Research Biosafety Plan
(EHS Program 3.2)

1.0 Overview

Environmental Health and Safety (EHS) at Weill Cornell Medicine (WCM) has developed this Research Biosafety Program Manual, which is periodically reviewed to include revisions to biosafety standards.

In all situations, the most recent version of the publications "*Biosafety in Microbiological and Biomedical Laboratories (BMBL),*" "*Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and Arthropod Containment Guidelines,*" as well as regulations issued by the Occupational Safety and Health Administration (OSHA) regulating biosafety will supplement and supersede this document.

The EHS program manuals covering Waste Disposal Procedures, Biological Spill Planning and Response, and the Bloodborne Pathogen Exposure Control Plan are also considered integral elements of the Research Biosafety Program.

All Principal Investigators conducting biomedical research at WCM are required to submit an Institutional Biosafety Committee (IBC) laboratory registration for review and approval by the IBC. EHS supports the IBC in carrying out WCM's Biosafety Program in the acquisition, use, training, transfer, storage, disposal, and emergency response procedures for all biosafety activities. The registration of research activities is completed within the Weill Research Gateway (WRG) and is described in the WRG Lab Registration Guide. EHS oversees the coordination of the IBC laboratory registration process, and the Research Safety Inspection Program Completion of these registrations provides a standardized submission process for PIs to meet the federal, state, and local laboratory safety requirements, including compliance with the National Institute of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

2.0 Emergency Contact Information

Please refer to the following contact list in the event of a fire or other emergency that may involve personnel while working with biological materials.

Environmental Health and Safety (Biological Spills)	**646-962-7233 (1-7233)**
Workforce Health and Safety (8:00AM - 4:00PM)	**212-746-4370 (6-4370)**
Student Health Services (8:00AM - 4:00PM)	**212-746-1450 (6-1450)**
Emergency Medical Services (NYP ER)	**212-472-2222 (2-2222)**
Biosafety Officer	**646-962-7233 (1-7233)**
Engineering & Maintenance (ventilation, power, steam system failures)	
WCM	**212-746-2288 (6-2288)**
NYP	**212-746-1920 (6-1920)**
Security	**212-746-0911 (6-0911)**

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Weill Cornell Medicine
Environmental Health and Safety
TEL 646-962-7233 WEB weill.cornell.edu/ehs EMAIL ehs@med.cornell.edu
Weill Cornell Medicine | 402 East 67th Street, Room LA-0020 | New York, NY 10065

4.0 Applicability

All WCM faculty and research staff using biological agents must read and implement the practices outlined in this manual. Adherence to good microbiological practices and the development of specific research protocols will ensure safety and compliance with standards issued by OSHA, NIH, and the CDC.

5.0 Responsibilities

The WCM Research Biosafety Program establishes duties for individuals and groups as described below.

5.1 BIOLOGICAL SAFETY OFFICER

- Ensures that Department Chairs, Principal Investigators, Directors, and Managers comply with the Research Biosafety Program.

- Provides technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

5.2 ENVIRONMENTAL HEALTH AND SAFETY (EHS)

- Develops, maintains, and disseminates the Research Biosafety Program.
- Serves as a resource for biosafety procedures and containment.
- Trains laboratory personnel on the principles of this Program.
- Responds to emergency spills or releases.
- Maintains records of training, spills, emergencies, and exposures.
- Inspects laboratories for compliance with this Program.

5.3 PRINCIPAL INVESTIGATORS (PIS) AND OTHER LABORATORY SUPERVISORS

- Ensures that all personnel comply with the WCM Research Biosafety Program during the conduct of biological research.
- Be adequately trained in proper microbiological techniques.
- Provide laboratory research staff with protocols describing potential biohazards and necessary precautions.
- Instruct and train laboratory staff in the practices and techniques required to ensure safety and the procedures for dealing with accidents.
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- Supervise laboratory staff to ensure the use of all required safety practices and techniques.
- Correct work errors and conditions that may result in the release of recombinant or synthetic nucleic acid materials.
- Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment.
- Comply with permit and shipping requirements for hazardous materials, including recombinant or synthetic nucleic acid molecules.
- Adhere to the EHS Biological Spill Planning and Response Program for handling accidental spills and personnel contamination.

5.3.1 PI Responsibilities Prior to Conducting Research

- Submit a Laboratory Registration to the WCM IBC for review and approval.
- Determine whether the research is subject to Section III-A, B, C, D, E, or F of the NIH Guidelines
- Propose physical and biological containment levels in accordance with the CDC and NIH Guidelines.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.

5.3.2 PI Responsibilities While Conducting Research

- Consult the WCM IBC before modifying approved recombinant or synthetic nucleic acid research protocols.
- Submit periodic updates and any changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review.
- Report any significant problems regarding 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, OBA, and, as applicable, the Biological Safety Officer, Animal Facility Director, and other appropriate authorities.

5.4 LABORATORY PERSONNEL

- Follow all procedures outlined in this Plan.
- Adhere to recommendations made by the PI, Biological Safety Officer, and EHS.

5.5 INSTITUTIONAL BIOSAFETY COMMITTEE

- Conduct initial and periodic review of research conducted at or sponsored by the institution for compliance with the NIH Guidelines.
- Notify the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.
- Report any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OSP.

6.0 Occupational Health Support for Biomedical Research

The health screening/monitoring requirements for new research laboratory employees include an initial evaluation, which covers:

- Employee medical screening,
- Hepatitis Blood tests (B/C)/, Hepatitis B vaccination,
- Hepatitis B vaccination,
- TB screening,
- Respirator clearance, and
- Chemo or laser screening (as appropriate),
- Animal Handler Questionnaire.

The medical screening includes occupational and medical history, physical examination, and blood work for measles, mumps, rubella, and varicella immunity, as well as vision screening. Research laboratory employees will complete a yearly medical monitoring questionnaire, undergo TB screening, complete chemo screening as required, and complete the animal handler questionnaire.

An additional level of protection for at-risk personnel may be achieved with appropriate prophylactic immunizations. Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risks (local or systemic reactions) should be required for all clearly identified at-risk personnel. Examples of such preparations include vaccines against hepatitis B, yellow fever, rabies, and poxviruses (e.g., vaccinia). If special hazards exist for an individual's work, the IBC, EHS, and the Director of Workforce Health and Safety should be consulted for other tests.

Individuals with work-related illnesses and injuries or accidental exposures to blood, body fluids, and other potentially infectious materials should follow posted Exposure and Spill Response Guide and report directly to Workforce Health and Safety so that the exposure can be documented, and appropriate preventive measures initiated.

The Principal Investigator is responsible for reporting illness among laboratory personnel that affects single individuals repeatedly or multiple individuals, either at the same time or in some close sequence. The PI is also responsible for reporting even sporadic instances of unusual or life-threatening diseases such as leukemia, lymphoma, or chronic disabilities due to nervous, respiratory, renal, or gastrointestinal illness to the IBC.

7.0 Risk Assessment and Biosafety Level Determination

The selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a comprehensive risk assessment of factors associated with the research.

Risk assessment is the process used to identify hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory-Acquired Infection (LAI), and the probable consequences of such an exposure. The NIH Guidelines has established a classification and assigned human etiological agents into four risk groups based on the hazard. The descriptions of the NIH risk group classifications are presented in **Section 7.1.**
They correlate with but do not equate to biosafety levels. The risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level.

Laboratory directors and principal investigators should use the risk assessment to alert their personnel to the hazards of working with biological agents, and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building and the public. Principal Investigators should make their laboratory personnel aware of the etiological signs and symptoms associated with infection or toxicity of any agents used or stored in their laboratory.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. The capability of the laboratory staff to control hazards must be considered as well. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. Laboratory directors or PIs should train and retrain new personnel to the point where aseptic techniques and safety precautions become second nature.

First, identify hazardous characteristics of the agent and perform an assessment of the inherent risk, which is the risk in the absence of

mitigating factors. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible host, severity of disease, and the availability of preventive measures and effective treatments.

Other hazardous characteristics of an agent include possible routes of transmission of laboratory infection, infective dose, stability in the environment, host range, whether the agent is indigenous or exotic to the local environment, and the genetic characteristics of the agent. It is important to remember that the nature and severity of disease caused by an LAI and the probable route of transmission of the infectious agent in the laboratory may differ from the route of transmission and severity associated with the naturally acquired disease.

Reports of LAIs are also a clear indicator of hazard and often are sources of information helpful for identifying the agent and procedural hazards, and the precautions for their control.

The predominant routes of transmission in the laboratory are:

1. Direct skin, eye, or mucosal membrane exposure to an agent;
2. Parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors;
3. Ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and
4. Inhalation of infectious aerosols.

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents also require careful consideration of a risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an essential indicator of hazard. The death of a primate center laboratory worker from Cercopithecine herpesvirus 1 (CHV-1, also known as Monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard.

The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments.

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment, and procedures that generate small particle aerosols and larger airborne particles (droplets) and the use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

A procedure's potential to release microorganisms into the air as aerosols and droplets is the most critical operational risk factor that supports the need for containment equipment and facility safeguards. Procedures that impart energy to a microbial suspension will produce aerosols. Equipment used for handling and analyzing infectious agents in laboratories, such as pipettes, blenders, centrifuges, sonicators, vortex mixers, cell sorters, and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometers are potential sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods.

Procedures and equipment that generate respirable size particles also generate larger size droplets that settle out of the air rapidly, contaminating hands, work surfaces, and possibly the mucous membranes of the persons performing the procedure. An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable component is relatively small; in contrast, hand and surface contamination can be substantial. The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Small-particle aerosols have respirable size particles that may contain one or several microorganisms. These small particles stay airborne for protracted periods and easily disperse throughout the laboratory. When inhaled, the human lung will retain those particles creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to airflow from the laboratory. Larger particle droplets rapidly fall out of the air, contaminating gloves, the immediate work area, and the mucous membranes of unprotected workers.

There may be hazards that require specialized Personal Protective Equipment (PPE) in addition to safety glasses, laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training in the proper use of PPE may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator worn incorrectly may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as biological safety cabinets (BSCs), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets. Safety equipment that is not working properly is hazardous, especially when the user is unaware of the malfunction. Poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique compromise the containment capability of a BSC.

Facility safeguards help prevent the accidental release of an agent from the laboratory. For example, one facility safeguard is directional airflow, which helps prevent aerosol transmission from a laboratory into other areas of the building. Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity.

Consideration of facility safeguards is an integral part of the risk assessment. A biological safety professional, building and facilities staff, and the IBC, can and do help assess the facility's capability to provide appropriate protection for the planned work and recommend changes as necessary. Risk assessment may support the need to include additional facility safeguards in the construction of new or renovation of old facilities.

Third, make a determination of the appropriate Biosafety Level and select additional precautions indicated by the risk assessment. The selection of the appropriate Biosafety Level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards.

There will be situations where the intended use of an agent requires greater precautions than those described in the agent's summary statement. These situations will require the careful selection of additional precautions. An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols.

Fourth, before implementation of the controls, review the risk assessment and selected safeguards with a biosafety professional, the IBC, and, if needed, a subject matter expert.

Fifth, as part of an ongoing process, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. Staff at all skill levels need to know how to identify hazards in the laboratory and how to obtain assistance in protecting themselves and others in the laboratory. An evaluation of a worker's training, experience in handling infectious agents, proficiency in following good microbiological practices, correct use of safety equipment, consistent use of standard operating procedures (SOPs) for specific laboratory activities, ability to respond to emergencies, and willingness to accept responsibility for protecting oneself and others is an important indication that a laboratory worker is capable of working safely.

Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for laboratory staff in order to reduce the risks associated with working with hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials. Laboratory directors or principal investigators should consider the use of competency assessment(s) to train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.

Sixth, revisit regularly and verify risk management strategies and determine if changes are necessary. Continue the risk management cycle and adjust and adapt as the need arises. This includes a regular update of biosafety manuals and SOPs when changes in procedures or equipment occur. A cyclical, adaptable risk management process forms the basis for a robust culture of safety in the biological laboratory.

A sound risk communication strategy is also critical for both hazard identification and successful implementation. Ultimate success will be measured by whether you establish, strengthen, and sustain a culture of safety while encouraging communication about risks between management and staff to prevent accidents before they happen. The regular review of all hazards, prioritization of risk, multidisciplinary review of priority risks, and establishment of risk mitigation measures demonstrate our institution's commitment to a safe and secure working environment and form the cornerstone of a biosafety program.

This approach to risk assessment is not static and benefits from active participation by all relevant stakeholders. The goal of the biological safety program is for ongoing evaluation and periodic readjustments to stay aligned with the changing needs of the institution and to protect all persons from potential exposure to biological materials in laboratories and associated facilities.

7.1 RISK GROUP CLASSIFICATION OF BIOLOGICAL AGENTS

The Risk Group (RG) of an agent is an important factor to be considered during the biosafety safety risk assessment process. Biological agents are assigned to their relevant Risk Groups based on their ability to cause disease in healthy human adults and spread within the community. However, just because a biological agent is listed as a Risk Group 3 agent, it does not mean the activities conducted with that biological agent must occur in a BSL-3 laboratory. NIH Appendix B (page 42) reflects the current state of knowledge and should be considered a resource document.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease, which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are 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 (RG4)	Agents that are 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)

Risk Assessment Examples: Factors that can elevate the likelihood of an exposure to and/or release of biological agents during work in the laboratory, and/or escalate its associated consequences are given in the tables below:

Table 7.1.1 Factors that affect the likelihood of an incident occurring

Factors associated with high likelihood of incidents occurring	Rationale
Laboratory activities associated with aerosolization (for example, sonication, homogenization, centrifugation)	When aerosols are generated by these methods, the likelihood of exposure through inhalation is increased, as is the likelihood of release of these aerosols into the surrounding environment, where they might contaminate laboratory surfaces and also spread into the community.
Laboratory activities associated with sharps materials	When activities involve work with sharps, the likelihood of percutaneous exposure to a biological agent through a puncture wound is increased.
Low competency of personnel carrying out the work	Low proficiency of personnel in laboratory processes and procedures, through lack of experience, understanding, or failure to comply with SOPs and GMPP, can lead to errors in performing the work, which is more likely to result in exposure to and/or release of a biological agent. Cleaning and maintenance personnel must be trained before working close to a biological agent.
Highly environmentally stable biological agents	Biological agents that have settled on laboratory surfaces (for example, contamination caused by poor technique that allowed settling of aerosol or droplets after release) can be a source of inadvertent exposure as long as they remain stable in the environment, even if the contamination cannot be seen.
Inadequate or poor availability of electrical power, dilapidated laboratory facilities, and building systems, malfunctioning equipment, damage from frequent severe weather, and access of insects and rodents to the laboratory.	All these factors may result in partial breaches in, or complete failure of, biocontainment systems designed to reduce the likelihood of exposure to and/or release of biological agents.

Table 7.1.2 Factors that affect the consequences of an incident if it were to occur

Factors associated with greater consequences if an incident were to occur	Rationale
Low infectious dose	For infection to occur in an exposed individual, a certain quantity (volume, concentration) of biological agent must be present. Even a small amount of an agent could result in severe consequences, such as a laboratory-associated infection. Furthermore, exposure to larger quantities of that agent (greater than the infectious dose) may result in a more severe presentation of the infection.
High communicability	Even one single exposure (causing carriage or a laboratory-associated infection) could rapidly spread from laboratory personnel or fomites to many individuals.
High severity and mortality	Low proficiency of personnel in laboratory processes and procedures, through lack of experience, understanding, or failure to comply with SOPs and GMPP, can lead to errors in performing the work, which is more likely to result in exposure to and/or release of a biological agent. Cleaning and maintenance personnel must be trained before working close to a biological agent.

Limited availability of effective prophylaxis or therapeutic interventions	The symptoms or outcomes of a laboratory-associated infection cannot be effectively prevented, reduced, or eliminated by a medical intervention. This may also include situations where medical intervention is not available, or emergency response capacity is limited.
Large susceptible population (including laboratory personnel at increased risk)	The larger the susceptible population, the more likely a laboratory-associated infection could rapidly spread and infect larger numbers of people.
Lack of endemicity (such as exotic disease)	When an agent is not endemic in the surrounding population, the population is more likely to be susceptible to the agent, leading to an increased likelihood of a laboratory-associated infection spreading to the community.

Table 7.1.3 Factors associated with both a high likelihood of and greater consequences from a potential incident

Factors associated with both a high likelihood of and greater consequences from a potential incident higher likelihood and greater consequence	**Rationale**
High concentration or volume of the biological agent	The more biological agent there is in the substance being handled, the more infectious particles there will be available for exposure, and the more likely the exposure volume will contain the infectious dose of that agent. Furthermore, being exposed to a higher concentration of the agent could result in a more severe infection, illness, or injury.
Airborne route of transmission	Biological agents with an airborne route of transmission may be capable of remaining airborne in aerosols for prolonged periods of time and may disseminate widely in the laboratory environment, increasing the likelihood that personnel may be exposed to the agent. Furthermore, following an exposure event, aerosolized biological agents may be inhaled and deposit on the respiratory tract mucosa of the exposed individual, possibly leading to a laboratory-associated infection.

7.2 PRINCIPLES OF BIOSAFETY

A fundamental objective of the WCM biosafety program is the containment of potentially hazardous biological agents. The term containment describes a combination of primary and secondary barriers, facility practices and procedures, and other safety equipment, including personal protective equipment (PPE), for managing the risks associated with handling and storing hazardous biological agents in a laboratory environment. The purpose of containment is to reduce the risk of exposure to staff and the unintentional release of hazardous biological agents into the surrounding community and environment.

Primary barrier or primary containment is defined as physical containment measure(s) placed directly at the level of the hazard. Safety equipment such as biological safety cabinets (BSCs), enclosed containers, and other biosafety controls are designed to protect personnel, the surrounding community, and the environment from possible exposure to hazardous biological agents. Primary barriers can function to either provide containment (e.g., BSCs) or direct personal protection from the hazardous biological agents used. The BSC is the standard device used to provide containment of hazardous biological agents when conducting microbiological activities.

Additional primary containment devices may include sealed containers (e.g., sealed rotors and centrifuge safety cups). These enclosed containers are designed to contain aerosols, droplets, and leakage of hazardous biological agents that may result during certain activities (e.g., centrifugation). Sealed containers provide containment for transfers between laboratories within a facility, between facilities, and depending upon risk assessment, within a laboratory.

Personal protective equipment (PPE) helps protect the user's body from injury from a variety of sources (e.g., physical, electrical, heat, noise, chemical) or potential exposure to biological hazards and airborne particulate matter. PPE includes gloves, coats, gowns, shoe covers, closed-toe laboratory footwear, respirators, face shields, safety glasses, goggles, or ear plugs. PPE is usually used in combination with other biosafety controls (e.g., BSCs, centrifuge safety cups, and small animal caging systems) that contain hazardous biological agents, animals, or materials being handled. In situations where a BSC cannot be used, PPE may become the primary barrier between personnel and hazardous biological agents.

Secondary Barriers utilized in the design and construction of the laboratory facility provide a means of secondary containment of hazardous biological agents. The secondary barriers, together with other biosafety controls, help provide protection of personnel, the surrounding community, and the environment from possible exposure to hazardous biological agents.
When the risk of infection by aerosol or droplet exposure is present, higher levels of secondary containment and multiple primary barriers may be used in combination with other controls to minimize the risk of exposure to personnel and the unintentional release into the surrounding community or the environment.

Such design features may include, but are not limited to the following:

- Ventilation strategies to ensure containment of the hazards;
- Effluent decontamination systems; and
- Specialized building/suite/laboratory configurations, including:
  - Controlled access zones to support the separation of the laboratory from office and public spaces
  - Anterooms;
  - Airlocks.

Facility Practices and Procedures established at WCM are essential to support the implementation and sustainability of a successful biosafety program. Persons working in WCM facilities that handle and store hazardous biological agents must be able to properly identify all potential hazards and be trained and proficient in necessary, safe practices and procedures. Laboratory management and leadership are responsible for providing and arranging the appropriate training of all personnel based on their functional roles and responsibilities in support of the biosafety program. Strict adherence to documented laboratory best practices and procedures is an essential element of a robust biosafety program since failure to follow the established procedures could result in an accidental exposure to personnel or unintentional release of hazardous biological agents into the surrounding community or the environment.

Laboratory Biosecurity In recent years, with the passing of federal legislation regulating the possession, use, and transfer of biological Select Agents with high adverse public health and/or agricultural consequences (DHHS, USDA APHIS Select Agents), a much greater emphasis has been placed in the emerging field of biosecurity. Biosecurity and Select Agent issues are covered in detail in Appendix C of this plan. While biosafety focuses on the protection of personnel, the surrounding community, and the environment from the unintentional release of hazardous agents, the field of biosecurity is focused on the prevention of the theft, loss, and misuse of hazardous biological agents, equipment, and/or valuable information by an individual(s) for malicious use. Principles of Laboratory Biosecurity are described in Section VI of the BMBL (6th edition).

8.0 Laboratory Biosafety Level Criteria

Descriptions of the four Biosafety levels for activities involving infectious agents, recombinant microorganisms, and laboratory animals are summarized here. The levels are designated in ascending order, by the degree of protection provided to personnel, the environment, and the community. The standard and special practices, safety equipment, and facilities that apply to agents assigned to Biosafety Level 1-4 are described in the BMBL.

Table 8-0: Summary of Recommended Biosafety Levels for Biological Agents

Biosafety Level	Agent Types	Special Practices	Primary Barriers and PPE	Facilities (Secondary Barriers)
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	***Standard microbiological practices**	▪ No primary barriers required. ▪ PPE: laboratory coats and gloves; eye, face protection, as needed.	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	▪ Agents associated withhuman disease and hazards to personnel and the environment ▪ Limited access; occupational ▪ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	**Standard microbiological practices plus;** ▪ Limited access ▪ Occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; ▪ All procedures that may generate an aerosol or splash conducted in a BSC; ▪ Decontamination process needed for laboratory equipment	**Primary barriers:** ▪ BSCs or other physical containment devices used for all manipulationsof agents that cause splashes or aerosols ▪ PPE: Laboratory coats, gloves, face, and eye protection, ▪ Including respiratory protection, as needed	**BSL-1 plus:** ▪ Self-closing doors ▪ sink located near exit; windows; ▪ Autoclave available
3	Indigenous or exotic agentsthat may cause serious or potentially lethal disease through the inhalation route of exposure	**Standard microbiological and BSL-2 practice plus:** ▪ Controlled access ▪ viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; ▪ all procedures with infectious materials performed in a BSC ▪ Decontamination of all waste	**Primary Barriers:** ▪ BSCs or other physical containment device for all procedures with viable agents <u>PPE</u> ▪ solid front gowns, scrubs, or coveralls; ▪ two pairs of gloves, when appropriate; ▪ protective eyewear, ▪ respiratory protection, as needed	

4	▪ Dangerous/exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections thatare frequently fatal, for which there are no vaccines or treatments ▪ Related agents withunknown risk of transmission	**Standard microbiological, BSL-2 and BSL-3 practices plus:** ▪ Clothing change beforeentry; ▪ daily inspections of essential containment and life support systems; ▪ all wastes decontaminated prior to removal from laboratory; ▪ shower on exit	**Primary barriers:** ▪ BSCs for all procedures with viable agents; ▪ solid front gowns, scrubs, or coveralls in a Cabinet laboratory ▪ gloves; full-body, air-supplied, positive-pressure suit in a Suit laboratory	**BSL-3 plus:** ▪ Entry sequence; entry through airlock with airtight doors (suit lab) ▪ walls, floors, ceilings form sealed internal shell; ▪ dedicated, non-recirculating ventilation system required; ▪ double-door, pass-through autoclave required

**Standard Microbiological Practices: A basic laboratory code of practice applicable to all types of laboratory activities with biological agents, including general behaviors and aseptic techniques that should always be observed in the laboratory. This code serves to protect laboratory personnel and the community from infection, prevent contamination of the environment, and provide protection for the work materials in use.*

8.1 BIOSAFETY LEVEL 1 (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment. Adeno-associated virus, Escherichia coli K-12, Saccharomyces cerevisiae, and Baculovirus are representative of microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals.

BSL-1 work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or related science. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no particular primary barriers but includes laboratory doors, a sink for handwashing, and a laboratory bench.

Biosafety Level 1

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

A. Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety and access to the laboratory.
- The laboratory supervisor ensures that all laboratory personnel receives 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.
- The personnel receives 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, 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.

B. Special Practices

None required

C. 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.

D. 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.

8.2 BIOSAFETY LEVEL 2 (BSL-2)

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

1. Laboratory personnel have 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 Biosafety Cabinets (BSCs) or other physical containment equipment.

Biosafety Level 2 practices, equipment, and facility design and construction apply to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With proper microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, Salmonella, and Toxoplasma are representative of microorganisms assigned to this containment level.

BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the WCM Bloodborne Pathogen Exposure Control Plan for specifically required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures and ingestion of potentially infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or

high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Furthermore, the use of primary containment equipment is also recommended when high-risk infectious agents are suspected to be present in any human, animal, or plant-derived specimens. **Personal protective equipment (PPE) should be used as appropriate, such as splash shields, face protection, gowns, and gloves.** Secondary barriers, such as hand washing sinks and waste decontamination facilities (i.e., autoclave), must be available to reduce potential environmental contamination.

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

A. Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety and access to the laboratory.
- The laboratory supervisor ensures that laboratory personnel receives 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.
- The personnel receives 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, 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 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 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.

B. Special Practices

- Access to the laboratory is controlled when work is being conducted.
- The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrates proficiency in standard microbiological practices and techniques for working with agents requiring BSL-2 containment.
- Laboratory personnel are provided with 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 methods).

- 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.

C. 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.

D. Laboratory Facilities (Secondary Barriers)

- Laboratory doors are self-closing and have locks that work in accordance with 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. Refer to section 13.5 for additional details. 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. See Appendix A.
  - 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.

8.3 BIOSAFETY LEVEL 3 (BSL-3)

Biosafety Level 3 practices, safety equipment, and facility design and construction apply to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii are representative of the microorganisms assigned to this level.

The primary routes of exposure to personnel working with these types of biological agents relate to accidental exposure via the percutaneous or mucosal routes and inhalation of potentially infectious aerosols. At BSL-3, more emphasis is placed on primary and

secondary barriers to protect personnel, the surrounding community, and the environment from exposure to potentially infectious aerosols. All procedures involving the manipulation of infectious materials are conducted within a BSC or other primary containment device. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other primary containment strategies (e.g., centrifuge safety cups, sealed rotors or soft wall containment enclosures) are implemented based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.

Secondary barriers for BSL-3 laboratories include those previously mentioned for BSL-1 and BSL-2 laboratories. They also include enhanced ventilation strategies to ensure inward directional airflow, controlled access zones to limit access to only laboratory-approved personnel, and may contain anterooms, airlocks, exit showers, and/or exhaust HEPA filtration.

BSL-3 laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures.

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

A. Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety and access to the laboratory.
- The laboratory supervisor ensures that all 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.
- All personnel receives 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, 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 references and 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 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 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).
- 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.

B. 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.
- Laboratory personnel are provided medical surveillance, 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 demonstrates 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 methods).
- 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. The 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.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.
- Based on the work being performed, additional PPE may be required.
- Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or another type of 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.

D. 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.
- 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. See Appendix A, Figure 11. 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 recirculated 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.
- The laboratory exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA filtered.
- 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.
- The 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 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-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.
- A BSL-3 laboratory has special engineering and design features and a biosafety manual specific to the laboratory and organisms in use. The laboratory director prepares or adopts this manual and incorporates biosafety precautions into standard operating procedures.

The Director of EHS, the Biosafety Officer, and the Senior Associate Dean of Research and Office of Sponsored Research Administration (OSRA) must be consulted before initiating research at this level.

8.4 BIOSAFETY LEVEL 4 (BSL-4)

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL- 4 agents also should be handled at this level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4. The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent the release of viable agents into the environment. ***There are no BSL-4 research facilities at WCM.***

9.0 Vertebrate Animal Biosafety Level

If experimental animals are used, institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, and care.

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security, and care for the laboratory animal.

Laboratory animal facilities are a particular type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents in vivo and in vitro are comparable. However, the animal room can present unique problems, as the activities of the animals themselves can present hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite andscratch, and they may be infected with a zoonotic agent.

The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a WCM IACUC protocol-driven risk assessment, and the specific practices and containment are documented on the WCM Research Animal Resource Center(RARC) Protection and Control form. For additional information, please refer to the RARC User Guide.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. The publication bythe Institute for Laboratory Animal Research (ILAR), "Occupational Health and Safety in the Care and Use of Research Animals", is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, "Occupational Health and Safety in the Care and Use of Nonhuman Primates."

Personnel receive specific training in humane animal care and handling in accordance with the appropriate regulatory requirements and guidance documents (e.g., Animal Welfare Regulations, Guide for the Care and Use of Laboratory Animals, and taxon-specific publications for wild/exotic animals) as well as animal facility procedures, and are supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures. This includes training on proper use of engineering controls, including biosafety cabinets (BSCs) or downdraft tables, as well as personal protective equipment (PPE) appropriate to the ABSL as determined by a risk assessment. The biosafety officer (BSO), the IBC, or equivalent resource, and/or other applicable committees are responsible for the review of protocols and policies to protect personnel who manipulate and care for animals from hazardous exposures.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations, designated ABSL-1–4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimum standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents requiring BSL-1–4 containment, respectively. Investigators who are inexperienced should seek help in designing their experiments from individuals experienced in this specialized work.

Table 9.0 – Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals Are Used

Animal Biosafety Level	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in health adults	Standard animal care and management practices, including appropriate medical surveillance programs	▪ As required for standard care of each species ▪ PPE laboratory coats and gloves; eye, face protection, as needed	▪ Standard animal facility ▪ No recirculation of exhaust air ▪ Directional airflow recommended ▪ Hand washing sink is available
2	▪ Agents associated with human disease ▪ Hazard: percutaneous injury, ingestion, mucous membrane exposure	**ABSL-1 practice plus:** ▪ Limited access ▪ Biohazard warning signs ▪ "Sharps" precautions ▪ Biosafety manual ▪ Decontamination of all infectious wastes and animal cages prior to washing	**ABSL-1 equipment plus primary barriers:** ▪ Containment equipment appropriate for animal special ▪ PPE: Laboratory coats, gloves, gloves, face, eye, and respiratory protection, as needed	**ABSL-1 plus:** ▪ Autoclave available ▪ Hand washing sink available ▪ Mechanical cage washer recommended ▪ Negative airflow into animal and procedure rooms recommended
3	Indigenous or exotic agentsthat may cause serious or potentially lethal disease through the inhalation route of exposure	**ABSL-2 practice plus:** ▪ Controlled access ▪ Decontamination of clothing before laundering ▪ Cages decontaminated before bedding is removed ▪ Disinfectant foot bath as needed	**ABSL-2 equipment plus:** ▪ Containment equipment for housing animals and cage dumping activities ▪ Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols ▪ PPE: Appropriate respiratory protection	**ABSL-2 facility plus:** ▪ Physical separation from access corridors ▪ Self-closing double-door access ▪ Sealed penetrations ▪ Sealed windows ▪ Autoclave available in the facility ▪ Entry through anteroom or airlock ▪ Negative airflow into animal and procedure rooms ▪ Hand washing sink near the exit of animal or procedure room
4	Dangerous/exotic agents which pose a 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 data are available to re-designate the level.	Related agents with unknown risk of transmission. ABSL-3 Practice plus: • Entrance through change room where personal clothing is removed, and laboratory clothing is put on; shower on exiting.	All wastes are decontaminated before removal from the facility SBSL-3 equipment plus.

In addition to the animal biosafety levels described in this section, the USDA has developed facility parameters and work practices for handling agents of agriculture significance. Appendix D in the BMBL includes a discussion on Animal Biosafety Level 3 Agriculture (BSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Appendix D in the BMBL also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with specific veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not addressed explicitly in this section; however, the Arthropod Containment Guidelines are available online via the American Society of Tropical Medicine and Hygiene (ASTMH).

9.1 ANIMAL BIOSAFETY LEVEL 1 (ABSL1)

Animal Biosafety Level 1 is suitable for work in animals 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.

Standard practices, safety equipment, and facility requirements apply to ABSL-1. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required. Personnel must have specific training in animal facility procedures and must be 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.

A. 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. 8–12
  - 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 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.13 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. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
- 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.

B. Special Practices

None required.

C. 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, 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.

D. 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
- Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
- 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, opening and 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.

- Furniture can support anticipated loads and uses.
- 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.
- 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.

9.2 ANIMAL BIOSAFETY LEVEL 2 (ABSL2)

Animal Biosafety Level 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 pose moderate hazards to personnel and the environment. I**t also addresses hazards from ingestion, as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that:

1. Access to the animal facility is restricted;
2. Personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents;
3. Personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and
4. BSCs or other physical containment equipment is used when processes involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. An employee occupational health program is in place for researchers conducting work with animals.

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

A. 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.8–12
- 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.13 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.

B. 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.

C. 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.
- Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.

D. 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, 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 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. See Appendix A.
- 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.

9.3 ANIMAL BIOSAFETY LEVEL 3 (ABSL3)

Animal Biosafety Level 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 severe or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirementsof ABSL-2. The ABSL-3 laboratory 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.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment and an employee occupational health program is implemented.

Animal Biosafety Level 3 requires a specially designed facility, with unique engineering and design features, a biosafety manual specific to the laboratory and organisms in use, which includes written standard operating procedures, and specialized training in handling these agents. Prior consultation with the Director of the Research Animal Resource Center (RARC), the Director of EHS, the Biosafety Officer and the Senior Associate Dean of Research and Office of Sponsored – Research Administration (OSRA) must be made before initiating research at this level.

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

A. 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 consul-tation 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.8–12
    - 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.13 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.
- 20. 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.

B. 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 surveil-lance 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. See Appendix K. 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.

C. 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 micro-organisms 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.

D. 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.

9.4 ANIMAL BIOSAFETY LEVEL 4 (ABSL4)

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a highindividual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.

There are no ABSL-4 research facilities at Weill Cornell Medicine.

10.0 Working with Human, NHP and Mammalian Cells and Tissue Culture

Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency, the inadvertent transmission of disease to organ recipients, and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) withininfected individuals in the U.S. population. Potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HIV, HCV, HTLV, EBV, HPV and CMV. Cells immortalized with viral agents such as SV-40, EBV adenovirus or HPV, as well as cells carrying viral genomic material also present potential hazards to laboratory workers. Tumorigenic human cells also are potential hazards due to self-inoculation. There has been one reported case of development of a tumor from an accidental needle-stick. **Laboratory workers should never handle autologous cells or tissues.**

Work with blood and OPIM involves risk of exposure not only to these agents, but also other opportunistic pathogens transmitted primarily by other routes (e.g., contact, droplet, and airborne) that may be present in blood or the sample material at the time it is being handled. For example, Mycobacterium tuberculosis may be transmitted via the airborne route and primarily present in human lung tissues, while bacterial species such as Staphylococci may be contact transmitted but present in localized tissues or blood during acute infections. Prions, responsible for spongiform encephalopathies and other diseases, may be more concentrated in neural tissues rather than blood, whereas viral hemorrhagic fever-causing viruses can be considered bloodborne pathogens but are often present in other body fluids, such as urine.

Numerous pathogens can be present in human materials and each agent may have a number of different characteristics to consider pertaining to the process of infection. For this reason, a risk assessment must be performed that takes into account material source, type, characteristics, and the procedures being performed with the material.

In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple laboratory-acquired infections. The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to bloodborne pathogens final rule would include primary human cell lines and explants.

Other primate cells and tissues also present risks to laboratory workers. Non-human primate (NHP) cells, blood, lymphoidand neural tissues should always be considered potentially hazardous. Old World non-human primate (NHP) specimens (i.e., macaques) may contain Macacine herpesvirus (Herpes B) and Simian Immunodeficiency Virus (SIV). This material should always be considered potentially infected and should be handled with strict barrier precautions and with swift occupational responses for potential exposures. Herpes B virus infection in macaques is usually symptom-free, or causes only mild oral lesions, but in humans, the infection can be fatal. Personnel working in the laboratory with potentially infected cells or tissues from macaques must always follow additional precautions (**access to NHP exposure kit, NHP training**). Exposure of mucous membranes or through skin breaks provides herpes B virus access to a new host, whether the virus is being shed from a macaque or present in contaminated cells or tissues.

At a minimum, human and other primate cells should be treated as potentially infectious and handled using BSL-2 practices, engineering controls, and facilities.The use of a biological safety cabinet (BSC) for culturing activities is the universally accepted best practice. Higher containment must be considered for cell lines harboring Risk Group 3 and 4 pathogens as indicated by the risk assessment; higher containment must be considered if the agents present become airborne when energy is imparted on the biological sample. Personal protective equipment (PPE) such as laboratory coats, gloves, and eye protection should be worn in tissue culture laboratories and additional PPE should be added as indicated by risk assessment. All waste culture material must be decontaminated before disposal.

All laboratory staff working with human cells and tissues must be enrolled in an occupational medicine program specificfor bloodborne pathogens and should operate under the policies and guidelines established in the WCM Bloodborne Pathogen Exposure Control Plan. Laboratory staff working with human cells and tissues, NHP blood/tissues, other body fluids, and other tissues should be offered hepatitis B immunization and must be evaluated by a health care professional following an exposure incident.

11.0 Recombinant DNA Research

11.1 RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

The purpose of the NIH Guidelines is to specify the practices for constructing and safe handling of research involvingrecombinant and synthetic nucleic acid molecules, by determining appropriate biosafety practices and procedures forresearch involving the construction and handling of recombinant or synthetic nucleic acid molecules, as well as cells, organisms, and viruses that contain such molecules. Recombinant or synthetic nucleic acid molecules are defined in the *NIH Guidelines* as:

1. Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell (i.e., recombinant nucleic acids);
2. Nucleic acid molecules that are chemically, or by other means, synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e., synthetic nucleic acids); or
3. Molecules that result from the replication of those described in (1) or (2).

As an NIH-funded institution, Weill Cornell Medicine adopts all practices recommended under the Guidelines for ResearchInvolving Recombinant or Synthetic Nucleic Acid Molecules, as published by the National Institutes of Health.
Compliance with the NIH Guidelines is mandatory for all investigators conducting recombinant or synthetic nucleic acid molecule research funded by the NIH, or performed at or sponsored by any public or private entity that receives any NIH funding. This broad reach of the NIH Guidelines aims to instill biosafety practices throughout theinstitution.

The Weill Cornell Medicine Institutional Biosafety Committee (IBC) is a faculty-led committee of experts in biosafety- related fields. The IBC, established under the NIH Guidelines, is responsible for providing review and oversight to ensurethat all forms of research are conducted at Weill Cornell Medicine (WCM) in compliance with the applicable Federal, State, and local health and safety standards and Institutional policies.

The IBC oversight of research includes projects involving:

- Recombinant or synthetic nucleic acid molecules,
- Biological agents classified as Risk Group 2, 3, and 4 in the NIH Guidelines,
- Research involving select agents as listed by the USDA/CDC,
- Human gene transfer research, and
- Clinical trials involving the use of biohazardous agents in human subjects.

The guidelines have specific sections addressing risk assessment concerning the hazards associated with recombinant and synthetic nucleic acid molecules and manipulations of microorganisms. Appendix B of the guidelines: *"CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD"*

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document.

Experiments involving recombinant and synthetic nucleic acid molecules lend themselves to a third containmentmechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either:

(i) The infectivity of a vector or vehicle (plasmid or virus) for particular hosts, or
(ii) Its dissemination and survival in the environment.

Vectors, which provide the means for recombinant and synthetic nucleic acid molecules and/or host cell replication, canbe genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant andsynthetic nucleic acid molecules outside the laboratory.

Since these three means of containment are complementary, different levels of containment can be established that applyvarious combinations of the physical and biological barriers along with constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the NIH Guidelines.

For research involving plants, there are four biosafety levels (BL1-P through BL4-P) described in the Guidelines for Research Appendix L, Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants. **If your research involves plants, plant pathogens, or related recombinant DNA, EHS m be contacted prior to conducting experiments.**

For research involving animals which are of a size or have growth requirements that preclude the use of conventionalprimary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in the Guidelines for Research Appendix M.

Copies of the NIH Guidelines are available online in the NIH Website. For additional information, please contact the Officeof Sponsored Research Administration (OSRA) or Environmental Health and Safety.

12.0 Principles of Cleaning, Disinfectants, and Sterilization

12.1 CLEANING

Cleaning is the removal of gross contamination from a surface to the extent necessary for further processing for intended use. In these cases, cleaning can be used to remove microorganisms and other associated contaminants (e.g., blood, tissues, culture media) from a surface by physical means but may not provide any antimicrobial activity. Cleaning is often an essential pre-requisite to disinfection or sterilization processes to ensure the optimal activity of the antimicrobial effects of disinfectants or sterilization processes. Biofilms may be present in the laboratory (e.g., sinks, plumbing fixtures, fluid-filled lines of laboratory equipment, water containing reservoirs, incubator humidification systems) and are often difficult to treat/disinfect. Most biofilms require physical cleaning (e.g., scrubbing) and the use of compatible oxidative disinfectants (e.g., chlorine dioxide, peroxyacetic acid, ozone). In some situations, replacing tubing and distribution lines may be necessary.

Disinfection is generally a less-lethal process than sterilization; it eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g., bacterial spores) present on inanimate objects. Disinfection does not ensure a kill level and lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled by several factors, each one of which may have a pronounced effect on the end results. Factors affecting disinfection include the following:

1. Nature and number of contaminating microorganisms (especially the presence of bacterial spores);
2. Amount of organic matter present (e.g., soil, feces, blood);
3. Type and condition of surfaces, instruments, devices, and materials to be disinfected;
4. Temperature; and
5. Contact (exposure) time.

By definition, chemical disinfection, especially high-level disinfection, differs from chemical sterilization by the lack of sporicidal power. Some disinfectants rapidly kill only the ordinary vegetative forms of bacteria, such as staphylococci and streptococci, some forms of fungi, and lipid-containing viruses; others are effective against such relatively resistant organisms as Mycobacterium bovis or Mycobacterium terrae, non-enveloped viruses, and most forms of fungi.
In general, most laboratories use a disinfectant that has a broad range of activity; thus, most labs should select a product with a tuberculocidal/mycobactericidal claim for routine purposes. Many of these products will also have claims that meet the OSHA Bloodborne Pathogens Standard.

Sterilization: Any item, device, or solution is sterile when it is completely free of all forms of living microorganisms, including spores and viruses. This definition is categorical and absolute; an item is either sterile or it is not. Sterilization can be accomplished by dry or moist heat, gases, and vapors (e.g., chlorine dioxide, ethylene oxide, formaldehyde, hydrogen peroxide, methyl bromide, nitrogen dioxide, ozone, propylene oxide), plasma sterilization technology, and radiation (e.g., gamma, e-beam in industry).

The sterilization procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million. Laboratories use sterilization techniques for producing media, sterilizing glassware, and other items, and for decontaminating waste.

Decontamination renders an area, device, item, or material safe to handle in the context of being reasonably free from a risk of disease transmission. The primary objective of decontamination is to reduce the level of microbial contamination so that transmission of infection is prevented. The decontamination process may involve the cleaning of an instrument, device, or area with ordinary soap and water. In laboratory settings, decontamination of items, used laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, which may be the most cost-effective way to decontaminate a device or an item.

When steam sterilization is used to decontaminate laboratory waste that contains items that have a high bio-burden and there is no pre-cleaning (i.e., infectious waste), the cycle times are generally longer and should be verified and validated for the typical load. Validation involves the combined use of thermocouples and biological indicators (BIs) placed throughout the load to ensure penetration of steam into the waste. Verification can be accomplished by routine monitoring of the steam sterilization cycles (i.e., cycle times, pressure, temperature) and by placing BIs within the load. In addition to time, temperature may also be increased to ensure inactivation of pathogens. Decontamination in laboratory settings often requires longer exposure times because pathogenic microorganisms may be protected from contact with steam.

Chemical disinfectants used for decontamination range in activity from high-level disinfectants (e.g., high concentrations of sodium hypochlorite [chlorine bleach]), which might be used to decontaminate spills of cultured or concentrated infectious agents in research or clinical laboratories, to low-level disinfectants or sanitizers for general housekeeping purposes or spot decontamination of environmental surfaces in healthcare settings. Resistance of selected organisms to decontamination is presented in descending order in Figure 1. If dangerous and highly infectious agents are present in a laboratory, the methods for decontamination of spills, laboratory equipment, biological safety cabinet, or infectious waste are very significant and may include prolonged autoclave cycles, incineration, or gaseous treatment of surfaces.

Figure 1. Descending Order of Relative Resistance to Disinfectant Chemicals

Prions

↓

Bacterial Spores *Bacillus subtilis*, *Clostridium sporogenes*, *Clostridium difficile*

↓

Mycobacteria *Mycobacterium bovis*, *M. terrae*, and other Nontuberculous mycobacteria

↓

Non-enveloped or Small Viruses Poliovirus, Coxsackievirus, Rhinovirus

↓

Fungi Trichophyton spp., Cryptococcus spp., Candida spp.

↓

Vegetative Bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella choleraesuis*, Enterococci

↓

Enveloped or Medium-size Viruses Herpes simplex virus, CMV, Respiratory syncytial virus, HBV, HCV, HIV, Hantavirus, Ebola virus

Space decontamination is a specialized activity and should be performed by individuals with proper expertise, training, and personal protective equipment. Procedures for decontamination of large spaces such as incubators or rooms are varied and influenced significantly by the type of etiologic agent involved, the characteristics of the structure containing the space, and the materials present in the space. The primary methods for space decontamination follow. Fumigants that are currently used are either gases, vapors, mists, or fogs (dry mists). Fumigants that are gases obey gas laws, can evenly distribute throughout the room, and are easily scalable by increasing the volume of gases used. Fumigants applied as mists or fogs do not behave like gases and are particles (<1–12 μ in size) that settle onto surfaces being treated.

Decontamination of Surfaces: Liquid chemical disinfectants may be used for decontamination of large surface areas. For the most part, intermediate and low-level disinfectants can be safely used and, as with all EPA-registered disinfectants, the manufacturer's instructions should be followed. Disinfectants that have been used for decontamination include: sodium hypochlorite solutions at concentrations of 500 to 6000 parts per million (ppm); oxidative disinfectants, such as hydrogen peroxide and peracetic acid; phenols; and iodophors. Procedures for the use of chemical disinfectants should include safety precautions, the use of appropriate personal protective equipment, hazard communication, and training on spill response.

Table 1. Activity Levels of Selected Liquid Chemical Disinfectants

Chemical[a]	Concentration	Activity level
Glutaraldehyde	Variable	Sterilization
Glutaraldehyde	Variable	Intermediate to high-level disinfection
Ortho-phthalaldehyde (OPA)	0.55%	High-level disinfection
Hydrogen peroxide	6–30%	Sterilization
Hydrogen peroxide	3–6%	Intermediate to high-level disinfection

Formaldehyde[b]	6–8%	Sterilization
Formaldehyde	1–8%	Low- to high-level disinfection
Chlorine dioxide	Variable	Sterilization
Chlorine dioxide	Variable	High-level disinfection
Peracetic Acid	0.08%–0.23% with peroxide concentrations of 1–7.35%	Sterilization
Peracetic acid	Variable	High-level disinfection
Hypochlorites[c]	500–6000 mg/L Free available	Intermediate to high-level disinfection
Alcohols (ethyl, Isopropyl)[d]	70%	Intermediate-level disinfection
Phenolics	0.5–3%	Low- to intermediate-level disinfection
Iodophors[e]	30–50 mg/L Free	Low- to intermediate-level disinfection
Quat. Ammonium Compounds	Variable	Low-level disinfection

a. This list of chemical disinfectants centers on generic formulations. A large number of commercial products based on these generic components can be considered for use. Users should ensure that commercial formulations are registered with EPA or by the FDA. Users can search for EPA-registered products at https://www.epa.gov/ pesticide-labels.

b. Because formaldehyde is classified as a known human carcinogen and has a low permissible exposure limit (PEL), the use of formaldehyde is limited to certain specific circumstances under carefully controlled conditions (e.g., for the disinfection of certain hemodialysis equipment). There are no FDA-cleared liquid chemical sterilant/ disinfectants that contain formaldehyde.

c. Generic disinfectants containing chlorine are available in liquid or solid form (e.g., sodium or calcium hypochlorite). The indicated concentrations are rapid-acting and broad-spectrum (i.e., tuberculocidal, bactericidal, fungicidal, and virucidal). Note: Common household bleach is an excellent and inexpensive source of sodium hypochlorite. Concentrations between 500 and 1000 ppm chlorine are appropriate for the vast majority of uses requiring an intermediate-level of germicidal activity; higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where there may be spores or there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory). In situations where there is an excessive amount of organic material present, the surfaces should be thoroughly cleaned to remove as much organic material as possible before applying sodium hypochlorite solution to disinfect the surface (see product label instructions). The concentration of the sodium hypochlorite should be determined in advance of use and the solution should be made fresh each day.

d. The effectiveness of alcohols as intermediate-level germicides is limited because they evaporate rapidly, resulting in short contact times, and because they lack the ability to penetrate residual organic material. They are rapidly tuberculocidal, bactericidal, and fungicidal, but may vary in spectrum of virucidal activity. Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time.

e. Only those iodophors registered with EPA as hard-surface disinfectants should be used, closely following the manufacturer's instructions regarding proper dilution and product stability. Antiseptic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments.

When using chemical agents for decontamination, pay attention to instructions for their use and Safety Data Sheets (SDS); ensure they are used safely, and that appropriate precautions and protections are used. Exposures to disinfectants have resulted in occupational injuries such as cancer, hypersensitivities, dermatitis, and asthma.

The laboratory is responsible for selecting an appropriate EPA-registered product and using it according to the manufacturer's instructions on the product label. The more commonly used public health antimicrobial products are described in the Glossary (e.g., sporicides, disinfectants, and sanitizers). The lists of selected EPA-registered disinfectants are available at https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants

12.1.2. Autoclave

Autoclaving at temperature 121^{O}C (steam under pressure), at 20 psi, is one of the most convenient and effective means ofsterilization available. Care must be taken to ensure that the steam can circulate articles in order to provide even exposure to heat. The success of sterilization is very time-dependent in liquid media, with large volumes requiring longer times to reach the effective temperature within the media itself.

Additionally, there should be no empty spaces in the load that could insulate against the steam. This condition couldprevent the steam from reaching the container and transferring the heat to the vessel. In dry loads, small amounts ofwater should be included inside the autoclave bag to ensure moisture content within the open bag.

A Diack or commercial Bacillus stearothermophilus or Bacillus subtilis var. niger test strips to check for autoclave efficiency should be used on each terminal sterilization of pathogenic cultures. Autoclave tape (non-lead containing) canbe used for routine runs involving sterile media and glassware.

12.2 USEFUL DILUTIONS OF WESCODYNE AND COMMON HOUSEHOLD BLEACH

12.2.1 Standard Wescodyne Solution

3 OZ=90cc (1.2 cc/5 gallons = 1ppm)

90cc	=	36cc	=	18cc	=	2.37cc	= 75 ppm available iodine 500ml
		5gal		2gal		1gal	
180cc	=	72cc	=	36cc	=	4.8cc	= 150 ppm available iodine 500ml
		5gal		2gal		1gal	
1800cc	=	720cc	=	360cc	=	48.0cc	= 500 ppm available iodine 500ml
		5gal		2gal		1gal	

12.2.2 Bleach Solutions

1/100 dilution of 5.25% Bleach ~ 525ppm

1/10 dilution of 5.25% Bleach ~ 5,250ppm

1.0 (straight) of 5.25% Bleach = 52,500ppm available chlorine1/8 = 6562.5ppm ~ Dakin solution

(500 ppm recommended for most uses)

12.2.3 Phenolics

Follow the directions of the manufacturer for proper dilution of the concentrate. Any deviation from the quantities recommended will result in less than satisfactory results. The preparation as formulated at a given concentration wastested using A.O.A.C. protocols, and proven effective against the organisms listed on the table.

13.0 Biological Safety Cabinet (BSC)

Biological safety cabinets (BSC) are among the most effective, as well as the most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Class I, II, III) have performance characteristics and applications which are described below. The type of cabinet used will depend on application, type of agents used in the lab, and whether product sterility, personal protection, or both are critical considerations in the research environment. BSCs are designed to provide personnel and environmental protection when appropriate practices and procedures are followed.

In the early 1960s, the laminar flow principle evolved. Unidirectional air moving at a fixed velocity along parallel lines was demonstrated to reduce turbulence resulting in predictable particle behavior. Biocontainment technology also incorporated this laminar or uniform, directional flow principle with the use of the HEPA filter to aid in the capture and removal of airborne contaminants from the air stream. This combination of technologies that exists in the Class II BSC serves to help protect the laboratory worker from potentially infectious aerosols4 generated within the cabinet and also provides necessary product protection. Class II BSCs are partial barrier systems that rely on the directional movement of air to provide containment. Properly maintained Class I and II BSC s, when used in conjunction with **proper microbiological techniques,** provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents).

As with any other piece of laboratory equipment, **personnel must be trained in the proper use of the biological safetycabinet.** Of particular note are those activities which may disrupt the inward directional airflow through the work opening of Class I and II BSCs. Repeated insertion of the worker's arms in and from the work chamber, or briskly walking past theBSC while it is in use are demonstrated causes of the escape of aerosolized particles from within the cabinet. Class I andII BSCs should be located away from traffic patterns and doors. Fans, heating, and air conditioning registers, and other air handling devices can also disrupt airflow patterns if located adjacent to the BSC. Strict adherence to recommended practices for the use of BSCs and proper placement in the laboratory is essential in attaining the maximum containment capability of the equipment, as is the mechanical performance of the equipment itself.

It is imperative that BSCs are tested and certified in situ at the time of installation within the laboratory, any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supersede the critical certification prior to use in thelaboratory. A list of BSC certifiers is available on the EHS website.

13.1 CLASS I BSC

The Class I BSC provides **personnel and environmental protection, but no product protection**. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class IBSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are explicitly used to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g., cage dumping, culture aeration or tissue homogenization).

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system, and the building exhaustfan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the cabinet exhaust plenum. A second HEPA filter may be installed in the terminal end of the building exhaustprior to the exhaust fan.

Some Class I models used for animal cage changing are designed to allow recirculation of air into the room after HEPAfiltration and may require more frequent filter replacement due to filter loading and odor from organic materials captured on the filter. The re-circulating Class I BSC should be annually certified for sufficient airflow and filter integrity.

13.2 CLASS II BSC

As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation ofviruses, cabinets were needed that also provided product protection.

The Class II (Types A1, A2, B1, and B2) BSCs provide **personnel, environmental and product protection**. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection. Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a hard connection.

HEPA filters are effective at trapping particulates and thus infectious agents, but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1and B2 BSCs exhausting to the outside should be used when working withvolatile, toxic chemicals, but amounts must be limited.

Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and may be used for theformulation of nonvolatile antineoplastic or chemotherapeutic drugs.

13.2.1 The Class II, Type A1 BSC

An internal blower draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 lfpm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Laminar airflow reduces turbulence in the work zone and minimizesthe potential for cross-contamination.

The downward moving air “splits” as it approaches the work surface; the blower draws part of the air to the front grille andthe remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.

The air is discharged through the front and rear grilles under negative air pressure into a blower and pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the airpasses through the exhaust HEPA filter, and 70% recirculates through the supply HEPA filter back into the work zone.

13.2.2 The Class II, Type A2 BSC

The Type A2 cabinet has a minimum calculated or measured inflow velocity of 100 lfpm. All positive pressure biologically contaminated plenums within the cabinet are surrounded by a negative air pressure plenum, thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment. Small quantitiesof volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy with exhaust alarm.

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, and thereby disturbing the internal cabinet airflow.

The proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood, which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing. The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The canopy must be removable or be designed to allow for operational testing of the cabinet. Class II Type A1 orA2 cabinets should never be hard-ducted to the building exhaust system.

13.2.3 The Class II, Type B1 BSC

Some biomedical research requires the use of small quantities of hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood, or a static-air glove boxequipped with a double-door airlock. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 2 (later called Type B) BSC, and was designed for manipulations of minute quantities of these hazardous chemicals with in vitro biological systems. The NSF International NSF/ANSI Standard 49 - 2002 definition of Type B1 cabinets includes this classic NCI design Type B, as well as cabinets without supply HEPA filters located immediately below the work surface, and/or thosewith exhaust/recirculation down flow splits other than exactly 70/30%.

The cabinet supply blowers draw room air (plus a portion of the cabinet’s recirculated air) through the front grille and thesupply HEPA filters located immediately below the work surface. This particulate-free air flows upward through a plenumat each side of the cabinet and then down to the work area through a back-pressure plate. In some cabinets there is an additional supply HEPA filter to remove particulates that may be generated by the blower-motor system.

The room air is drawn through the face opening of the cabinet at a minimum measured inflow velocity of 100 lfpm. As withType A1 and A2 cabinets, there is a split in the down-flowing air stream just above the work surface. In the Type B1 cabinet, approximately 70 percent of the downflow air exits through the rear grille, passes through the exhaust HEPA filter,and is discharged from the building. The remaining 30 percent of the downflow air is drawn through the front grille. Since the air flowing to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet work area.

Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system, or a properly designed laboratory building exhaust. Fans for laboratory exhaust systems should be located at the terminal end of the ductwork. Afailure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate.

A pressure-independent monitor and alarm should be installed to provide warning and shut off the BSC supply fan, shoulda failure in exhaust airflow occur. Since not all cabinet manufacturers supply this feature, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B1 BSCs should connect the

exhaust blower to the emergency power supply.

13.2.4 The Class II, Type B2 BSC

This BSC is a total-exhaust cabinet; no air is recirculated within it. It provides simultaneous primary biologicaland chemical containment.

Consideration must be given to the chemicals used in BSCs, as some chemicals can destroy the filter medium, housingsand/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills, capturing the supply air, plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted andpasses through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the workbeing performed) prior to discharge to the outside.

The Class II, Type B2 cabinet exhausts as much as 1200 cubic feet per minute of conditioned room air, making this cabinet expensive to operate. The higher static air pressure required to operate this cabinet also results in additional costs associated with heavier gauge ductwork and higher capacity exhaust fan. The need for using this type of BSC should,therefore, be justified by the research to be conducted.

Should the building exhaust system fail, the cabinet will be pressurized, resulting in a flow of air from the work area backinto the laboratory. Cabinets built since the early 1980's usually have an interlock system, installed by the manufacturer,to prevent the supply blower from operating whenever the exhaust flow is insufficient; but systems can be retrofitted if necessary. A pressure-independent device, such as a flow monitor, should monitor exhaust air movement.

13.2.5 Special Applications

Class II BSCs can be modified to accommodate particular tasks. For example, the front sash can be modified by the manufacturer to accommodate the eyepieces of a microscope, or the work surface can be designed to accept a carboy, acentrifuge, or other equipment that may require containment. A rigid plate with openings for the arms can be added if needed.

Good cabinet design, microbiological aerosol tracer testing of the modification, and appropriate certification are required to ensure that the basic systems operate properly after modification.

13.3 CLASS III BSC

The Class III BSC was designed for work with highly infectious microbiological agents and the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, thatis accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminatedbetween uses. Reversing that process allows materials to be removed from the Class III BSC safely.

Both supply and exhaust air are HEPA-filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or aHEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by an exhaust system exterior tothe cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.) "The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility ventilation system."

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation ofthe materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials, so the trade-off is clearly on the side of maximizing personal safety. Depending on the design of thecabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment. Class III BSCs do not have laminar airflow.

13.4 HORIZONTAL / VERTICAL LAMINAR FLOW "CLEAN BENCH"

Horizontal laminar flow "clean benches" are not BSCs. These devices discharge HEPA-filtered air from the back of thecabinet across the work surface and toward the user. They only provide product protection and can be used for certainclean activities, such as the dust-free assembly of sterile equipment or electronic devices.

Clean benches should never be used when handling cell culture material, drug formulations, or when manipulating potentially infectious materials. The worker will be exposed to the materials being manipulated on theclean bench potentially resulting in hypersensitivity, toxicity, or infection; depending on the materials being handled.

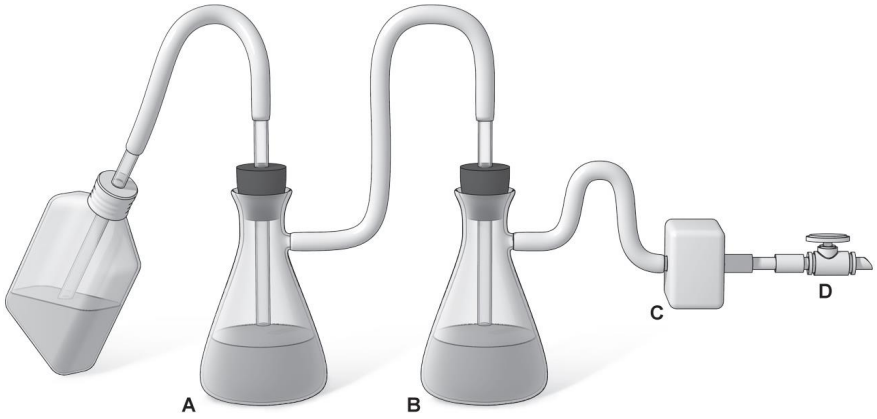
Horizontal/Vertical laminar flow "clean benches" must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these devices.

13.5 RECOMMENDATIONS FOR EFFECTIVE USE OF BSC'S

- The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated. Relocation may break the HEPA filter seals or otherwise damage the filters or the cabinet.
- Each BSC must be tested and certified at least annually to ensure continued, proper operation.
- It is strongly recommended that NSF accredited field certifiers are used to test and certify BSCs.
- **Adequate space use of the cabinet should be planned** to prevent over-crowding or restriction of movement in thecabinet.
- All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front and back grille of the cabinet.
- Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split pattern.
- Bulky items such as biohazard bags, discard pipette trays, and vacuum collection flasks should be placed to one side of the interior of the cabinet.
- **The biological safety cabinet should be allowed to operate five minutes before manipulations are initiated.** This step allows removal of any possible contaminated air that may have entered while breaching the air-barrier. Insertion ofhands and equipment causes turbulence at the point of entry, mixing clean air with dirty air.
- **Interior surfaces must be wiped down with 70% alcohol at the beginning and at the end of the day.**
- **All equipment to be used should be brought inside the cabinet before starting up the cabinet's internal airbarrier.**
- **Good microbiological techniques should always be used when working in a BSC. For example, techniques used to reduce splatter and aerosol generation will also minimize the potential for personnel exposure to infectious materials manipulated within the cabinet.**
- **Do not place anything over the front grill, especially if sterility is necessary.** A substantial portion of the air is contaminated (make-up air for the exhaust), therefore this practice defeats one of the key features of the cabinet.
- **Learn to work deep in the interior of the cabinet, at least four inches from the intake grill.** This prevents contamination of the work area and eliminates spillage of liquids into interior surfaces of the cabinet through the grills.
- **Personnel movement near the cabinet front should be kept to a minimum.** Ideally, a separate room with a door will reduce the chances of disturbing air-barrier flow. Movement at the hood face should be minimal, with all movementsmade slowly so that the airflow at the face of the BSC is not disturbed.
- **The use of centrifuges and shakers must be performed with care** since these activities disturb airflow in thecabinet and can breach the air-barrier.
- **Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant and to an in-line HEPA or equivalent filter (see figure below)**. This combination will protect the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution (bleach, 1/10 of volume) into the flask to inactivate the microorganisms as they are collected.
- **Biosafety cabinet users should refrain from using natural gas and other flammable substances within a recirculating BSC.** Open flames are not required in the near microbe-free environment of a biological safety cabinet.
- **Ultraviolet Lamps Ultraviolet (UV) lamps should not be used as the sole disinfection method in a BSC. If installed;**
  - **UV lamps should be cleaned regularly to remove any film that may block the output of the lamp.**
  - **The lamps should be evaluated regularly and checked with a UV meter to ensure that the appropriate intensity of UV light is being emitted.**
  - **Replace the bulb when the fluence rate is below 40 uW/cm2.**
  - **Unshielded UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure.**
  - **If the cabinet has a sliding sash, close the sash when operating the UV lamp. Most new BSCs use sliding sashes that are interlocked when operating the UV lamp to prevent exposure**.
- **When finished working within the BSC, it should be surface decontaminated.** With the cabinet blower running, all containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the

cabinet's sides and back and the interior of the glass.

- **The biological safety cabinet is not a substitute for good microbiological technique.**
- **Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant and to an in-line HEPA or equivalent filter. Commercial equivalents are acceptable once validated for specific laboratory use. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing a volume of a chemical decontamination solution having a concentration of chemical sufficient to decontaminate microorganisms when the flask is filled to its maximum capacity into the flask to inactivate the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste. The flask material should be resistant to the decontamination solution used.**



Protection of the house vacuum system during aspiration; The left suction flask (A) is used to collect the contaminatedfluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-lineHEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

14.0 Biological Spill Response

The procedures for containing, mitigating, reporting, and treatment following accidental spills or personnel contaminationwith biological materials are detailed in the Biological Spill Planning and Response Manual.

A Biological Spill Response Flowchart for staff , posted within the lab, will assist staff in assessing a spill and then taking the appropriate steps in case of an accidental spills within a biological safety cabinet, centrifuge, incubator or in the laboratory. Biological spills that do not involve injury, are contained, or pose little hazard to personnel, can be cleaned up so long asthe person cleaning the spill:

- Spill kits, including disinfectant, are easily accessible.
- Has received the proper training on how to clean up the spill,
- Is wearing the proper protective equipment to do the cleanup, and
- Performs the cleanup by following the procedure described within the Biological Spill Planning and Response Manual.

For other types of spills, i.e., larger spills or spills of a biological agent that may cause injury or illness, personnel should hold their breath and leave the lab immediately and notify Environmental Health and Safety (ext. 2-7233). In reporting of a biological spill or incident, principal investigators and laboratory personnel should follow the notification procedures posted within the lab on the Exposure and Spill Response Guide.

15.0 Biological Waste Disposal

Biological/regulated medical waste is generated in diagnosis, treatment and immunization of humans or animals, in research pertaining thereto, or in production and testing of biologicals. Biological waste may include animal waste, cultures and stocks, human blood, blood products, tissues, cell lines and body fluids, human pathological waste, recombinant DNA, infectious agents, isolation waste and sharps. A summary of biological waste disposal procedures is available on the EHS website.

The proper management of biological waste must adhere to applicable local, state, and federal regulations. Prior to disposal of your biological waste, please refer to Section 7 of the EHS Waste Disposal Procedure Manual; which detailsthe appropriate procedures for all biological waste streams encountered in the research laboratory.

16.0 Resources and References

Biological Agent(s) resources:

- Health Canada Pathogen Safety Data Sheets
- Biosafety in Microbiological Biomedical Laboratories (BMBL) 6th Edition:
  - Section VIII-A: Bacterial Agents
  - Section VIII-B: Fungal Agents
  - Section VIII-C: Parasitic Agents
  - Section VIII-D: Rickettsial Agents
  - Section VIII-E: Viral Agents
  - Section VIII-F: Viral Agents Arboviruses and Related Zoonotic Viruses
  - Section VIII-G: Toxin Agents
  - Section VIII-H: Prion Agents
- EHS Print-And-Go Sheets
  - Levtivirus
  - Poxivirus
  - Bloodborne Pathogen
- Disinfection, Sterilization, and Preservation, 5th Edition, Block, S.S.; (2001) Lippincott Williams & Wilkins,Philadelphia.
- Class II (laminar flow) biosafety cabinetry, NSF/ANSI 49 – (2002), NSF International Standard/AmericanNational Standard.
- Manual of Clinical Microbiology; 6th Edition, Murray, P.R., et al.; (1995) American Society for Microbiology,Washington DC.

- Biological Safety Principals and Practices, 3rd edition, (2000), Fleming, D.O., Hunt, D.L., American Societyfor Microbiology, Washington DC.
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines),April 2019, Amendment Effective April 25, 2019, Federal Register, April 26, 2019 (84 FR 17858)
- Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention, National Institutes ofHealth, HHS Publication No. (CDC), (2020)
- Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 3rd Edition, DEPARTMENT OF HEALTH & HUMAN SERVICES, Centers for Disease Control and Preventionand National Institutes of Health, (September 2007)
- Arthropod Containment Guidelines (Version 3.1), A project of The American Committee of MedicalEntomology of the American Society of Tropical Medicine and Hygiene
- World Health Organization, Laboratory Biosafety Manual 4th edition, 2020

Appendix A: Biological Material Shipments - Transportation of Hazardous Materials

Shipping of biological materials must be in compliance with the Biological Materials and Dry Ice Shipments Manual available on the EHS website. Additional information on the transportation of dangerous goods, importation, and interstateshipment of etiologic agents of human disease, diagnostic specimens, and other related materials may be obtained by contacting Environmental Health and Safety.

Transportation of infectious substances and materials that are known or suspected to contain them, as well as the shipping of recombinant DNA molecules, are regulated as hazardous materials by the United States Department of Transportation (DOT), foreign governments, and the International Civil Aviation Organization. For this reason, their transportation is subject to regulatory controls. For transport purposes, the term “infectious substance” is understood to include the term “etiologic agent.”

Only trained shipping personnel are authorized to prepare packages and related shipping documents. EHS provides specialized shipper training for biological materials and dry ice, which enables laboratory and clinical personnel to initiate shipments of these materials. All other shipments of chemical and radiological materials must be completed by Environmental Health and Safety, unless otherwise directed by EHS.

The import, export and shipment of chemical, biological and radioactive material is highly regulated by federal and international agencies including:

- International Air Transport Association (IATA)
- U.S. Department of Transportation (DOT)
- Federal Aviation Administration (FAA)
- United States Postal Service (USPS)
- Centers for Disease Control and Prevention (CDC)
- Occupational Health and Safety Administration (OSHA)
- U.S. Department of Human and Health Services (HHS)
- U.S. Department of Agriculture (USDA)
- U.S. Department of Commerce
- U.S. Department of Fish and Wildlife

Further information on shipping etiologic agents is available from:

(i) The Centers for Disease Control and Prevention, ATTN: Biohazards Control Office, 1600 Clifton Road,Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883;

(ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or

(iii) (iii) U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), VeterinaryServices, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Appendix B: Regulated Pathogens

The US Department of Commerce regulates the export of biological agents, pathogens, genetic elements, and vaccines and are controlled under Export Control Classification Number (1C351, 1C352, 1C353, 1C354).

ECCN controls all biological agents and "toxins," regardless of quantity or attenuation, that are identified in the List of Items Controlled for this ECCN, including small quantities or attenuated strains of select biological agents or "toxins" that are excluded from the lists of select biological agents or "toxins" by the Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture, or the Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services, in accordance with their regulations in 9 CFR part 121 and 42 CFR part 73, respectively.

Biological agents and pathogens are controlled under ECCN 1C351, when they are an isolated live culture of a pathogen agent, or a preparation of a toxin agent that has been isolated or extracted from any source or material, including living material that has been deliberately inoculated or contaminated with the agent. Isolated live cultures of a pathogen agent include live cultures in dormant form or in dried preparations, whether the agent is natural, enhanced or modified.

ECCN also controls genetic elements or genetically modified organisms for all biological agents and "toxins," regardless of quantity or attenuation, that are identified in the List of Items Controlled for this ECCN, including genetic elements or genetically modified organisms for attenuated strains of select biological agents or "toxins" that are excluded from the lists of select biological agents or "toxins" by the Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture, or the Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services, in accordance with the APHIS regulations in 7 CFR part 331 and 9 CFR part 121 and the CDC regulations in 42 CFR part 73.

Pathogens of human, livestock, poultry, fish, and plants may require special laboratory design, operation, and containment features. This may be BSL-3, BSL-3 plus enhancements or BSL-4 and for animals ABSL-2, ABSL-3, or BSL-3-Ag.

The importation, possession, or use of the following agents is prohibited or restricted by law, CDC, USDA, APHIS regulations, or administrative policies.

For the agents listed, an Export license is required by the Department of Commerce, or they may be agentsregulated as Select Agents under the Bioterrorism Act of 2002. Possession of these agents requires registration with either the CDC or APHIS, and a permit issued for interstate movement or importation by APHIS-VS.

The importation, possession, use, or interstate shipment of animal pathogens other than those listed may also besubject to regulations of the U.S. Department of Agriculture.

- VIRUSES;
- African horse sickness virus;
- African swine fever virus;
- Andes virus;
- Avian influenza (AI) viruses identified as having high pathogenicity (HP), as follows:
    - AI viruses that have an intravenous pathogenicity index (IVPI) in 6-week-old chickens greater than 1.2; *or*
    - AI viruses that cause at least 75% mortality in 4- to 8-week-old chickens infected intravenously.
- ***Note:*** *Avian influenza (AI) viruses of the H5 or H7 subtype that do not have either of the characteristics described in 1C351.a.4 (specifically, 1C351.a.4.a or a.4.b) should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0). If the amino acid motif is similar to that observed for other HPAI isolates, then the isolate being tested should be considered as HPAI and the virus is controlled under* 1C351.a.4.
- Bluetongue virus;
- Chapare virus;
- Chikungunya virus;
- Choclo virus;
- Classical swine fever virus (Hog cholera virus);
- Crimean-Congo hemorrhagic fever virus;
- Dobrava-Belgrade virus;
- Eastern equine encephalitis virus;
- Ebolavirus (includes all members of the Ebolavirus genus);
- Foot-and-mouth disease virus;
- Goatpox virus;
- Guanarito virus;
- Hantaan virus;
- Hendra virus (Equine morbillivirus);

- Japanese encephalitis virus;
- Junin virus;
- Kyasanur Forest disease virus;
- Laguna Negra virus;
- Lassa virus;
- Louping ill virus;
- Lujo virus;
- Lumpy skin disease virus;
- Lymphocytic choriomeningitis virus;
- Machupo virus;
- Marburgvirus (includes all members of the Marburgvirus genus);
- Middle East respiratory syndrome-related coronavirus (MERS-related coronavirus);
- Monkeypox virus;
- Murray Valley encephalitis virus;
- Newcastle disease virus;
- Nipah virus;
- Omsk hemorrhagic fever virus;
- Oropouche virus;
- Peste-des-petits ruminants virus;
- Porcine Teschovirus;
- Powassan virus;
- Rabies virus and all other members of the Lyssavirus genus;
- Reconstructed 1918 influenza virus,
- Tick-borne encephalitis virus (Siberian subtype, formerly West Siberian virus)
- BACTERIA
- Bacillus anthracis;
- Brucella abortus;
- Brucella melitensis;
- Brucella suis;
- Burkholderia mallei (Pseudomonas mallei);
- Burkholderia pseudomallei (Pseudomonas pseudomallei);
- Chlamydia psittaci (Chlamydophila psittaci);
- Clostriduim argentinense (formerly known as Clostridium botulinum Type G), botulinum neurotoxin producing strains;
- Clostridium baratii, botulinum neurotoxin producing strains;
- Clostridium botulinum;
- Clostridium butyricum, botulinum neurotoxin producing strains;
- Clostridium perfringens, epsilon toxin producing types;
- Coxiella burnetii;
- Francisella tularensis;
- Mycoplasma capricolum subspecies capripneumoniae (“strain F38”);
- Mycoplasma mycoides subspecies mycoides SC (small colony) (a.k.a. contagious bovine pleuropneumonia);
- Rickettsia prowazekii;
- Salmonella enterica subspecies enterica serovar Typhi (Salmonella typhi);
- Shiga toxin producing Escherichia coli (STEC) of serogroups O26, O45, O103, O104, O111, O121, O145, O157, and other shiga toxin producing serogroups;
    - **Note:** *Shiga toxin producing Escherichia coli (STEC) includes,*
    - **Technical Note:** *1C351.a.41 includes reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments.*
- Rift Valley fever virus;
- Rinderpest virus;
- Rocio virus;
- Sabia virus;
- Seoul virus;
- Severe acute respiratory syndrome-related coronavirus (SARS-related coronavirus);
- Sheeppox virus;
- Sin Nombre virus;
- St. Louis encephalitis virus;
- Suid herpesvirus 1 (Pseudorabies virus; Aujeszky's disease);
- Swine vesicular disease virus;
- Tick-borne encephalitis virus (Far Eastern subtype, formerly known as Russian Spring-Summer encephalitis virus
- Variola virus;
- Venezuelan equine encephalitis virus;
- Vesicular stomatitis virus;
- Western equine encephalitis virus;
- Yellow fever virus
    - *inter alia, enterohaemorrhagic E. coli (EHEC), verotoxin producing E. coli (VTEC) or verocytotoxin producing E. coli (VTEC).*
- Shigella dysenteriae;
- Vibrio cholerae; or
- Yersinia pestis.
- TOXINS
- Abrin;
- Aflatoxins;
- Botulinum toxins;
- Cholera toxin;
- Clostridium perfringens alpha, beta 1, beta 2, epsilon and iota toxins;
- Conotoxins;
- Diacetoxyscirpenol;
- HT-2 toxin;
- Microcystins (Cyanginosins);
- Modeccin;
- Ricin;
- Saxitoxin;

- Shiga toxins (shiga-like toxins, verotoxins, and verocytotoxins);
- Staphylococcus aureus enterotoxins, hemolysin alpha toxin, and toxic shock syndrome toxin (formerly known as Staphylococcus enterotoxin F);
- T-2 toxin;
- Tetrodotoxin;
- Viscumin (Viscum album lectin 1); *or*
- FUNGI
- Coccidioides immitis; or
- Coccidioides posadasii.

Appendix C: Select Agents and Toxins

Following the anthrax attacks of 2001 that resulted in five deaths, Congress significantly strengthened federal oversight of biological agents and toxins that have the potential to pose a severe threat to public health; animal and plant health; and animal and plant products (Select Agents and Toxins). The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (Bioterrorism Response Act) required the Department of Health and Human Services (HHS) to regulate the possession, use, and transfer of select biological agents and toxins that have the potential to pose a severe threat to public health and safety. Subtitle B of Title II of the Bioterrorism Response Act (cited as the Agricultural Bioterrorism Protection Act of 2002) granted comparable regulatory authorities to the U.S. Department of Agriculture (USDA) over select biological agents and toxins that have the potential to pose a severe threat to animal and plant health or products. The Bioterrorism Response Act also requires HHS and USDA to coordinate activities regarding the zoonotic agents regulated by both Departments.

These activities are implemented through the Federal Select Agent Program (FSAP). FSAP is managed jointly by the Centers for Disease Control and Prevention's (CDC) Division of Select Agents and Toxins (DSAT) and the Animal and Plant Health Inspection Service's (APHIS) Agriculture Select Agent Services (AgSAS). FSAP regulates the acquisition, use, storage and transfer of Select Agents and Toxins through the development, implementation, and enforcement of the federal Select Agent regulations—7 CFR Part 331 (APHIS-PPQ), 9 CFR Part 121 (APHS-VS), and 42 CFR Part 73 (CDC).

FSAP provides national oversight of the safety and security of potentially dangerous biological Select Agents and Toxins. Key elements of the Select Agent regulations include:

- *All entities that possess, use, or transfer Select Agents and Toxins must be registered with FSAP.*
- *All individuals who have access to Select Agents and Toxins must first be approved by FSAP after a security risk assessment (SRA) performed by the Federal Bureau of Investigation's (FBI) Criminal Justice Information Services Division (CJIS) to help guard against access to the agents and toxins by those who may wish to misuse them.*
- *Enforcement actions for regulatory violations may be taken to address present risks and increase future compliance through administrative actions and/or civil monetary penalties. An entity may be referred to the HHS Office of the Inspector General (OIG) or APHIS Investigative and Enforcement Services (IES), or the FBI may be notified of the incident for potential further investigation, as appropriate.*
- An entity's registration may be denied, suspended, or revoked if it is determined that such action is necessary to protect human, animal, or plant health, or animal or plant products.
- Each registered entity must designate a Responsible Official (RO), an individual with the authority and responsibility to act on behalf of the entity and charged with ensuring compliance with the Select Agent regulations. The RO is able to respond to onsite incidents involving Select Agents in a timely manner, ensures annual inspections are conducted for each space where Select Agents are stored or used, reviews the entity's validated inactivation procedures and investigates any failures, and reports the identification and final disposal of any Select Agent or Toxin in a diagnostic specimen or proficiency test. Alternate Responsible Official(s) (ARO) may be designated to serve when the RO is not available; AROs have the same responsibilities as ROs.
- Each registered entity must develop and implement a written security plan sufficient to safeguard their Select Agents and/or Toxins against unauthorized access, theft, loss, or release.
- Each registered entity must develop and implement a written biosafety plan commensurate with the risk of their Select Agents and/or Toxins, given their intended use.
- A registered entity must receive pre-approval for *Restricted experiments* that pose heightened safety and security risks. See Section 13 of the Select Agents and Toxins regulations for additional information.
- Each registered entity must develop and implement a written incident response plan specific to the hazards associated with their Select Agents and/or Toxins.
- Each registered entity must provide information and training on biosafety, security, and incident response to individuals with access to Select Agents and Toxins.
- Any instances of the theft, loss, or release of a Select Agent or Toxin must be promptly reported to FSAP in accordance with the Select Agent and Toxin regulations.
- An entity may only transfer a Select Agent or Toxin to another entity registered to possess that agent or toxin, and the transfer must be preauthorized by FSAP.
- Each registered entity must maintain complete records and documentation including, but not limited to: inventories, exposures, lists of individuals with approved access, and entry into areas containing Select Agents or Toxins.

- FSAP may conduct inspections of an entity without prior notification and prior to issuing a certificate of registration.

There are specific exemptions or exclusions to the regulations including specific attenuated strains or Select Toxins modified to be less potent or toxic. Entities must use validated inactivation procedures to inactivate Select Agents.

As of January 2017, FSAP regulates 66 Select Agents and Toxins. The list of Select Agents and Toxins is reviewed at least every two years to determine if agents or toxins need to be added to or deleted from the list. For more information on the regulations and guidance documents for implementation of a Select Agent program, please visit https://www.selectagents.gov .

All work with select agents must be approved by the Federal Select Agent Program, the designated Responsible Official, Environmental Health and Safety, the WCM Research Dean; and must conform to the WCM Federal Select Agent policies. Contact EHS for more information.