiency of 99.99%.



Classes of Biological Safety Cabinets:

- **Class I** cabinets are negative pressure cabinets with room air being drawn in from the front of the cabinet across the work area and HEPA filtered as it is exhausted back to the surrounding room. This type of cabinet provides no protection for the research materials but does protect the researcher(s) and the surrounding laboratory environment from materials worked with in the cabinet.

- **Class II** cabinets are the most used and versatile cabinets. Airflow is drawn into the front grille of the cabinet. Exhaust air and the air supplied to the work surface pass through HEPA filters providing protection of the research materials, research personnel, and the environment.

- **Class III** cabinets are gas-tight enclosures with non-opening view windows. Operations in the cabinet are conducted through attached rubber gloves. The cabinet is maintained under negative air pressure of at least 0.5 inch (12.7 mm) water gauge (w.g.). Supply air is drawn into the cabinet through HEPA filters. The exhaust air is treated by double HEPA filtration, or by HEPA filtration and incineration.

BSC class	Face velocity	Airflow pattern	Nonvolatile toxic chemicals /radionuclides	Volatile toxic chemicals/ radionuclides
I	75	In at front; exhausted through HEPA to the outside or room.	Yes	Yes (when exhausted outside)
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a canopy unit.	Yes (minute amounts)	No
II, A2	100	Same as II, A1, but higher face velocity and plenums are under negative pressure to room; exhausted air can be canopy-ducted to the outside through a HEPA filter.	Yes	Yes (minute amounts)
II, B1	100	30% recirculated, 70% exhausted through dedicated HEPA-filtered duct to the outside.	Yes	Yes (minute amounts)
II, B2	100	No recirculation; total exhaust to the outside through hard-duct and a HEPA filter.	Yes	Yes (small amounts)
III	N/A	Supply air inlets and hard duct exhausted to outside through two HEPA filter in series.	Yes	Yes (small amounts)

Proper Use of a Biological Safety Cabinet

Before beginning work:

- Monitor alarms, pressure gauges, or flow indicators for any changes.
- If the UV light is on, shut off the UV light.
- Turn the cabinet on and let it run for 3-5 minutes.
- Wipe the work surface with an appropriate disinfectant, such as 70% ethanol.
- Plan your work and place everything needed for the procedure inside the BSC.
- Wipe items with disinfectant before placing them in BSC.

During work:

- Avoid airflow disruption that could affect the level of protection provided by the BSC.
- Keep the BSC free of clutter.
- Don't place objects over the front air intake grille.
- Don't block the rear air intake grille.
- Limit traffic in the area when the BSC is in use.
- Make sure the lab door is closed and avoid opening/closing the door if located near the BSC.
- Move arms slowly when removing or introducing items. Avoid sweeping arm motions.
- When working with infectious materials, change gloves when moving in and out of the cabinet.
- Keep all materials at least 4 inches inside the front sash.
- Keep clean materials at least one foot away from any aerosol generating activities to minimize the potential for cross contamination.
- Place any equipment that creates air turbulence in the back 1/3 of the cabinet and stop other work while the equipment is running.
- Don't operate an open flame in the cabinet as this can disrupt air flows within the cabinet.
- Segregate contaminated and clean items. Work from "clean to dirty."
- Clean up all spills in the cabinet immediately. Allow the cabinet to run for 3-5 minutes before resuming work.

After completion of work:

- Don a new pair of gloves and wipe down all items with an appropriate disinfectant before removing.
- Remove all materials and wipe all interior surfaces of the BSC with an appropriate disinfectant.
- Wash hands after completion of work.
- Periodically decontaminate and clean under work grilles.

Biosafety Levels

Specific biosafety levels are described later in this manual.

Biosafety levels consist of combinations of:

- Laboratory practices and techniques
- Safety equipment
- Laboratory facilities

Each combination is specifically appropriate for:

- The operations performed
- The documented or suspected routes of transmission of the infectious agents
- The laboratory function or activity

The BSLs described in this manual should be differentiated from Risk Groups.

Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

Summary of Recommended Biosafety Levels for Infectious Agents:

Biosafety Level (BSL)	**Agent Characteristics**
BSL1	• Not known to consistently cause diseases in healthy adults.
BSL2	• Agents associated with human disease. • Routes of Transmission include percutaneous injury, ingestion, mucous membrane exposure.
BSL3	• Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.
BSL4	• Dangerous/exotic agents which pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments. • Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to re-designate the level. • Related agents with unknown risk of transmission.

Biosafety Level 1

Biosafety Level 1 (BSL-1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans and that present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open benchtops using standard microbiological practices. Special containment equipment or facility design is not generally required but may be used as determined by appropriate risk assessment. Laboratory personnel receive specific training in the procedures conducted in the laboratory and are supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility specifications are recommended for BSL-1.

Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
- The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance
- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    - The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
- A sign is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
- Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials.
    - Glove selection is based on an appropriate risk assessment.
    - Gloves are not worn outside the laboratory.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.

- Persons 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 are not permitted in laboratory areas. Food is stored outside the laboratory area.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    - Plasticware is substituted for glassware whenever possible.
    - Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
        - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
    - Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    - Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    - Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    - Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
- An effective integrated pest management program is implemented.
- Animals and plants not associated with the work being performed are not permitted in the laboratory.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

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

- Protective laboratory coats, gowns, or uniforms are worn to prevent contamination of personal clothing.
- Protective eyewear is worn by personnel when conducting procedures that have the potential to create splashes and sprays of microorganisms or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
- In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

Laboratory Facilities (Secondary Barriers)

- Laboratories have doors for access control.
- Laboratories have a sink for handwashing.
- An eyewash station is readily available in the laboratory.
- The laboratory is designed so that it can be easily cleaned.
    - Carpets and rugs in laboratories are not appropriate.
    - Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Laboratory furniture can support anticipated loads and uses.
    - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    - Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior are fitted with screens.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

Biosafety Level 2

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work with agents associated with human disease and pose moderate hazards to personnel and the environment. BSL-2 differs from BSL-1 primarily because: 1) laboratory personnel receive specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility specifications are recommended for BSL-2.

Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
- The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance
- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary
    - The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
- A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
- Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials.
    - Glove selection is based on an appropriate risk assessment.
    - Gloves are not worn outside the laboratory.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

    - Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.

- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons 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 are not permitted in laboratory areas. Food is stored outside the laboratory area.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    - Plasticware is substituted for glassware whenever possible.
    - Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
        - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
    - Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    - Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    - Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.

    - Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.

- An effective integrated pest management program is implemented.
- Animals and plants not associated with the work being performed are not permitted in the laboratory.

Special Practices

- Access to the laboratory is controlled when work is being conducted.
- The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-2 containment.
- Laboratory personnel are provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
- Properly maintained BSCs or other physical containment devices are used, when possible, whenever:
    - Procedures with a potential for creating infectious aerosols or splashes are conducted. These include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotors or centrifuge safety cups with loading and unloading of the rotors and centrifuge safety cups in the BSC or another containment device.
    - If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment and administrative controls are used, based on a risk assessment.
- Laboratory equipment is decontaminated routinely; after spills, splashes, or other potential contamination; and before repair, maintenance, or removal from the laboratory.
- A method for decontaminating all laboratory waste is available (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
- Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the laboratory supervisor and any other personnel designated by the institution. Appropriate records are maintained.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Protective laboratory coats, gowns, or uniforms designated for laboratory use are worn while working with hazardous materials and removed before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Protective clothing is disposed of appropriately or deposited for laundering by the institution. Laboratory clothing is not taken home.
- Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
- The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.
- In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

Laboratory Facilities (Secondary Barriers)

- Laboratory Doors are self-closing and have locks in accordance with the institutional policies.
- Laboratories have a sink for handwashing. It should be located near the exit door.
- An eyewash station is readily available in the laboratory.
- The laboratory is designed so that it can be easily cleaned.
    - Carpets and rugs in laboratories are not appropriate.
    - Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Laboratory furniture can support anticipated loads and uses.
    - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    - Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment.
- There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
- BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
    - BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
    - BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
    - BSCs are certified at least annually to ensure correct performance.

Biosafety Level 3

Biosafety Level 3 (BSL-3) is suitable for work with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel receive specific training in handling pathogenic and potentially lethal agents, and they are supervised by scientists competent in handling infectious agents and associated procedures.

A BSL-3 laboratory has special engineering and design features.

The following standard and special practices, safety equipment, and facility specifications are recommended for BSL-3.

Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
- The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
    - The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
- A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
- Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials.

  - Glove selection is based on an appropriate risk assessment.
  - Gloves are not worn outside the laboratory.
  - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - Do not wash or reuse disposable gloves and dispose of used gloves with other contaminated laboratory waste.

- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons 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 are not permitted in laboratory areas. Food is stored outside the laboratory area.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
  - Plasticware is substituted for glassware whenever possible.
  - Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
    - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
    - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
    - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
  - Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with

appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    - Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    - Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
- An effective integrated pest management program is implemented.
- Animals and plants not associated with the work being performed are not permitted in the laboratory.

Special Practices

- All persons entering the laboratory are advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or laboratory areas is required for scientific or support purposes are authorized to enter.
- All persons who enter operational laboratory areas are provided information on signs and symptoms of disease and receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
- The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-3 containment.
- A system is established for reporting and documenting near misses, laboratory accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
- Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
- Biological materials that require BSL-3 containment are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the laboratory. Once removed, the primary container is opened within a BSC in BSL-3 containment unless a validated inactivation method is used. The inactivation method is documented in-house with viability testing data to support the method.
- All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. No work with open vessels is conducted on the bench. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of personal protective equipment and other administrative and/or engineering controls, such as centrifuge safety cups or sealed rotors, are used, based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.
- Laboratory equipment is routinely decontaminated after spills, splashes, or other potential contamination, and before repair,

maintenance, or removal from the laboratory.

    - Equipment or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor method.

- A method for decontaminating all laboratory waste is available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
- Decontamination of the entire laboratory is considered when there has been gross contamination of the space, significant changes in laboratory usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on a risk assessment.
- Decontamination processes are verified on a routine basis.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Laboratory workers wear protective clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
- Based on work being performed, additional PPE may be required.
    - Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splash guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
    - Two pairs of gloves are worn when appropriate.
    - Respiratory protection is considered. Staff wearing respiratory protection are enrolled in a properly constituted respiratory protection program.
    - Shoe covers are considered.
    - In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

Laboratory Facilities (Secondary Barriers)

- The laboratory is separated from areas that are open to unrestricted traffic flow within the building.
    - Laboratory access is restricted. Laboratory doors are lockable in accordance with institutional policies. Access to the laboratory is through two consecutive self-closing doors. A clothing change room and/or an anteroom may be included in the passageway between the two self-closing doors.
- Laboratories have a sink for handwashing. The sink is hands-free or automatically operated and should be located near the exit door. If a laboratory suite is segregated into different zones, a sink is also available for handwashing in each zone.
- An eyewash station is readily available in the laboratory.
- The laboratory is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping.
    - Carpets and rugs are not permitted.

  - Spaces between benches, cabinets, and equipment are accessible for cleaning.
  - Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and ventilation openings are capable of being sealed to facilitate space decontamination.
  - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases.
  - Walls and ceilings are constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

- Laboratory furniture can support anticipated loads and uses.
  - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant.
- All windows in the laboratory are sealed.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. Vacuum lines not protected as described are capped. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
- A ducted mechanical air ventilation system is required. This system provides sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory is designed such that under failure conditions the airflow will not be reversed at the containment barrier.
  - A visual monitoring device that confirms directional airflow is provided at the laboratory entry. Audible alarms to notify personnel of airflow disruption are considered.
  - The laboratory exhaust air is not re-circulated to any other area in the building.
  - The laboratory exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA filtered.
- BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
  - BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
  - BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
  - BSCs are certified at least annually to ensure correct performance.
  - Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the room.
- Equipment that may produce infectious aerosols is used within primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters are tested annually

and replaced as needed.

- Facility is constructed to allow decontamination of the entire laboratory when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on the risk assessment.
    - Facility design consideration is given to means of decontaminating large pieces of equipment before removal from the laboratory.
- Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These laboratory enhancements may include one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas-tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; containment of other piped services; or advanced access control devices, such as biometrics.
- When present, HEPA filter housings have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. All HEPA filters are located as near as practicable to the laboratory to minimize the length of potentially contaminated ductwork. The HEPA filter housings allow for leak testing of each filter and assembly. The filters and housings are certified at least annually.
- The BSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience.
- Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress are developed and implemented.

Animal Biosafety Level 1

Animal Biosafety Level 1 (ABSL-1) is suitable for animal work involving well-characterized agents that are not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to personnel and the environment.

Special containment equipment or facility design may be required as determined by risk assessment.

Personnel receive specific training in animal facility procedures and are supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following standard practices, safety equipment, and facility specifications are recommended for ABSL-1.

Standard Microbiological Practices

- The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
- Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
- Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC), as appropriate.
- The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- Personal health status may affect an individual's susceptibility to infection or ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
- Appropriate occupational medical services are in place, as determined by risk assessment.
    - An animal allergy prevention program is part of the medical surveillance.
    - Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    - The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.

    - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

- A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
- Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
    - Glove selection is based on an appropriate risk assessment.
    - Consider the need for bite and/or scratch-resistant gloves.
    - Gloves worn inside the animal facility are not worn outside the animal facility.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, supervisors adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions are always taken with sharp items. These include:
    - Plasticware is substituted for glassware whenever possible.
    - Use of needles and syringes or other sharp instruments is limited in the animal facility and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are used whenever possible.
        - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, or the use of forceps to hold the cap when recapping a needle).

        - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.

    - Non-disposable sharps (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.

    - Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

- All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.

- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:

    - Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.

    - Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.

- An effective integrated pest management program is required.

- Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Specialized devices or equipment for restraint or containment may be required as determined by appropriate risk assessment.

- Laboratory coats, gowns, or uniforms are the minimum recommended to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the animal facility.

- Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.

- Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.

- Additional PPE is considered for persons working with large animals.

Animal Facilities (Secondary Barriers)

- ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.

    - External facility doors are self-closing and self-locking.

  - Access to the animal facility is restricted.
  - Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and never propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

- The animal facility has a sink for handwashing.
  - Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
  - If open floor drains are provided, the traps are filled with water and/ or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, ceilings) are water-resistant.
  - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
  - It is recommended that penetrations in floors, walls, and ceilings be sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
  - Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - External windows are not recommended; if present, they are resistant to breakage. Where possible, windows are sealed. If the animal facility has windows that open, they are fitted with fly screens.
  - Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Furniture can support anticipated loads and uses.
  - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
- Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals.*
  - Ventilation system design considers the heat and high moisture load produced during the cleaning of animals rooms and the cage wash process.
- Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washers have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.

Animal Biosafety Level 2

Animal Biosafety Level 2 (ABSL-2) builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and posing a moderate hazard to personnel and the environment. It also addresses hazards from ingestion and from percutaneous and mucous membrane exposure.

ABSL-2 requires that, in addition to the requirements for ABSL-1, a BSC or other physical containment equipment is used when procedures involve the manipulation of infectious materials or where aerosols or splashes may be created.

Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment. An appropriate occupational health program is in place, as determined by risk assessment.

The following standard and special practices, safety equipment, and facility specifications are recommended for ABSL-2.

Standard Microbiological Practices

- The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
- Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
- Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC), or equivalent resource, as appropriate.
- The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
- Appropriate occupational medical services are in place, as determined by risk assessment.
    - An animal allergy prevention program is part of the medical surveillance.

  - Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.

- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
  - The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, biological materials in use, and the work performed.
  - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
- A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunization, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
- Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - Glove selection is based on an appropriate risk assessment.
  - Consider the need for bite and/or scratch-resistant gloves.
  - Gloves worn inside the animal facility are not worn outside the animal facility.
  - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, supervisors adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions are always taken with sharp items. These include:

- Plasticware is substituted for glassware whenever possible.
- Use of needles and syringes or other sharp instruments is limited in the animal facility and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
    - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
    - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, or the use of forceps to hold the cap when recapping a needle).
    - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
- Non-disposable sharps (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
- Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

- All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Decontaminate all potentially infectious materials before transport or disposal using an effective method. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility. 18.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    - Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    - Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
- An effective integrated pest management program is required.
- Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

Special Practices

- Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.

- All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.

    - Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.

    - Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas. Cages are decontaminated prior to washing.

- Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.

    - Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.

    - Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.

    - Decontamination processes are verified on a routine basis.

- Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the animal facility supervisor and any other personnel designated by the institution. Appropriate records are maintained.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Properly maintained BSCs and other physical containment devices or equipment are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include the necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. A risk assessment dictates the type of other physical containment devices used when BSCs may not be suitable.

    - When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with micro-isolator lids or other equivalent primary containment systems for larger animals.

    - If used, actively ventilated caging systems are designed to contain microorganisms. Exhaust plenums for these systems are sealed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positively pressurized if the exhaust fan fails. The system is also alarmed to indicate operational malfunctions. Exhaust HEPA filters and filter housings are certified annually.

- Protective clothing, such as gowns, uniforms, scrubs, or laboratory coats, and other PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated.

  - Scrubs and uniforms are removed before leaving the animal facility.
  - Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
  - Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.

- Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials when the animal or microorganisms is handled outside the BSC or another containment device. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
- Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
- Additional PPE is considered for persons working with large animals. 6. Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.

Animal Facilities (Secondary Barriers)

- ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - External facility doors are self-closing and self-locking.
  - Access to the animal facility is restricted.
  - Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and are never to be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
- A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink is also available for handwashing at the exit from each segregated area.
  - Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
  - If open floor drains are provided, the traps are filled with water and/ or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
  - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.

  - Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, door frames, outlets, and switch plates to facilitate pest control and proper cleaning.
  - Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - External windows are not recommended; if present, they are sealed and resistant to breakage.
  - Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

- Furniture is minimized and can support anticipated loads and uses.
  - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

- Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals.*
  - Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
  - The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways.
  - A ducted exhaust air ventilation system is provided.
  - Exhaust air is discharged to the outside without being recirculated to other rooms.

- Mechanical cage washers have a final rinse temperature of at least 180°F. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.

- BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
  - BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
  - BSCs can be connected to the animal facility exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the animal facility environment if no volatile toxic chemicals are used in the cabinet.
  - BSCs are certified at least annually to ensure correct performance.
  - Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or on a replacement schedule determined by a risk assessment.

- An autoclave is present in the animal facility to facilitate decontamination of infectious materials and waste. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.

Animal Biosafety Level 3

Animal Biosafety Level 3 (ABSL-3) involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

The ABSL-3 facility has special engineering and design features.

ABSL-3 requires that in addition to the requirements for ABSL-2, all procedures are conducted in BSCs or by use of other physical containment equipment. Inward airflow at the containment boundary is maintained. Handwashing sinks are capable of hands-free operation.

Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment.

The following standard and special safety practices, safety equipment, and facility specifications are necessary for ABSL-3.

Standard Microbiological Practices

- The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
- Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
- Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC), or equivalent resource, as appropriate.
- The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- Personal health status may affect an individual's susceptibility to infection, ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
- Appropriate occupational medical services are in place, as determined by risk assessment.
    - An animal allergy prevention program is part of medical surveillance.
    - Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
    - a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, biological materials in use, and the work performed.

  - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

- A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunization, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
- Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - Glove selection is based on an appropriate risk assessment.
  - Consider the need for bite and/or scratch-resistant gloves.
  - Gloves worn inside the animal facility are not worn outside the animal facility.
  - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated facility waste.
- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, supervisors adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions are always taken with sharp items. These include:
  - Plasticware is substituted for glassware whenever possible.
  - Use of needles and syringes or other sharp instruments is limited in the animal facility and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
    - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
    - Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, or the use of forceps to hold the cap when recapping a needle).
    - Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.

  - Non-disposable sharps (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
  - Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
- All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
  - Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
  - Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
- An effective integrated pest management program is required.
- Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

Special Practices

- Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
- A system is established for reporting and documenting near misses, animal facility accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
- Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the animal facility director, facility supervisor, institutional management, and appropriate facility safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
- Only necessary equipment and supplies are recommended to be taken inside the animal facility.
- All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
  - Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
  - Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas.
- Biological materials that are to remain in a viable state during removal from the animal facility are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the facility by authorized personnel. Once removed, the primary container is opened within a BSC in BSL-3 or ABSL-3

containment unless a validated inactivated method is used. The inactivation method is documented in-house with viability testing data to support the method.

- Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, state, and federal requirements.
    - Equipment is decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or manipulated. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
    - Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
    - Decontamination processes are verified on a routine basis.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Properly maintained BSCs and other physical containment devices or equipment are used for manipulations of infectious materials and animals as determined by risk assessment.
    - The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with micro-isolator lids, open cages placed in inward flow ventilated enclosures, HEPA filter isolators and caging systems, or other equivalent primary containment systems.
        - Actively ventilated caging systems are designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems are sealed to prevent the escape of microorganisms if the ventilation system becomes static, and the exhaust is HEPA-filtered. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system is alarmed to indicate operational malfunctions.
    - When animals cannot be housed in ventilated containment cages/ units, certain features of the animal room act as the primary barriers. The procedures in place include how workers are protected from agents shed by the animals (e.g., PPE enhancements) as well as how the environment is protected from such agents through the use of biocontainment enhancements such as some combination of boot or PPE change or surface decontamination at the door, a personal shower at the room level, and/or other procedures.
- Special consideration is given to the potential for cross-contamination when open caging is used.
- Personnel within the animal facility wear protective clothing, such as uniforms or scrubs.
    - Disposable PPE such as non-woven, olefin cover-all suits, or wrap-around or solid-front gowns are worn over this clothing before entering areas where infectious materials and/or animals are housed or manipulated. Front-button, laboratory coats are unsuitable.
    - Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
    - Disposable PPE is removed when leaving the areas where infectious materials and/or animals are housed or manipulated. Scrubs and uniforms are removed before leaving the animal facility.

    - Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.

- All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate head covering, eye, face, and respiratory protection. To prevent cross-contamination, boots, shoe covers, or other protective footwear are used where indicated and disposed of or decontaminated after use.
- Head covering, eye protection, and face protection are disposed of with other contaminated animal facility waste or decontaminated after use.
- Procedures may require wearing two pairs of gloves (i.e., double-glove). Change outer gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
- Additional PPE is considered for persons working with large animals.

Animal Facilities (Secondary Barriers)

- ABSL-3 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
    - External facility doors are self-closing and self-locking.
    - Access to the animal facility is restricted.
    - Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and are never propped open.
    - Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Exit showers may be considered based on risk assessment. An additional double-door anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.
- A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a handwashing sink is also available near the exit from each segregated area.
    - The sink is hands-free or automatically operated.
    - Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
    - Sink traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
    - Floor drains are maintained and filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
    - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases. Floors slope to drain, if present.

  - Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, outlets, switch plates, and doorframes, to facilitate pest control, proper cleaning, and decontamination. Walls, floors, and ceilings form a sanitizable and sealed surface.
  - Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - External windows are not recommended; if present, they are sealed and resistant to breakage.
  - Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

- Furniture is minimized and can support anticipated loads and uses.
  - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

- Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.
  - Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
  - The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A visual monitoring device, which confirms directional airflow, is provided at the animal room entrance.
  - A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.
  - The exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA-filtered.
  - The ABSL-3 animal facility is designed such that under failure conditions the airflow will not be reversed at the containment barrier. Alarms are considered to notify personnel of ventilation and HVAC system failure.

- Cages are decontaminated prior to removal from the containment barrier and prior to washing in a mechanical cage washer. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.

- BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
  - BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
  - BSCs can be connected to the animal facility exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can

be safely recirculated back into the animal facility environment if no volatile toxic chemicals are used in the cabinet.

  - BSCs are certified at least annually to ensure correct performance.
  - Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the animal room.

- Equipment that may produce infectious aerosols is contained in primary barrier devices that exhaust air through HEPA filtration, or other equivalent technology, before being discharged into the animal facility. These HEPA filters are tested annually and replaced as needed.
- All vacuum lines are protected with HEPA filters, or their equivalent, or are capped. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
- An autoclave is available within the containment barrier. The autoclave is utilized to decontaminate infectious materials and waste before moving these materials to the other areas of the facility. If not within the containment barrier, special practices are developed for the transport of infectious materials to designated alternate locations for decontamination. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.
- The ABSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience.
- Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These enhancements may include one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas-tight dampers to facilitate animal room isolation; final HEPA filtration of the animal room exhaust air; animal room effluent decontamination; containment of other piped services; or advanced access control devices, such as biometrics.

Biological Spill Procedures

Spills involving recombinant DNA and/or infectious cultures, should be **reported immediately** to the Institutional Biosafety Officer.

Biosafety Level 1 and Biosafety Level 2 Biological Spills

Inside the Biosafety Cabinet

- Remove contaminated clothing and wash exposed skin, if applicable.
- Wear gloves and a lab coat.
- Pick up any sharp items, e.g., broken glass or needles, with forceps placed in a sharps container.
- Cover spill with paper towels and pour an appropriate disinfectant such as 10% bleach, around and over the spill.
- Allow suitable contact time, 20 minutes at a minimum.
- Wipe up the spill from the outside edge working towards the center of the spill.
- Discard disposable materials used to clean up the spill in a red biological waste bag.
- Wipe the surrounding area and the spill area again with disinfectant.
- Disinfect or autoclave any non-disposable materials used.
- Report the spill to your supervisor.

Outside of the Biosafety Cabinet

- Alert others in the area, and request they vacate the area.
- Remove contaminated clothing and wash exposed skin with soap and water.
- Exit the lab and place “Do Not Enter” signage on the door.
- Report the spill to your supervisor.
- Allow aerosols to settle for at least 30 minutes before re-entering the lab.
- Wear gloves, lab coat, and face protection.
- Pick up any sharp items, e.g., broken glass or needles with forceps or dustpan and brush and place in a sharps container.
- Cover spill with paper towels and pour disinfectant, e.g., 10% bleach, around and over the spill.
- Allow suitable contact time, at a minimum 30 minutes.
- Wipe up the spill from the outside edge working towards the center of the spill.
- Discard disposable materials used to clean up the spill in a biological waste bag.
- Wipe the surrounding area and the spill area again with disinfectant.
- Disinfect or autoclave any non-disposable materials used.
- Report the spill to your supervisor.

Biosafety Level 3 Biological Spill
Follow your laboratory-specific SOP for BSL3 biological spills.

Biological Waste Management

Biological waste includes but is not limited to the following: potentially infectious materials to humans, animals, and plants, genetically modified materials, animal carcasses, and human and animal tissues (pathological waste).

Always use proper PPE when collecting and handling biological waste.

Solid Biological Waste Collection and Handling:

- Collect BSL1 and BSL2 waste in red-lined Stericycle biological waste boxes for pick-up and off-site treatment.
- When containers are 3/4 full, tie off the red bag liner with a single knot and tuck bag completely inside the container. If using cardboard boxes, tape the cover or box closed with packaging tape. For the plastic reusable containers, snap the cover flaps shut.
- Contact sean.fitzgerald@hmhn.org if you plan on performing on-site treatment of solid biological waste.

Mixed Biohazardous Waste

- Animal or human tissue treated with chemical fixatives are processed as solid chemical waste. Label the hazardous waste, with the appropriate chemical hazard and identify the waste as non-infectious animal/human tissue.
- Mixed biological, chemical and/or radioactive waste requires special procedures for decontamination and disposal.

Liquid Biological Waste

- Liquid biological waste must be treated with an appropriate disinfectant at the appropriate concentration and contact time before sink disposal.
    - For example, allow a minimum contact time of 30 minutes at a final concentration of 10% bleach before drain disposal.
- Carefully pour the disinfected liquid waste down the sink drain and flush with generous amounts of water.
- Autoclaving genetically modified cultures, BSL1, and BSL2 liquid waste may also be acceptable. Follow lab-specific procedures for autoclaving liquid waste.

BSL3 Biological Waste

- Biological waste from BSL3 laboratories must be decontaminated and the process validated following established lab specific procedures prior to removal containment.

Laboratory Aerosols

Significant amounts of laboratory procedures can create biological aerosols that can be potentially hazardous to the personnel and the surrounding environment. Standard microbiological practices provide aerosol minimization techniques, but some special precautions can help increase the level of prevention.

Standard Precautions

- When working with infectious or potentially infectious materials, aerosol-generating procedures must be performed in a biological safety cabinet.
- Utilize respiratory protection whenever required.
- When spills occur, leave the area and allow any aerosols to settle before addressing the spill.

Specific precautions for laboratory procedures with aerosol potential

Pipetting:

- Dispense liquids as close to the reservoir as possible.
- Make sure to rinse with the appropriate disinfectant before disposing pipettes or pipette tips.
- Take caution when removing pipette or pipette tips.

Syringe usage:

- Make sure to avoid air bubbles in liquid discharged.

Inoculation loops:

- Try to use pre-sterilized, disposable loops when possible.
- When heat-sterilizing reusable loops, allow the loop to cool before introducing it to any potentially infectious materials.

Liquid decanting:

- Pour carefully and as close to the vessel receiving as possible.

Animal inoculation:

- Remove syringe needles from animals slowly, with a smooth and steady motion.

Vortexing:

- Make sure all substances being vortexed are in a durable container with a tight-fitting lid.

Blending

- Use a laboratory grade blender with a tight-fitting lid.
- Operate the blender in a biosafety cabinet if infectious material is used.
- Allow aerosols to settle before opening the lid.

Sonication:

- Operate the sonicator in a biosafety cabinet or aerosol containment if infectious material is being used.

Centrifuging:

- Use aerosol-proof rotors or safety buckets with caps that seal with O-rings.
- Before use, inspect O-rings and safety caps for cracks, chips, and erosion.
- Use tubes with threaded caps.
- Avoid overfilling the tube and getting caps/closures wet.
- Wipe tubes down with disinfectant after filling.
- Load and unload rotors and buckets inside the BSC.
- Balance buckets, tubes and rotors before centrifuging.
- Wait a few minutes after the centrifuge completes to allow aerosols to settle.
- Disinfect the centrifuge after use.

Vacuum flask and aspiration set-ups:

- House vacuum lines and vacuum pumps must be protected by using a hydrophobic filter installed between the collection flask and vacuum source.
- In some cases, a HEPA filter may be required to protect against infectious aerosols.

Sharps Precaution and Disposal

A sharp is any instrument that can cause punctures, cuts, or scrapes to the body. Sharps includes but is not limited to: needles, syringes, scalpels, razor blades, slides, coverslips, Pasteur pipettes, capillary tubes, sharp or broken glass, lancets, suture needles, and microtome blades. Use of sharps should be restricted to trained personnel and instances in which no alternative is available. In addition, sharps are covered under regulatory waste guidelines and must not be disposed of with regular trash.

Sharps Precautions

- Avoid the use of needles and other sharps whenever possible. Use plastic alternatives to glass if available.
- Minimize any contact with sharps by disposing or storing them immediately after use.
- Needles must never be recapped, removed from the syringe, sheared, bent or broken.
- Use a mechanical device to remove scalpel blades. Never use your fingers.

Disposal of Laboratory Sharps

- All sharps must be disposed of into an approved, puncture resistant, sharps container.
- Sharps used with genetically modified and biological materials must be collected in red biohazard sharps containers for disposal.
- Do not overfill the sharps container.
- Never force materials into a sharps container.
- Never reach into the sharps container.
- Do not remove the lid from the container.
- When a sharps container is ¾ full, close it and dispose of it in the solid biological waste stream.

Select Agents and Exempt Quantities of Select Agent Toxins

Select Agents are federally regulated agents that have potential use in biological warfare. Health and Human Services (HHS) regulates select agents targeting humans, the United States Department of Agriculture (USDA) regulates select agents targeting animals, and the USDA Plant Protection and Quarantine (PPQ) regulates select agents targeting plants. Before possessing, using, sending, or receiving select agents, the institution and Principal Investigator must register with CDC, APHIS, and/or USDA to receive official authorization for each individual requesting access to select agents. Requirements include background checks on those authorized to access select agents, security plans and inventories. Immediately notify the Office of Biosafety if you discover select agents in your laboratory that have not been registered.

A few common examples are listed below:

- Botulinum neurotoxins
- Botulinum neurotoxin producing species of *Clostridium*
- Conotoxins (Short, paralytic alpha)
- *Francisella tularensis*
- Lassa fever virus
- Ricin
- SARS-associated coronavirus (SARS-CoV)
- Tetrodotoxin
- Variola major virus (Smallpox virus)
- Variola minor virus (Alastrim)
- *Yersinia pestis*
- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella melitensis*
- Avian influenza virus
- Classical swine fever virus
- Foot-and-mouth disease virus
- Newcastle disease virus

The select agent regulations also apply to Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms as described below:

- Nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the select agent viruses.
- Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed if the nucleic acids are in a vector or host chromosome and/or can be expressed in vivo or in vitro.
- Listed viruses, bacteria, fungi, and toxins that have been genetically modified.

Exclusions:

The select agent regulations do not apply to:

- Any select agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
- Non-viable select agent organisms or non- functional toxins.
- The HHS secretary may exclude attenuated strains or toxins if it is determined that they do not pose a public health threat.

Exempt Quantities of Select Agent Toxins:
The US federal government identifies Select Agent Toxins as toxins with the potential to be used in biological warfare. Only toxins listed here are controlled as Select Agent Toxins. CDC allows for the storage and use of these toxins in limited amounts without the full regulatory burden of registration with the CDC/USDA and the associated security requirements.

HHS Toxins [§73.3(d)(3)]	*Amount*
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Select Toxin Transfer under Permissible Amounts Due Diligence Form and Instructions

The purpose of this form is to document due diligence performed in the transfer of select toxins in any amount under the excluded amounts as defined by the select agent regulations from one laboratory to another laboratory or institution.

In accordance with the Federal Select Agent Program Requirements, a registered entity transferring an amount of select toxin otherwise excluded under the provisions of 42 CFR 73.3(d) must:

- Transfer the amounts only after the transferor uses due diligence and documents that the recipient has legitimate need (i.e., reasonably justified by a prophylactic, protective, bona fide research, or other peaceful purpose) to handle or use such toxins
- Report to CDC if they detect a known or suspected violation of Federal law or become aware of suspicious activity related to a toxin listed in 42 CFR 73.3(d)

To ensure that only individuals who have a legitimate purpose to handle and use select toxins, the transferor needs to document the recipient's intended use of the toxins and document the legitimate need for the select toxins.

Instructions:

1. Recipient and Transferor make contact to determine what select toxins they would like to transfer and when they would like to complete the transfer.
2. Recipient completes all of Section A of the Select Agent Toxin Transfer Due Diligence Form.
3. Recipient submits the completed/signed form to the Transferor.
4. Transferor completes all of Section B of the Select Agent Toxin Transfer Due Diligence Form.
5. Transferor submits the completed/signed form to the institutional biosafety officer for approval.
6. The Biosafety Officer reviews the transfer request and signs the form when approved.
7. Completed form is provided to sender.
8. Sender transfers toxin to recipient (in accordance with hazardous material shipping regulations) utilizing a shipping method which can be tracked. The shipper includes a copy of the form inside the shipment. Sender notifies the recipient of the tracking information and anticipated date of arrival.
9. Recipient notifies Sender upon receipt of shipment. Both shipper and recipient retain records for three years or as required by hazardous materials shipping requirements.

Permissible Toxin Amounts

HHS Toxins [§73.3(d)(3)]	*Amount*
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Select Toxin Transfer - Permissible Amounts

Due Diligence Form

Section A

Section A (Completed by Recipient)			
Name of Institution:			
Address:			
City:	**State:**	**Zip Code:**	
Recipient Name:			
Phone Number:	**Email Address:**		
Name of Select Toxin(s) Requested:		**Amount of Select Toxin(s) Requested:**	
1.			
2.			
3.			
4.			
Current Amount of Select Toxin(s) in Recipient Facility:			
1.			
2.			
3.			
4.			
Intended Use of Select Toxin(s):			
By signature on this form, I, _____, declare the intended use of this toxin is for legitimate need (e.g., prophylactic, protective, bona fide research or other peaceful purpose). **Signature of Recipient:**			
Date:			

Select Toxin Transfer - Permissible Amounts

Due Diligence Form

Section B

Section B (Completed by Sender)			
Name of Institution:			
Address:			
City:	**State:**	**Zip Code:**	
Sender Name:			
Phone Number:	**Email Address:**		
Name of Select Toxin(s) Shipped:		**Amount of Select Toxin(s) Shipped:**	
1.			
2.			
3.			
4.			

I hereby certify that the select toxins listed above were packaged, labeled, and shipped in accordance with all federal and international regulations. All information in this form is true and correct to the best of my knowledge. I understand that violations of 7CFR 331, 9CFR 121, and 42 CFR 73 may result in civil or criminal penalties, including imprisonment.

Signature of Sender:
Printed Name of Sender:
Title:
Date:

The above transfer of excluded quantities of select toxins has been reviewed and approved by the institutional biosafety officer.

Signature of Biosafety Officer:
Printed Name of Biosafety Officer:
Date:

Import Permits

Contact the Institutional Biosafety Officer at (201) 250-1024 or sean.fitzgerald@hmhn.org for assistance if an import permit is required. All agencies listed below may require an inspection of the importer's facility and safety validation.

Items Requiring CDC Import Permits:

- Any infectious (etiologic) agent known or suspected to cause disease in humans.
- Unsterilized specimens of human and animal tissues (such as blood, body discharges, fluids, excretions or similar material) containing an infectious or etiologic agent.
- Hosts and Vectors:
    - Animals: Any animal known or suspected of being infected with an organism capable of causing disease in humans may require an import permit.
    - Arthropods: Any living insect or other arthropod that is known or suspected of containing an etiologic agent (human pathogen).
    - Snails: Snail species capable of transmitting a human pathogen.

Importation permits are issued only to the importer, who must be in the United States. A CDC Import Permit can be obtained on their website: http://www.cdc.gov/od/eaipp/importApplication/agents.html

CDC Contact Information
Phone: (404) 718-2077; Fax: (404) 471-8333
Email: ImportPermit@cdc.gov

United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA/APHIS/VS) regulate the importation of animals and animal-derived materials to ensure that exotic animal and poultry diseases are not introduced into the United States.

Animal Products Requiring a USDA Import Permit

Generally, a USDA veterinary permit may be needed for materials derived from animals or exposed to animal-source materials. Materials which require a permit include, animal tissues, blood, cells or cell lines of livestock or poultry origin, antibodies for in vivo use in non-human species, and bulk shipments of test kit reagents. Veterinary biological products (vaccines, antisera, diagnostic kits, and other products of biological origin) produced in other countries may be imported into the United States for research and evaluation if a permit is obtained.

To obtain and import permit or for more information visit: https://www.aphis.usda.gov/aphis/resources/permits

Fish & Wildlife Service and National Marine Fisheries Service
Fish and Wildlife Service permits are required for marine mammals, certain fish, and certain live animals, including bats. Call 1-800-344-WILD for further information
Contact information:
http://www.fws.gov/permits/ImportExport/ImportExport.html

Permit Division, Office of Protected Resources, National Marine Fisheries Service (301) 713-2355 or 713-2289 Fish and Wildlife Service, Office of Management Authority (703) 358-2104.