



Institutional Biosafety Manual

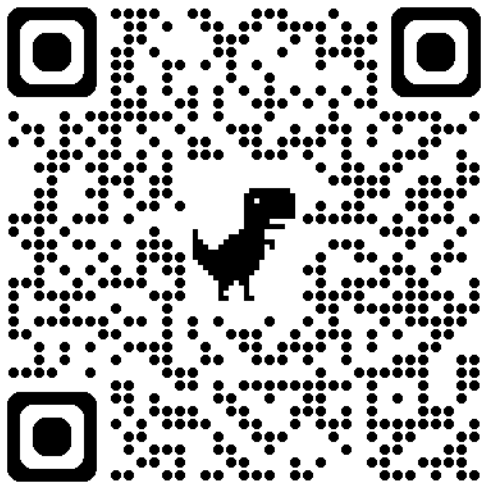
BIOSAFETY GUIDELINES



Requirements and Guidelines for the
Safe Handling of Infectious Agents and Biological Toxins

Revised: September 2026

Scan the QR code to visit the ASU website (www.alasu.edu)



Scan the QR code to visit the IBC website
(<https://alasu.edu/academics/programs-majors/cstem/physical-sciences-department/institutional-biosafety-committee-schedule.php>)

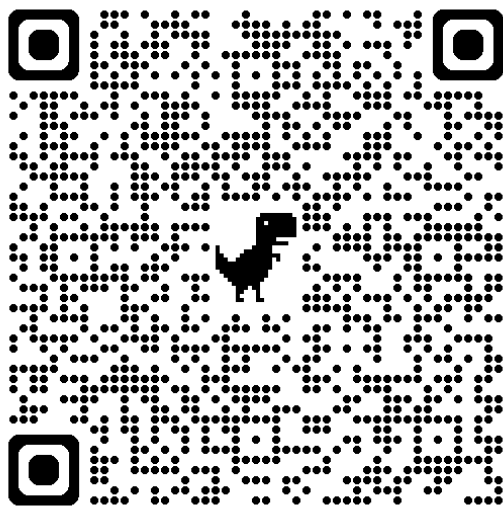


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BIOSAFETY FORWARD

This reference manual outlines the safe use, storage, handling, waste disposal, and emergency management of bio-hazardous materials for the Alabama State University (ASU) campus and for its students and workers in the field. This information supplements and reiterates information provided in the Biosafety Microbiological and Biomedical Laboratories and Occupational Safety and Health Administration (OSHA) Regulations. If there is a difference between this manual and the current BMBL and OSHA policy, those government policies take precedence.

ACKNOWLEDGEMENTS

The materials used to develop this manual were obtained from CDC and WHO websites. We thank the agencies for their published guidelines.

DISCLAIMER

An Institutional Biosafety Committee (IBC) manual disclaimer limits liability and clarifies that the institution and committee provide safety guidelines, but individual researchers remain responsible for compliance.

Emergency: 911

ASU Student Health Center: 334-229-4436

ASU Department of Public Safety: 334-229-4400

Secret Witness Hotline: 334-834-8293

Montgomery Police Department (MPD – Non-Emergency): 334-241-2651

Alabama Department of Public Safety (Trooper Division): 334-242-4128

Montgomery County Sheriff's Office: 334-832-4980

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Alabama State University Safety Policy

Purpose

The purpose of this manual is to clarify policies and procedures for the safe handling of biohazardous material within the Alabama State University (ASU) campus in a manner that will encourage research and further student interest in the field of science. In addition to protecting ASU personnel, these policies and procedures comply with applicable public regulations to protect the health of the general community and to prevent environmental contamination. This must be done in compliance with the applicable standards of the federal, state, and local regulations.

Policy

The University aims to provide a safe, healthy and secure environment in which to carry on the University's affairs. All possible preventive measures are taken to eliminate accidental injuries, occupational diseases and risks to personal security.

Definitions

An **administrative head of a unit** is a Director of a service unit, a Head of an academic department, a Director of a center, institute or school, a principal of a college, a Dean, an Associate Vice President, the Registrar, a Vice President or the President.

A **supervisor** is a person, not necessarily an administrative head of a unit, who has been delegated supervisory responsibility for others working or studying at ASU.

A **worker** is a person who is an employee, student or volunteer for ASU.

Duties and Responsibilities

The University:

It is the responsibility of the University, acting through administrative heads of unit to:

- Provide a safe, healthy and secure working environment.
- Ensure that regular inspections are made and action is taken as required to address unsafe conditions.
- Ensure that health, safety, and personal security considerations form an integral part of the design, construction, purchase and maintenance of all buildings, equipment and work processes.
- Provide first aid facilities/materials where appropriate.
- Support supervisors and safety committees in the implementation of an effective health, safety and security program.
- Ensure compliance with federal, state, and local regulations.
- Establish department or building safety committees.
- Communicate with the university community or affected groups about events or situations when potentially harmful conditions arise or are discovered.
- Ensure adequate resources are available to implement appropriate procedures.

The Supervisor:

It is the responsibility of supervisory staff to:

- Formulate specific safety rules and safe work procedures for their area of supervision.
- Ensure that all employees under their supervision are aware of safety practices and follow safety procedures.
- Provide training in the safe operation of equipment.
- Inspect their areas regularly for hazardous conditions.
- Correct unsafe work practices or hazardous conditions promptly.
- Be responsive to concerns expressed about personal safety and investigate any accidents, incidents or personal safety concerns which have occurred in their area of responsibility.
- Report any accidents or incidents involving personal safety to the appropriate University authority.

- Participate, if requested, on department or building safety committees.

Individual Students and Members of Staff and Faculty

It is the responsibility of individual students and members of faculty and staff to:

- Observe safety rules and procedures established by supervisory staff, administrative heads of unit and the University.
- Be safety-conscious in all activities, be they work, study, or recreation.
- Report as soon as possible any accident, injury, unsafe condition, insecure condition or threats to personal safety to a supervisor or administrative head of unit.
- Use proper and adequate care for personal protective equipment provided by the University.
- Participate, if elected or appointed, on departmental or building safety committees.

Institutional Biosafety Committee Members

The Institutional Biosafety Committee (IBC) oversees research and teaching involving biological agents to ensure safety for personnel, the environment, and the public. It does so by:

- Reviewing all research protocols involving biological materials (The review may lead to approval or disapproval based on safety compliance or concerns).
- Performing risk assessments to determine the risk potentials of research materials and procedures.
- Establishing containment levels and emergency plans for handling accidental spills and personnel contamination.
- Enforcing compliance with safety regulations;
- Investigating significant accidents or violations to ensure compliance with safety regulations.
- Inform university authorities of any recurring safety violations or noncompliance with biosafety regulations.

The appointment of IBC members is approved by the University administration through the office of the Provost and Vice-President for Academic Affairs.

Current Roster

Institutional Biosafety Committee Members

Name	Department/Unit	Contact Information
Dr. Vida A. Dennis, Chair	Biological Sciences	vdennis@alasu.edu
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Mr. Ron Dawsey	Alabama Department of Public Health (ADPH)	ron.dawsey@adph.state.al.us

Institutional Biosafety Committee Roles and Responsibilities

It is the responsibility of the IBC to:

- Formulate and implement policies related to the safe use of biological materials and some chemicals commonly used in biomedical research.
- Review research protocols involving biohazards and chemical hazards commonly used in biomedical research.
- Approve or disapprove such projects based on their potential hazard and proposed containment procedures.
- Establish, approve and monitor proper laboratory conditions and procedures required for such projects.
- Review the qualifications and training of investigators and laboratory personnel engaged in such research to ensure that appropriate laboratory safety techniques are used.
- Ensure adoption of proper disposal and decontamination procedures.
- Adopt emergency plans that cover accidental spills and personnel contamination resulting from research.
- Ensure investigation and reporting of any significant problems with or violations of the NIH and CDC Guidelines, as specified in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories.

The IBC meets monthly on the second Friday of the month during the Spring and Fall semesters, except in December, when schedules are adjusted for the holidays. A quorum for the IBC is the presence of at least 50% of the members. Members' presence may be in person, by phone, or by teleconference.

CHAPTER 1 – INTRODUCTION

Alabama State University is committed to providing a safe and healthy workplace for its faculty, staff, and students and to ensure the protection of the community and the environment. To meet this commitment, the Institutional Biosafety Committee has developed guidelines for the use of Infectious Agents and Biological Toxins.

The University Health and Safety Policy states that "the University shall be administered so as to ensure that health, safety and accident prevention form an integral part of the design, construction, purchase and maintenance of all buildings, equipment and work processes". These practices are concerned not only with the safety and health of faculty, staff, and students but also with protecting the community and the environment.

Human error and poor laboratory practices can compromise the best laboratory safeguards designed to protect laboratory workers. The primary factor in the prevention of laboratory accidents and laboratory-associated infections is a fully trained faculty and staff. To accomplish this, it is essential that faculty and staff receive the appropriate training in laboratory safety measures.

The Biological Waste Management Program (BWMP) has been developed to ensure that biological waste is handled safely and securely and properly disposed of. The program is designed to meet compliance with all federal, state, and local standards and regulations. Its purpose is to ensure the safe handling of biohazardous materials in all research and teaching facilities under the auspices of Alabama State University. The main focus of the program is the protection of faculty, staff, and students, and ways to protect research and the environment are also considered.

1.1 Definition of Biosafety

Biosafety, or Biological Safety, encompasses all aspects of containment to prevent unintentional exposure to hazardous biological materials and their accidental release into the environment. This also includes the containment of plants and animals.

1.2 Definition of Biosecurity and Writing a Plan

Biosecurity encompasses measures and procedures to prevent intentional misuse or theft of hazardous biological materials, focusing on safeguarding them from unauthorized access, diversion, or malicious use. A **biosecurity**

plan is implemented to prevent theft, misuse, or intentional release of pathogens. A pathogen is defined as an agent that can cause disease in humans or animals. The type of biosecurity plan that is created and implemented will depend on the nature of the facility, the type of research conducted, the type of biological material used, and the local environment. Personnel at various levels of administration can be involved in creating the biosecurity plan. Key features of a biosecurity plan should include facility security, inventory of pathogens, and emergency protocols for security incidents.

Facility security: In this part of the plan, strategies used to prevent the entry of unauthorized personnel and the theft of pathogens must be examined. For instance, is access to pathogens restricted somehow (i.e., kept under lock and key) and does the facility have specific security protocols in place to minimize the entry of unauthorized personnel (ie, Key card access, identity badges, protocols for locking doors).

Inventory of Pathogens: When working with pathogens, labs must have an inventory of all pathogens. Depending on the risk associated with the pathogen varying levels of information is necessary for the inventory. Access to the pathogens must be restricted to specific laboratory personnel and a tracking system must be established to determine if vials are unaccounted for. Personnel who do have access to the pathogens must be documented and kept on file.

Emergency Protocols for security incidents: In those cases where there have been unauthorized personnel entering the building or pathogen samples stolen, misused or intentionally released, an emergency protocol must be in place. In this protocol, a clear procedure must be stated for those who need to be contacted about theft or unauthorized entry (i.e., supervisor, security, law enforcement agencies, RMS, etc.).

1.3 Definition of a Biohazard

There is a distinction between biological materials and biohazardous materials.

- **Biological materials** encompass all materials containing genetic information and is capable of replication. Included in this are viruses and prions.
- **Biohazardous materials** are defined as infectious agents or biological materials that present a risk or potential risk to the health of humans

and/or animals in the environment. It should be noted that the following terms are used interchangeably throughout this document: biohazard, infectious material, infectious agent, and pathogen. The risk can be direct through infection or indirect through damage to the environment. Biohazardous agents can be classified into the following groups:

Bacteria: These single-celled organisms lack a nucleus and are classified in three phenotypes: Gram-positive (cocci or bacilli), Gram-negative (cocci or bacilli), and mycoplasmas (those that lack a cell wall). When bacteria cause harm and disease in human and/or animal hosts, they are referred to as bacterial pathogens. Some examples include: *Escherichia coli*, *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Staphylococcus aureus*, and *Bacillus anthracis*.

Viruses: These smallest replicating organisms have no metabolism of their own and are obligate parasites that depend on their host's machinery for replication. Viruses are classified according to their replication strategy, structure and the organization of their genome: double-stranded DNA, single-stranded DNA, RNA & DNA reverse transcribing, double-stranded RNA, negative-sense single-stranded RNA, and positive-sense single-stranded RNA. Some examples of viruses include: Influenza virus, Human Immunodeficiency Virus (HIV), Rabies virus, Ebola virus, and Coronavirus SARS-CoV-2.

- **Subviral agents:** These are infectious transmissible agents that are simpler than viruses in some respects.
- **Satellite viruses:** Morphologically similar to viruses, but cannot replicate in the absence of a host virus: examples are Hepatitis Delta Virus (HDV), which requires the Hepatitis B virus to replicate and infects humans; and the Adeno-Associated Virus (AAV), another human satellite that depends on adenoviruses.

Viroids: RNA-based infectious agents that primarily infect plants and do not encode any proteins; they rely on a helper virus for infectivity. Examples include **Potato Spindle Tuber Viroid (PSTVd)**, **Citrus Exocortis Viroid (CEVd)**, and **Apple Scar Skin Viroid (ASSVd)**.

Prions: Infectious proteins (without any associated nucleic acid) that can trigger normal protein to become abnormal and cause diseases in humans and animals. Examples of prion diseases are **Creutzfeldt-Jakob disease (CJD)**, a rare, fatal brain disease in humans; **Bovine spongiform encephalopathy (BSE)**, also known as "mad cow disease"; and **Fatal familial insomnia (FFI)**, an inherited prion disease that causes severe insomnia and death.

Fungi: These eukaryotic microorganisms are larger in size and have organelles, including a nucleus, vacuoles, and mitochondria. Two main categories, yeast and mold, are known to have species that cause disease in human and/or animal hosts. Molds have been known to release spores and some toxins, thus increasing the risk when using certain strains. Some examples include: *Aspergillus niger*, *Candida albicans*, and *Histoplasma capsulatum*.

Parasites: Protozoa and helminths that live on or within a larger host organism at the host's expense are considered parasites. There are two categories of parasites, endoparasites and ectoparasites. Endoparasites live within the tissues or cells, but cause infections that are generally treatable. Ectoparasites live on the external surface or within the skin of their host and cause infestation. Some examples of pathogenic protozoa include: *Plasmodium falciparum*, *Leishmania donovani*, *Toxoplasma gondii*, and *Giardia lamblia*. Some examples of pathogenic helminthes include: *Trichinella spiralis* (nematode), *Enterobius vermicularis* (pinworm), and *Hymenolepis nana* (tapeworm).

Zoonotic Pathogens: Zoonotic pathogens are those that are capable of infecting both animals and humans. Zoonoses are a greater risk with any activities involving first-generation wild-caught animals that may be infected and carry a pathogen indigenous to the animal's natural environment. Some examples include: Salmonellosis, Plague, Rabies, Toxoplasmosis, and prions.

Toxins: Toxins are poisonous substances that are produced by bacteria, animals or plants. They are usually active at very low concentrations and vary in size. They range from small molecules to larger molecules such as, peptides or proteins. In addition, they vary greatly in their severity, ranging from mildly poisonous to deadly. Some toxins are also able to cause illness upon contact

or absorption with body tissues.

Toxins of bacterial origin fall into two classes: endotoxins and exotoxins.

- **Exotoxins** are often heat-labile proteins and polypeptides that are produced and secreted or released by various species. Some examples include: Tetanus toxin, Cholera toxin, and Pertussis toxin.
- **Endotoxins** are structural molecules that are embedded in the outer layer of the cell wall of certain Gram-negative bacteria and can trigger an inflammatory response when released; lipopolysaccharides (LPS) are examples of endotoxins.

There are also some toxins that can be isolated from higher organisms, such as fungi (mycotoxins), plants (**phyco**toxins), and animals (**zoo**toxins like venom and tetrodotoxin).

Recombinant DNA: Recombinant DNA (rDNA) is formed when combining genetic material from more than one source, natural or synthetic, to produce novel DNA. A gene in its own natural genome may not pose a risk. However, the risk level can change when the gene is combined with another gene or modified in some way that affects its expression or function. Consequently, modifications that are done to the DNA must be examined and proper assessments must be done to determine the potential effects of the modified DNA. There are three kinds of rDNA: Genetically Modified Organisms (GMOs), Viral Vectors, and Synthetic DNA (sDNA).

- A **GMO** can be as simple as rDNA cloned into a bacterial or viral host to over-express a specific gene for further study. More complex GMOs include transgenic and knock-out animals, where their genome is altered by the insertion or removal of DNA segments, respectively.
- **Viral Vectors** are transfer vehicles used to deliver genetic material into host cells for subsequent gene expression. Genetic modifications are typically made to these vectors to improve gene delivery efficiency and to enhance the safety of the system. Some examples include: recombinant Adenovirus and HIV based lentivirus.

- **sDNA** refers to artificial segments of DNA that are chemically synthesized in vitro without an initial DNA template.

Cell Lines: While cultured cells on their own do not pose a risk to the worker, the biological agents that could potentially be contained within the cell or the media must be considered. In addition, the cell cultures could also contain other infectious agents that could be released into the environment. One of the primary hazards of manipulating any cell line is the expression of latent viruses. Endogenous viral sequences have been found in a variety of cell lines derived from mammalian species, including humans. Additional care should be taken with primary cultures.

Human blood and tissue: Like cultured cells, the risk with human blood and tissues is with what they may contain. There are numerous bloodborne pathogens such as HIV, hepatitis B & C, and TB. More details can be found in the Blood and Body Fluids: Exposure Control Plan.

1.4 Definitions

AUTOCLAVE: An apparatus for sterilization by steam pressure. This is the most commonly used method for decontaminating biohazardous material at ASU. Autoclaving provides heat and moisture as physical factors used to destroy organisms. Most organisms can be destroyed using a steam pressure of 121°C for a minimum of 15 minutes. Such laboratory wastes as Petri dishes, pipettes, culture tubes, glassware, etc., can be effectively decontaminated this way.

BIOHAZARD: Any material of a biological nature that poses a risk (hazard), including biologically active agents (e.g., venoms, allergens) and pathogens that can cause disease/death/illness in humans, animals or plants. It also refers to materials of a biological nature that cause damage to the environment (e.g., certain recombinant and synthetic DNA sequences). Any substance or material, including human/animal tissues, bodily fluids, cell cultures, etc. that are infected, synthesized or manipulated with a pathogen, is considered a biohazard.

BIOHAZARDOUS MATERIAL: Any substance which contains or potentially contains a biohazardous agent.

BIOLOGICAL: Refers to anything that relates to life, living and/or biology.

They can be natural processes or man-made preparations, such as drugs, vaccines, or antitoxins that are synthesized from living organisms or their products and used medically as a diagnostic, preventive, or therapeutic agents. Not all biological substances are considered pathogens. A biological substance becomes a biohazard only when it poses a threat to the health of organisms or the environment.

BIOLOGICAL SAFETY CABINET (HOOD): An enclosure designed for the containment of biological hazards. Special filters that remove potentially dangerous particles from the cabinet air provide various degrees of safety and sterility for users and cabinet contents.

BIOETHICS: The study of the ethical and moral implications of new biological discoveries and biomedical advances, as in the fields of genetic engineering, drug research, medicine, etc.

BIORISK: The effect of uncertainty expressed by the combination of the consequences of an event and the associated likelihood of occurrence, where biological material is the source of harm.

BIOSAFETY: A combination of containment principles, technologies, and practices implemented to prevent unintentional exposure to pathogens and toxins, or their unintentional release.

BIOSECURITY: Institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

CHEMICAL DISINFECTION: The use of chemicals to decontaminate materials and surfaces. This is an effective alternative to autoclaving for large spaces, surfaces and equipment as well as temperature-sensitive containers. The initial choice of chemical disinfectant depends upon the resistance of the microorganism of concern and can be a hypochlorite, phenolic, iodine, alcohol, acid or base. A common disinfectant is 1:9 dilution of household bleach or 70% ethyl or isopropyl alcohol.

CONTAINMENT: A mode of control of biohazard material by isolation and separation of an organism from the worker. It refers to measures taken to provide a barrier between the biohazard material and the worker.

DECONTAMINATION: The use of physical or chemical means to remove or inactivate biohazardous materials on a surface or item, rendering it safe to be handled. The most common agents of decontamination are liquid chemical disinfectants (Bleach, Virkon, Clydox) or physical methods

(radiation, filtration). These items are not to be considered sterile.

DISINFECTION: The removal of all or almost all pathogens from a surface. These items are not to be considered sterile.

DRY HEAT: A sterilization method that uses hot, moisture-free air to eliminate or kill all forms of microbial life, including bacteria, viruses, and spores. Dry heat is used for the sterilization of anhydrous oils, greases, powders, etc. that cannot be permeated by steam. Because it is less efficient than steam, it requires a temperature of 160 – 170°C for a period of 2 – 4 hours.

FUME HOOD: A chemical fume hood is a well-ventilated, enclosed chemical and fire-resistant work area that provides user access from one side. It isolates and removes toxic and noxious vapors from a working area and protects the user; it may contain liquids that are splashed or sprayed.

HAZARD: Is a source that has a potential for causing harm. A Hazard is not a risk without a specific environment or situation.

HEPA FILTER (High Efficiency Particulate Air Filter): a specialized mechanical air filter that removes nearly all (99.97%) particles 0.3 micrometers and above from the air that passes through it, including microscopic contaminants like bacteria, mold spores, and viruses.

PATHOGEN: A disease producing infectious agent. It can include such microorganisms as: virus, bacterium, prion, parasite or fungus that causes disease in its host. The host may be an animal, human, plant, or even another microorganism.

Pathogen Safety Data Sheet (PSDS): A technical document detailing the hazardous properties of a human or animal pathogen and providing safety guidance for working with it in a laboratory setting. It serves as a comprehensive guide for risk assessment, outlining information like the pathogen's identity and characteristics, its specific hazards (e.g., pathogenicity, infectious dose), recommended engineering controls and containment measures, personal protective equipment (PPE), first aid procedures, and methods for safe handling, storage, and disposal.

RISK: The likelihood of an event with a hazard that has consequences.

RISK ASSESSMENT: A management process that assesses the quantitative and qualitative value or risk related to a concrete situation and a recognized hazard. There are 3 key steps involved to ensure a quality assessment which include:

- Assessment: identifying all the hazards you will be working with and the environment in which you are working in.
- Mitigation: providing strategies to decrease the likelihood of an accident happening.
- Performance: documentation and follow-up of implemented mitigations to be sure they are continuously effective, monitored and modified when necessary.

RISK GROUPS (RGs): A classification system for infectious agents based on their potential to cause disease in humans and their potential to spread within the community. This system, which typically includes four levels, is used to determine the necessary laboratory precautions, equipment, and containment procedures needed to handle an agent safely.

PERSONAL CONTAINMENT: Standard worker practices used to reduce the spread of microorganisms.

PHYSICAL CONTAINMENT: Laboratory design features and physical barriers that workers use to isolate biohazards.

PRIMARY CONTAINMENT: Use of personal protective equipment (PPE) and other measures to prevent direct exposure of the personnel and the immediate laboratory environment to hazardous biological agents.

SECONDARY CONTAINMENT: Special facility building designs and the use of strict operational practices to protect the external environment from exposure to infectious materials used in a laboratory.

STERILE: Condition of an item that is free from living germs or microorganisms; aseptic.

STERILIZATION: A procedure that destroys virtually all viable organisms in a material or on a surface. The most common agents of sterilization are heat (autoclaving) or gas (ethylene oxide). When properly done, these items are considered sterile.

CHAPTER 2 – BIOSECURITY

Laboratory biosecurity refers to the institutional and personal measures aimed at preventing the loss, theft, misuse, diversion, or intentional release of infectious microorganisms and hazardous biological materials, thereby safeguarding human and animal health, the environment, and the economy. A biosecurity plan for a research institution outlines the security measures designed to protect high-risk biological agents from theft, loss, diversion, misuse, or intentional release. This plan is a critical component of a comprehensive biorisk management program, which works in tandem with biosafety practices to ensure the safe and secure handling of biohazardous materials. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), which jointly manage the Federal Select Agent Program (FSAP), require that research institutions have a biosecurity plan. Although this requirement mainly applies to institution that possesses, uses, or transfers specific biological select agents and toxins (BSAT) identified as potential severe threats to public health, a biosecurity plan is part of ASU's commitment to the safe and responsible conduct of research.

This plan encompasses Containment Level 1 & 2 Laboratories at ASU, and deals with precautions to ensure that there is no intentional compromise for the protection of laboratories and the materials that they contain and the risk to people, livestock and agriculture in the community.

Anyone using biohazardous materials must follow the institutional safety procedures and Laboratory Emergency Plan for reporting any incidents involving biohazardous materials.

2.1 Risk Assessment

A primary component of a biosecurity plan must be a detailed risk assessment. The biosecurity risk assessment should review and list the relevant assets, define the threats, outline the vulnerabilities, and determine the countermeasures or mitigation strategies specific for each facility. The biosecurity plan should then address the following factors: physical protection, personnel suitability/reliability, pathogen accountability, pathogen transport, and related incident and emergency response.

2.1.1 Physical Protection

This deals with the actual physical location of the laboratory and all the measures in place to ensure the access to the laboratory is secure and minimizes opportunities for unauthorized entry. Things to

consider in this area:

- **Controlled access:** Implement strict procedures for access to laboratories, particularly those with higher biosafety levels (BSL-2 and above): card access, keypads, locks, etc.
- **Secure storage:** Ensure that all potentially hazardous biological materials and research specimens are inventoried and stored securely at all times.
- **Back-up power:** Critical systems, especially in high-containment laboratories, must have emergency power to maintain security during an outage.

2.1.2 Personnel Suitability/Reliability

Personnel who have access to biohazardous materials must be carefully vetted and trained to prevent malicious or negligent acts. The following should be implemented when dealing with high-risk biohazards.

- **Background checks and screening:** For programs involving high-risk agents, participants must undergo background investigations and threat screenings.
- **Comprehensive training:** All personnel must receive documented, site-specific training in biosafety, biosecurity, and emergency incident response.
- **Supervisory oversight:** Supervisors are responsible for ensuring staff competency and compliance with all safety and security regulations.

2.1.3 Pathogen Accountability

Robust procedures must be in place to track and manage all potentially dangerous biological agents. These procedures include inventory requirements for proper labeling, tracking possession, inactivation, disposal after use and appropriate record keeping.

- Record keeping must include pathogen inventory and tracking: who has access to agents, where it is stored and under whose responsibility the pathogens lie.
- Inventories should be updated regularly.
- Clear protocols must be established for the safe transfer of ownership or responsibility for inventoried materials, especially when researchers leave the institution.

- A notification plan should be in place for identifying, reporting, and remediating security problems, i.e., inventory discrepancy, breach of security, release of agents, etc.

2.1.4 Transport security

Procedures for the safe and secure transport of biological materials, both on and off campus, must be followed to prevent accidental release or theft.

- **External procedures:** The transport of biological materials must conform with regulatory agencies like the CDC or USDA and must adhere to all applicable packaging and transport regulations.
- **Internal procedures:** Movement of hazardous materials within a facility or campus must use secure, leak-proof containers.

2.1.5 Information security

The plan must protect sensitive information related to pathogens, toxins, and research technologies, particularly for dual-use research of concern (DURC).

- **Data security:** All electronic data and records, such as notebooks and imaging data, should be backed up and securely stored.
- **Dual-use research oversight:** The plan must account for research that has a legitimate scientific purpose but could be intentionally misused. The NIH provides specific oversight frameworks for such research, including enhanced potential pandemic pathogens (ePPPs).

2.1.6 Biosecurity Incident and Incident Response Plan

The laboratory supervisor and the Principal Investigator are responsible for the implementation of the following procedures.

- Loss, theft, accidental release, unauthorized access or suspected tampering is to be immediately reported to the local ASU Department of Public Safety, the ASU Biosafety Committee Chair and Principal Investigator.
- This plan must be reviewed annually and revised as necessary.
- Unexplained, recurrent or extended illness of any laboratory personnel using or in the vicinity of biohazardous material must

be reported to the Biosafety Committee Chair.

The incident response plan must contain the following information:

- The name and full contact information for all necessary Safety Officers
- The laboratory facility supervisor's name
- ASU Security Office and phone number
- Procedures to be followed by employees performing rescue or medical duties
- Emergency treatment and first aid
- A list of Personal Protective Equipment and their locations
- Site security and control
- Procedures for emergency evacuation, including type of evacuation, exit route assignments, safe distances and places of refuge
- Decontamination procedures

2.1.7 Program management and oversight

Effective program management is essential for implementing and enforcing biosecurity policies.

- **Institutional Biosafety Committee (IBC):** The IBC reviews and approves all research involving potentially hazardous biological materials, including recombinant DNA. It provides oversight and ensures compliance with regulatory agencies' guidelines.
- **Regular review:** Biosecurity plans must be regularly re-evaluated and rehearsed through exercises and drills to ensure their validity and feasibility.
- **Incident reporting:** Any significant problems, violations, or accidents must be reported immediately to the NIH Office of Science Policy (OSP).

CHAPTER 3 – BIOSAFETY TRAINING

3.1 Training

All laboratory personnel must complete a Biosafety training course before working in a laboratory where biological materials may be used. A certificate is issued after successful completion of the course. A copy of the training certificate must be provided to the Institutional Biosafety Committee before the personnel start working in the laboratory.

Biosafety is regulated by the Occupational Safety and Health Administration (OSHA), which was created by the Occupational Health and Safety Administrative Act. Through its Bloodborne Pathogens standard (29 CFR 1910.1030) and the General Duty Clause, OSHA has established biosafety training requirements that apply to any employee with a “reasonably anticipated” exposure to human blood or other potentially infectious materials (OPIM).

Mandatory training requirements under this standard include:

- Initial training: Must be provided before an employee is assigned to tasks involving potential exposure.
- Annual retraining: Required at least once every 365 days.
- Supplementary training: Necessary when new or modified tasks or procedures introduce new exposure risks.
- Specialized training: Workers in laboratories that deal with HIV, HBV, Ebola or any other hazardous biohazard materials must receive specialized initial training in addition to general biosafety training.

All personnel working with or around biohazardous material must be instructed in:

- The laboratory-specific exposure control plan, including entry control procedures,
- The meanings of various signs, signals or other controls used,
- Applicable emergency procedures,
- The recognition and prevention of dangerous situations and exposure routes,
- The symptoms of possible acute/chronic exposures.

In addition, these personnel must receive documented training in:

- Basic biosafety controls and techniques,

- Waste disinfection, autoclaving and disposal,
- Biosafety cabinets and spills,
- Chemical and radioactive biohazards,
- Applicable directives which may not be covered in the regular biosafety training or not understood by the individual,
- Specific preventive control methods and requirements of their work and the work area.

General Biosafety training is given by the ASU Biosafety Committee Chair and follows the necessary requirements given by the Centers for Disease Control and Prevention, Laboratory Biosafety Manual. In addition to the General Biosafety training, each research personnel must complete the Biosafety training modules assigned to their category by the ASU Biosafety Committee.

Custodial and maintenance workers are given a separate course.

All training must be documented, and records must be available to the ASU Biosafety Committee Chair.

3.2 Special consideration when working with toxins

All laboratory workers handling toxins must be trained in the theory, practice and nature of the hazards of the material to be used.

A risk assessment for safe operation practices must be done before any work is performed, and it is recommended that practice runs with supervision, or no toxin be undertaken.

Risk and containment levels must be considered carefully when toxins and infectious agents are used together. If animals are used, there are also other safety issues to be considered.

Routine operations with dilute toxin solutions can be conducted under Biosafety Level 2 conditions with the aid of personal protection equipment and a certified biosafety cabinet or fume hood.

CHAPTER 4 – CONTAINMENT AND CLASSIFICATION

4.1 Identification of Biohazardous Material

All users of biohazardous material at ASU must be accountable for any biohazardous material that they possess. Biohazardous material encompasses, but is not limited to, biological agents, toxins, or products of these materials. The material must include an updated inventory and stored in an area with restricted access to authorized personnel.

The institution must know the following:

- Who is working with and storing biohazardous material
- Where the material is worked with and where it is stored
- When, how, and to whom the materials are transferred
- When and how the materials are destroyed

4.2 Access

Access to secure areas and biohazardous material is the responsibility of the area supervisor or the Principal Investigator.

- There must be controlled access to areas where biohazardous materials are used and stored.
- Laboratories and animal facilities are to be separated from the public and locked at all times.
- Storage rooms, cabinets, freezers and refrigerators containing biohazardous material are to be locked at all times when located in unattended storage areas.
- Access is restricted to the laboratory area, and the supervisors must know who is in this area.
- Visitors must be escorted and follow all facility policies and procedures.
- Laboratory keys are not to be shared with unauthorized personnel.
- Lost keys are to be reported to the ASU C-STEM Dean's Office.
- Access for routine cleaning, maintenance and repairs in restricted areas should be limited to hours when regular employees are present.

4.3 Risk Groups

Risk groups are recognized internationally by the WHO and usually match up with containment levels. A complete list can be found on the Centers for Disease Control and Prevention (CDC) website under infectious diseases (<https://www.cdc.gov/comec/resources/infectious-diseases.html>).

Pathogens and their associated Risk groups will no longer be a static part of the hardcopy guidelines. Instead, a dynamic list is maintained on the Occupational Health and Safety Administration (OSHA) website in order to update and accommodate ongoing risk assessments of risk or current pathogens and the addition of new pathogens to the list. In the existing guidelines, biological agents are classified according to risk.

The judgment of the inherent risks of pathogens is based on such factors as:

- The existence (or not) of effective therapies
- The availability of immunization against the specified pathogen
- The presence or absence of vectors
- The quantity of the biological agent being used
- Whether the pathogen is indigenous or exotic
- Whether it is an emerging pathogen with yet unknown characteristics

Classification of organisms according to risk group has traditionally been used to categorize the relative hazards of infective organisms. The factors used to determine which risk group an agent falls into is based upon its characteristics, including:

- Pathogenicity
- Infective dose
- Mode of transmission
- Host range
- Availability of effective preventative measures
- Availability of effective treatment

The Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) jointly publish the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, a key reference detailing these classifications. The following table summarizes the four risk groups.

Risk group	Risk to individual	Risk to community	Characteristics and Biosafety considerations	
Risk group 1 (RG1)	Low or no risk	Low or no risk	Agents are not known to cause disease in healthy workers, animals or plants Require only standard microbiological practices: basic laboratory, clean open bench, no Biosafety cabinet (BSC) needed.	• Non-pathogenic strains of <i>E. coli</i> • <i>Saccharomyces cerevisiae</i> (baker's yeast)
Risk group 2 (RG2)	Moderate	Low	Agents can cause human disease, but it is rarely serious and effective treatment or prevention measures are often available. The risk of community spread is limited. Agents spread via ingestion, inoculation and mucous membrane routes. Biological safety cabinet is required, and lab access must be restricted.	• Herpes viruses • <i>Salmonella</i> and <i>Shigella</i> species • Hepatitis B and C viruses
Risk group 3 (RG3)	High	Low	Agents can cause serious or potentially lethal disease, often transmitted by aerosols. Effective treatment and preventive measures may be available, but laboratory exposure is a high risk. HEPA filtration and respiratory protection are required. Controlled lab access, and strict decontamination protocols must be in place.	• <i>Mycobacterium tuberculosis</i> • West Nile virus • SARS-CoV-2 (COVID-19)
Risk group 4 (RG4)	High	High	Agents are likely to cause serious or lethal disease with a high risk of aerosol-transmitted lab infection. No effective treatment or preventive measures are usually available. Must be used in labs are in a separate building or isolated zone and require full-body, air-supplied suits for staff.	• Ebola virus • Marburg virus • Lassa fever virus

*Complete list of pathogens/level visit (<https://www.cdc.gov/comec/resources/infectious-diseases.html>). The table above summarizes how agents are classified by taking into consideration how much risk there is on the individual and the community as a whole. For example, Risk Group 2 has moderate individual risk, low community risk. This includes any pathogen that can cause human or animal disease, but unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Lab exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. The use of standard microbiological practices must be used in the laboratory with appropriate personal protective equipment such as gloves, lab coats, and protective eyewear.

Currently, no material designated as Risk Group 3 or 4 may be brought into the ASU for research or teaching purposes. Please contact the Biosafety Committee Chair for approval, if you are considering working with any of these agents.

Note: Currently, no material designated as Risk Group 3 or 4 may be brought into the campus of Alabama State University for research or teaching purposes. Please contact the Biosafety Committee Chair for approval, if you are considering working with any of these agents.

4.4 Containment levels

There are four physical containment levels, which are outlined in the Centers for Disease Control and Prevention. Microbiological agents are typically, but not always, handled in containment facilities at the same level as their risk group. (Risk Group $\neq$ Containment Level). The containment level may be changed if large volumes or aerosols are involved, but the Risk Group is never changed. The requirements for each containment level include all the requirements for the containment levels beneath it.

4.4.1 Containment or Biosafety level 1 (CL1, BSL1)

This refers to the basic laboratory that handles low-risk agents. BSL1 does not require any special design features beyond those suitable for a well-designed and functional laboratory. Work may be done on open bench tops. Containment is achieved by following basic microbiology laboratory procedures (refer to procedures section in this manual for a review of basic lab skills).

4.4.2 Containment or Biosafety level 2 (CL2, BSL2)

BSL2 labs must use 'primary containment' procedures, such as using appropriate personal protective equipment (i.e., gloves, lab coats, protective eyewear) and using containment devices such as Biological Safety Cabinets (BSCs)

If you are using one of the following, you must register as a level 2 lab:

- Microorganisms of low biohazard potential such as those in Risk Group 2 (RG 2),

- Recombinant DNA activity requiring BSL2 physical containment including animal studies that involve the construction of transgenic animals including human cells.
- Non-recombinant cell and/or tissue culture systems that require this level of containment.
- Oncogenic viral systems classified as low risk.
- Introduction of Risk Group 1 materials into experimental animals.
- Production activities with Risk Group 1 organisms.

Minimal physical requirements include the following:

- Biosafety cabinets: Class II, either Type A1 or A2 cabinet, properly maintained and certified annually or when moved or repaired.
- Clean, uncluttered benches, workspaces and floors.
- Sink for hand washing.
- Eyewash station readily accessible.
- Waste handling, autoclave, incineration, or chemical disinfection is available nearby.
- Centrifuges with sealed rotors or safety caps.

4.4.3 Containment or Biosafety level 3 (CL3, BSL3)

BSL3 labs use ‘primary and secondary’ containment barriers to minimize the release of infectious organisms. These secondary measures include using appropriate respiratory protection, HEPA filtration of exhausted lab air and strictly controlled laboratory access.

Note: There are currently no Level 3 laboratories on the ASU campus.

4.4.4 Containment or Biosafety level 4 (CL4, BSL4):

BSL4 labs are isolated units, structurally independent of other areas. Maximum containment of the agent is achieved by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the agent by using a positive pressure suit or the use of a Class III Biological Safety Cabinet.

Note: There are currently no Level 4 laboratories on the ASU campus.

CHAPTER 5 – BIOSAFETY LABORATORY PROCEDURES

5.1 INSPECTIONS AND PERMITS

5.1.1 Requirements for Laboratories using any Risk Group 2 Agents

- The laboratory must be separated from public areas by a door which must be lockable.
- Laboratory access must be limited to authorized personnel.
- Laboratory doors must have appropriate signs for biohazard level, containment level, contact information and entry requirements.
- Laboratory doors must be large enough for passage of anticipated equipment.
- Laboratory doors must be kept closed at all times.
- Office and paperwork areas must be located outside the containment laboratory.
- Working surfaces, doors and frames, and casework should be non-absorptive and any damaged surface repaired as soon as possible.
- Interior coatings must be resistant to gas and chemicals for disinfection and fumigation purposes.
- Windows must be screened.
- Hooks must be available for laboratory coats/coveralls and street clothes, separated from laboratory clothing.
- Hand washing sinks must be located near the laboratory exit, preferably “hands-free” capability.
- An emergency eyewash station and shower must be easily available nearby.
- Biological Safety Cabinets (Class II, either Type A1 or A2), must be located inside the laboratory away from high traffic areas and must be certified annually or when moved or filters changed.
- Waste handling: autoclaving, incinerating, or chemical disinfection must be available nearby.

5.1.2 Inspections

All laboratories working with Risk Group 2 must be inspected annually by the Biosafety Committee Chair.

5.1.3 Permits

Permits are issued by the Biosafety Committee Chair and must be displayed on the door to the laboratory.

5.2 Laboratory Criteria

Biosafety Level 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. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. 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 a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Personnel must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware, must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls

that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps is of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

None required.

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

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

- b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
- c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant.
5. Laboratory windows that open to the exterior should be fitted with screens.

Biosafety Level 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 BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Personnel must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
 - a. Careful management of needles and other sharps is of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

- c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- 6. Perform all procedures to minimize the creation of splashes and/or aerosols.
- 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
- 10. An effective integrated pest management program is required.
- 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these

conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All personnel entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

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

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices. Dispose of protective clothing appropriately or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Personnel who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

- c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- 5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be operated manually, hands-free, or automatically. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted in laboratories.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward airflow without recirculation to spaces outside of the laboratory.

10. HEPA-filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to ensure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 (BSL-3) is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation as the route of exposure. 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.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

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

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. All personnel must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps is of primary importance. Needles must not be bent, sheared,

- broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory waste should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required.
 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties,

the necessary precautions to prevent exposures, and exposure evaluation procedures.

Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All personnel entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided with medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC or other physical containment devices.

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 containment devices, such as a centrifuge safety cup or sealed rotor must be used.

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

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Personnel who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:

- a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- 5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

- 1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
- 2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different zones, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
- 3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
 - a. Floors must be slip-resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
 - b. Walls should be constructed to produce a sealed, smooth finish that can be easily cleaned and decontaminated.
 - c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shutdown. The selection of the appropriate materials and methods used to decontaminate the laboratory must be based on risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “ clean ” areas toward “ potentially contaminated ” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
 - a. Laboratory personnel must be able to verify directional airflow.

A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of airflow disruption.

- b. The laboratory exhaust air must not recirculate to any other area of the building.
- c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

10. HEPA-filtered exhaust air from a Class II BSC can be safely recirculated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to ensure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. A method for decontaminating all laboratory waste should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
12. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, 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; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 (BSL-4) is required for work with dangerous and exotic agents that pose a high individual 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. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies, controls access to the laboratory.

There are two models for BSL-4 laboratories:

1. Cabinet Laboratory—Manipulation of agents must be performed in a Class III BSC; and
2. Suit Laboratory—Personnel must wear a positive-pressure supplied-air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices, equipment, and facilities apply to BSL-4.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
3. Mechanical pipetting devices must be used.
4. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

Precautions, including those listed below, must be taken with any sharp items. These include:

- a. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
 - b. Use of needles and syringes or other sharp instruments should be restricted in the laboratory, except when there is no practical alternative.
 - c. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal or decontamination. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal, located as close to the point of use as possible.
 - d. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
5. Perform all procedures to minimize the creation of splashes and/or aerosols.
 6. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.
 7. Decontaminate all wastes before removal from the laboratory by an effective and validated method.
 8. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's

biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

9. An effective integrated pest management program is required.

The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All personnel entering the laboratory must be advised of the potential hazards and meet specific entry requirements in accordance with institutional policies.

Only personnel whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all personnel entering and leaving the laboratory must be maintained. While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All personnel entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves (as appropriate). All personnel leaving the laboratory must take a

personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering.

After the laboratory has been completely decontaminated and all infectious agents are secured, necessary staff may enter and exit without following the clothing change and shower requirements described above.

2. Laboratory personnel and support staff must be provided appropriate occupational medical services including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired infections.
3. Each institution should consider the need for the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.
5. The laboratory supervisor is responsible for ensuring that laboratory personnel:
 - a. Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-4 containment.
 - b. Receive appropriate training in the practices and operations specific to the laboratory facility.
 - c. Receive annual updates and additional training when procedural or policy changes occur.
6. Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once

- removed, packaged viable material must not be opened outside BSL-4 containment unless inactivated by a validated method.
7. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory.
 - b. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the laboratory. The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.
 - c. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
 8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
 9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
 10. Supplies and materials that are not brought into the BSL-4 laboratory through the change room must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or air lock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or air lock. These doors must be secured after materials are brought into the facility. The doors of the autoclave or fumigation chamber are interlocked in a manner that prevents the opening of the outer door unless the

autoclave or fumigation chamber has been operated through a decontamination cycle. Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the laboratory.

11. Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.
12. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment (PPE)) Cabinet Laboratory

1. All manipulations of infectious materials within the laboratory must be conducted in the Class III biological safety cabinet. Double-door, pass-through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

The Class III cabinet must have a HEPA filter on the air supply intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas-tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor

decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves.

Class III cabinet gloves must be inspected for damage prior to use and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.

The Class III cabinet must be certified at least annually.

2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. All protective clothing must be removed in the dirty side of the change room before showering. Reusable clothing must be autoclaved prior to removal from the laboratory for laundering.
3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eyeglasses must be decontaminated before removal through the personal body shower.

4. Disposable gloves must be worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Cabinet Laboratory

1. All procedures must be conducted by personnel wearing a one-piece positive pressure supplied air suit. All manipulations of infectious agents must be performed within a BSC or other primary barrier system. Equipment that may produce aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as needed. HEPA-filtered exhaust air from a Class II BSC can be safely recirculated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's specifications.
2. Workers must wear laboratory clothing, such as scrub suits, before entering the room used for positive-pressure suits. All laboratory clothing must be removed in the dirty side change room before entering the personal shower.
3. Inner disposable gloves must be worn to protect against breaks or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to entering the personal shower. Dispose of used gloves with other contaminated waste.
4. Decontamination of outer suit gloves is performed during laboratory operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers) Cabinet Laboratory

1. The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the facility must be arranged to ensure sequential passage through an inner (dirty) changing area, a personal shower and an outer (clean) change room upon exiting the room(s) containing the Class III BSC(s). An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on an uninterrupted power supply (UPS). A double-door autoclave, dunk tank, fumigation chamber, or ventilated air lock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the door of the cabinet room(s) and the inner change room. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to the chemicals used for cleaning and decontaminating the area. Floors must be monolithic, sealed and covered.

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

Drains in the laboratory floor (if present) must be connected directly to the liquid waste decontamination system.

Services and plumbing that penetrate the laboratory walls, floors, or ceilings must be installed to prevent backflow from the laboratory. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shutdowns, and in other situations, as determined by risk assessment.

The selection of the appropriate materials and methods used for decontamination must be based on risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate a malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.

All HEPA filters should be located as near as practicable to the cabinet and laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housing should be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and the ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. If BSC exhaust is to be recirculated to the outside, BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a hard ducted, direct connection ensuring that cabinet exhaust air passes through two (2) HEPA filters—including the HEPA in the BSC—prior to release outside. Provisions to ensure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid waste must be documented. The decontamination process for liquid waste must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double door, passing through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bio-seal must be durable, airtight and capable of expansion and contraction. Positioning the bio-seal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress¹ must be developed and implemented.

Suit Laboratory

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

¹ The action of going out of or leaving the place

Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

Entry into the BSL-4 laboratory must be through an air lock fitted with airtight doors. Personnel who enter this area must wear a positive-pressure suit supplied with HEPA-filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup.

A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity-fed supply of chemical disinfectant, is needed.

An automatically activated emergency power source must be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets.

Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.

2. Sinks inside the suit laboratory should be placed near procedure areas and be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and covered.

All penetrations¹ in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

Drains, if present, on the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents must have protection against insect and animal intrusions.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners should be avoided. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.

¹ electrical conduits, HVAC ductwork, or plumbing

5. Windows must be break-resistant and sealed.
6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the BSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.
9. A dedicated, non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag (agriculture) labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housing must be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA-filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to ensure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid waste must be documented. The decontamination process for liquid waste must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double door, passing through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory.

Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bio-seal must be durable, air-tight, and capable of expansion and contraction. Positioning the bio-seal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress ¹ must be developed and implemented.

5.3 Inventory Control Procedures

- Written records of biohazardous materials must be updated each time they are used, received and destroyed.
- Inventories of biohazardous material must be current and available for review by the IBC Chair and other inspection agencies.
- The Biosafety Officer (BSO) is responsible for having secure duplicate inventory records of biohazardous material and keeping them indefinitely.
- There must be adequate training for those who are working with biohazardous materials.
- Anyone working with biohazardous material must have a laboratory emergency plan.

The inventory shall keep track of the following:

- Name of material
- Catalogue number and lot number if known

- Quantity
- Source, commercial or private
- Date material received
- Date material used
- Name of person using
- Record of transfers within and outside of the institution
- Labeling of samples
- Date material destroyed and decontamination method
- Where material may be stored

CHAPTER 6 – BIOHAZARDOUS WASTE MANAGEMENT

For disposal of all non-biohazardous and ethidium bromide waste please refer to the Chemical Laboratory Safety and Methodology Manual

A biohazard is a biological agent or substance present or arising from the work environment which presents or may present a hazard to the health or well-being of the worker or community.

ASU follows the Laboratory Biosafety Manual Guidelines which require that any biohazardous material must not be disposed of with regular waste and must be decontaminated before disposal or cleaning for reuse. This is to protect individuals and the community from unnecessary exposure to such material. The laboratory using this material is responsible for proper treatment and disposal.

Biohazardous waste encompasses the following material:

- Animal anatomical waste such as body parts, carcasses, organs.
- Non-anatomical waste such as sharp and disposable instruments and equipment which have contacted animal or human blood, biological fluids, or tissues.
- Tissue or microbial cultures and material contaminated by such cultures.
- Live vaccines.
- Containers or materials saturated with blood products.
- Please see the ASU Biosafety Committee Chair regarding the disposal of any human anatomical waste.

6.1 Biohazardous Waste Handling

- Biohazard waste must not be stored for any length of time and should be disposed of frequently to reduce the accumulation of biohazardous waste in work areas. Hazardous biological waste must be segregated from non-hazardous waste in appropriate containment and is essential in reducing pollution.
- If waste material must be stored before decontamination, it must be in a locked area at 4 °C or lower and treated as soon as possible, with no longer than 30 days of storage.
- All stored biohazard waste must be in sturdy leak-proof and puncture-proof containers and identified as to the contents, the

name of the user and the date of storage. A Biohazard symbol must be displayed on the outer container.

6.2 Biohazardous Waste Containment

Unlike chemicals, infectious material has the ability to replicate, thus there is no “safe” level for release into the environment.

Each lab must have well defined procedures for waste disposal to protect workers, maintenance and cleaning staff from exposure to biohazardous materials.

Principal Investigators and Supervisors are responsible for ensuring that all employees and students are trained and familiar with proper waste disposal procedures and that all lab procedures are in conformance with these requirements.

- There are approved, specified containers in each lab for segregating waste at ASU.
- The designated sharps container on each lab bench. Sharps include needles and syringes, scalpel and razor blades, and clinical items that may puncture, such as Pasteur pipettes.
- Waste containing liquids must be in leak-proof, unbreakable containers prior to disposal.

Biohazard Contaminated Waste NON-TISSUE	Debris Contaminated with Biohazardous or Nuisance Biological Material, but no Tissue	Yellow Biomedical Waste Bucket
Biohazardous Waste TISSUE	Biohazardous and Nuisance Biological Waste Associated Debris	Red Biomedical Waste Bucket

6.3 Decontamination of Biohazardous Waste

Decontamination is considered a procedure in the containment barrier to prevent occupational exposure to infectious agents and/or the accidental release of agents. Approved decontamination methods include autoclaving (heat sterilization), dry heat or gas sterilization, incineration or disinfection with the appropriate chemical. Each laboratory is responsible for their decontamination procedures, which should be part of the laboratory

procedures, and lab workers must be trained in these procedures.

Mixed waste such as infectious waste that is radioactive should be chemically inactivated with 10% chlorine bleach before being labeled and disposed of as radioactive waste. **Do not autoclave radioactive waste.** For decontaminated biohazard waste disposal, please contact the radioactive committee at ASU.

6.4 Transport of Biohazardous Waste

Precautions must be taken whenever biohazardous waste is moved from one area to another to control the risks of leakage or spillage and to contain any leaks or spills. Limit the number of moves, and use leak-proof and break resistant containers that are closed securely. Use carts that have guard rails or raised edges for secure transport. The SOP (Appendix D) was implemented by IBC at ASU.

CHAPTER 7 – EQUIPMENT

7.1 Biological Safety Cabinets (BSC)

Types and Classes of BSCs

There are 3 classes of BSCs Class I, Class II (with many subtypes within) and Class III. Please refer to the Biosafety Officer to ensure you are working with the correct Class of BSC. More detailed information can be found in the Laboratory Biosafety Guidelines.

Class I cabinets have un-recirculated airflow away from the operator that is discharged to the atmosphere after filtration through a HEPA filter. They provide good operator protection but do not protect the material within the cabinet (the product) from contamination.

Class II cabinets are designed for personnel, product and environmental protection. They are designed for work involving microorganisms in containment levels 2, 3 and 4 laboratories and are divided into two types (A and B) on the basis of construction type, airflow velocities and patterns, and exhaust systems.

Class III cabinets are totally enclosed and gas-tight with HEPA-filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. The cabinet is kept under negative pressure of at least 120 Pa (0.5 in. w.g.), and airflow is maintained by a dedicated exterior exhaust system.

Containment

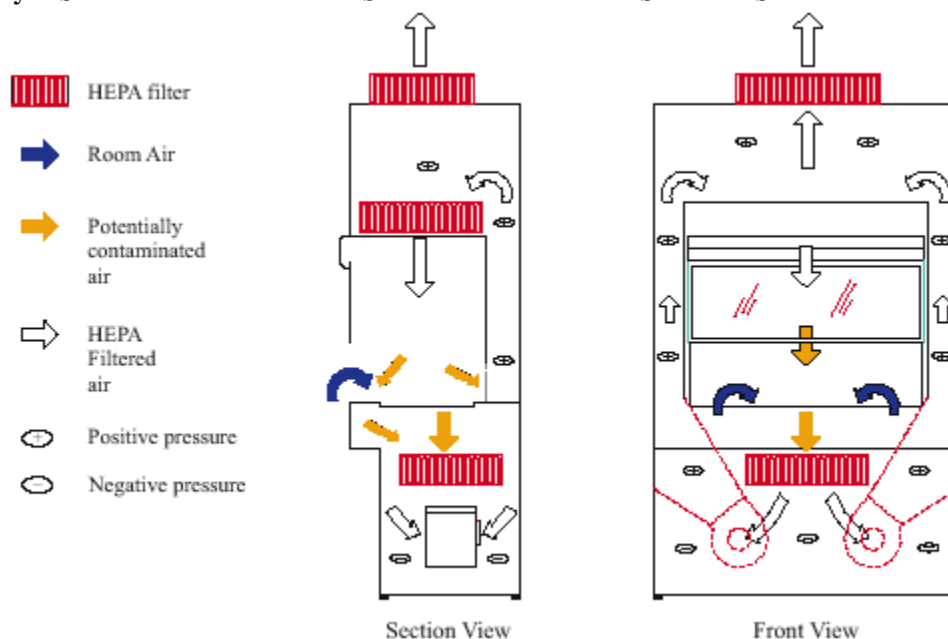
Biological safety cabinets provide containment for aerosols and separate the work material from the worker and the environment while providing clean air within the enclosed area.

How it works

These cabinets contain fans that direct air into a non-turbulent curtain which is called a laminar air flow. They provide effective containment and are designed to provide protection for workers, the material and the environment when users observe basic guidelines for their use.

Filtered air is drawn into the cabinet across the work surface and the air is re-filtered before it is vented out. Thus, it will maintain a sterile work area by providing a curtain of air between the worker and the material. Most of these cabinets have a UV light for extra protection which is turned off when the cabinet is being used.

Some BSCs are designed to provide only a sterile work surface and do not protect the worker. One must understand what level of cabinet is being used and how to use and operate it correctly for maximum protection. Within type (A), there are two subtypes, A1 (formerly designated type A) and A2 (formerly designated type B3). Within type (B), there are two subtypes, B1 and B2. Class II cabinets are most commonly used in biomedical research laboratories because of their characteristics.



Use of a Biological Cabinet

Class II cabinets are appropriate for both microbiology and tissue culture work. They provide effective containment and a sterile air supply inside.

- Understand how the BSC works and know what level cabinet is being used.
- Minimize the storage of materials inside and around the BSC.
- Plan the work to be done inside.
- Assemble all materials that will be needed before starting work to avoid disrupting the air flow.
- Do not overload the cabinet.
- Avoid disrupting the air flow inside the BSC with items such as a micro centrifuge.
- Be sure that all vents and airfoils are clean and unblocked.
- Segregate contaminated items from clean items.
- Use a horizontal pan with disinfectant inside the BSC for pipette discard.
- Do not use a Bunsen burner in the cabinet; alternatively, use a micro-incinerator for sterilizing inoculation loops.
- The cabinet must be turned on at least 5 minutes before starting work in

- order to purge the air and remove any particulates.
- The operator should wear a closed front lab coat or surgical gown and gloves which should overlap the cuffs.
 - All materials needed for the manipulations should be placed in the cabinet before work is initiated to minimize in- and- out motions.
 - Do not cover the air intake grill in front or the side vents.
 - The operator should work well into the cabinet and at least 4 inches from the front grill.
 - When in use, the lab entry door (especially in small rooms) must be kept closed and traffic must be minimized.
 - Do not have electric fans blowing in the room when the BSC is in use.
 - Develop appropriate procedures for the collection and decontamination of waste material to avoid clutter.
 - The cabinet must be decontaminated with an appropriate disinfectant after each use.
 - Periodic use of 1-10% household bleach in water is acceptable, but it is corrosive, so 70% ethanol may be used more frequently if effective against the agent.

Validation

Biological safety cabinets must be inspected and certified annually or when they are moved or repaired. This must be done by a qualified technician and the certification notice displayed on the cabinet.

NOTE: BIOLOGICAL SAFETY CABINETS CANNOT BE USED AS FUME HOODS AS THE HEPA FILTERS WILL BE DESTROYED.

7.2 Laboratory Fume Hoods

Containment

This is the fume hood's ability to contain gases and vapors for effective exhaust. It is usually measured in parts per million. Consistent, uniform airflow across the fume hood opening is a critical performance requirement to deliver safety to laboratory workers.

How It Works

The most common type of fume hood is known as a ducted fume hood, and draws air into the partially contained space through

the use of a blower or fan, dilutes the contaminated air with the ambient air and exhausts it to the exterior. The hood usually sits on a storage cabinet and the opening is covered by a transparent sash which is movable vertically across the opening. Raising and lowering the sash changes the air flow into the hood.

Use of a Fume Hood

Fume hoods must be used properly for optimum protection. The fan may be checked with a piece of tissue paper dangled¹ at the hood opening. It is vital to ensure that the type of fume hood being used is appropriate for the hazards being controlled. All supplies and equipment needed in the hood should be placed there at the beginning of the work to avoid moving the sash while chemicals are being used. Do not use the hood as a storage area.

Validation

It must not be assumed that because the fan is running the fume hood is functioning properly. Fume hoods must be certified annually by a qualified inspector to ensure that there is maximum protection for the worker, and this certification notice must be displayed on the cabinet. They must be recertified if they are moved or repaired in any way.

NOTE: FUME HOODS CANNOT BE USED AS BIOLOGICAL CABINETS!

7.3 Centrifuges

Centrifuges pose an additional potential hazard and must be monitored and used appropriately to ensure the containment of biohazardous materials and to prevent aerosol contaminants.

Users must work to minimize the risk of aerosols when centrifuging biohazardous material. Aerosols can be generated when filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, resuspending sedimented pellets and by the very process of centrifugation.

The high-speed spins generated by centrifuges can create large amounts

¹ Hang or swing loosely

of aerosols if a spill, leak or tube breakage occurs. The greatest aerosol hazard occurs when a biohazardous tube breaks during centrifugation. Risk introduced by mechanical failure is avoided by maintaining and inspecting the centrifuge before use.

As ultimately safe centrifuge use is the responsibility of the end users, all users of centrifuges must be adequately trained and familiarized with the manufacturer's instructions prior to handling the instrument.

Safety Considerations

Catastrophic failures of centrifuges are extremely rare, even with high-speed ultracentrifuges, but when an event does occur, it generally means the loss of the sample, bottles, potentially the rotor, and in the worst-case scenario, the centrifuge. There can also be great danger to nearby equipment and risk to personnel. For this reason, the following steps should be taken to reduce the risk of damage. Extra precautions must be taken with the handling of any biohazardous material that may contaminate the rotor and/or centrifuge in the event of a bottle breakage.

- Before use, inspect tubes, O-rings, and buckets for cracks, chips, erosions, bits of broken glass, etc.
- Use unbreakable tubes whenever possible
- The use of aluminum foil to cap biohazardous tubes is forbidden.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. They are flammable and prone to shrinkage with age, distort on boiling and are highly explosive in an autoclave.
- Centrifuge tubes, rotors, and accessories must be prepped and handled in the Biosafety cabinet before and after centrifugation.
- Avoid filling tubes to the rim.
- Once filled, centrifuge tubes must be firmly capped appropriately and wiped with disinfectant before loading in the rotor.
- Always balance buckets, tubes and rotors properly before centrifugation
- Never walk away from a centrifuge until it has reached its maximum set run speed.

- Do not open the centrifuge lid during or immediately after operation, attempt to stop a spinning rotor by hand or with an object or interfere with the interlock safety device.
- To avoid producing aerosols, do not decant supernatants by hand. If you must, do so very carefully. Use a vacuum system with appropriate in-line reservoirs and filters.
- Avoid vigorous shaking of tubes when resuspending packed cells.
- When centrifugation is completed, wipe tubes down with disinfectant before removing cap.
- Clean up any spills promptly. When using high-speed or ultra-centrifuges, additional practices should include the following:
 - Connect the vacuum pump exhaust to a disinfectant trap.
 - Record each run in a logbook to keep a record of run time and speed for each rotor.
 - Install a HEPA filter between the centrifuge and the vacuum pump.
- Never exceed the specified speed limitations of the rotor.

7.4 Autoclaves

Autoclaving is the most reliable method of destroying microorganisms. The items to be sterilized are heated in a chamber (the autoclave) with saturated steam at a pressure of 15 psi or higher for at least 15 minutes or longer after the temperature reaches 121 °C. The material must be in contact with the steam for the required length of time to be effective.

Biohazard material must be labeled as such, contained securely and processed as soon as possible. All users must have successfully completed an authorized training session on the safe operating procedures of the autoclave.

Monitoring

The sterilization process must be monitored to ensure the integrity of the process. This is done with Biological Indicators which are used regularly to ensure that the process has been effective and whether the sterilization has been successful or not. Biological Indicators are strips that have a set amount of bacterial spores packaged individually in a glassine pouch and are placed in the autoclave with the material to be sterilized. The strips are then incubated and the color indicator in them changes color if the

results of autoclaving are unsatisfactory. A positive control is incubated at the same time to ensure that the incubator is working properly. The monitoring is done weekly, monthly or quarterly depending on how often the autoclave is used. Monitoring is also done after installation of a new autoclave, repair, relocation, malfunction, or suspected failure and a positive control indication. Chemical indicators, which are usually on autoclave tape or strips are useful for distinguishing between processed and unprocessed items only, and do not guarantee an item is sterile.

Risks

Associated risks with using an autoclave can be heat burns, steam burns, hot fluid scalds, hand and arm injuries from the autoclave door and body injury if there is an explosion. There is also some risk if there is exposure to human pathogens.

Autoclaves are inspected regularly and certified with biological indicators. The name of the person responsible for the autoclave shall be posted near the autoclave. PPE such as heat insulating gloves, lab coats and closed toed footwear must be worn when loading and unloading the autoclave.

Material Preparation

- Ensure that the material is able to be autoclaved.
- Do not autoclave material that contains solvents or substances that may emit toxic fumes.
- Do not autoclave any strong oxidizing agent (bleach) or material with organic material (paper, cloth, etc.) as there is risk of explosion.
- Always consult the Radiation Safety Officer before treating radioactive biological waste for disposal, as the material must be inactivated first before any sort of treatment for disposal.
- Do NOT overload autoclave with too many materials to allow steam circulation.
- Loosely close autoclave bags to allow steam penetration and leave caps or covers on containers loose.
- Do not mix incompatible materials in the autoclave.
- Glassware must be heat-resistant borosilicate and inspected for cracks prior to autoclaving.
- Plastics must be heat-resistant, e.g., polycarbonate (PC), PTFE and most polypropylene (PP) items.
- Sharps must be in a designated “Sharps” container.
- Containers of liquid must be no more than 2/3 full with lids loosened to allow steam to penetrate.

- Place material in low sided autoclavable containers. Place containers of liquid and other materials that may boil over or leak in a secondary pan in the autoclave. The pan must be large enough to handle any leaks or spills.
- Follow autoclave unit procedures carefully to ensure proper and safe sterilization.
- Use chemical indicators (i.e. autoclave tape) with every load to verify autoclaving operation.
- Add water to containers as appropriate.
- Every autoclave must have a Standard Operating Procedure in place with the correct operating procedures, safety concerns and a contingency plan.
- Wear appropriate heat-insulating gloves, lab coats and closed-toe shoes when loading and unloading the autoclave.
- Allow 10 minutes after the run is completed before opening the door carefully and then allow any trapped steam to escape from hot liquids.
- Let liquid loads stand for a full hour before touching with ungloved hands.
- Check autoclave log paper after each run to ensure that the program was successful.
- Record use of the autoclave in logbook provided, which should contain operators name, date, time, and duration of run.
- Do not attempt to operate the autoclave if there is a malfunction.
- Report any issues to the autoclave supervisor. Efficacy Monitoring Autoclaves must be monitored on a regular basis to ensure that they are decontaminating infective material properly. This is a typical procedure to check the efficacy.
- Biological indicators containing 10^4 to 10^6 cfu/ml of *B. stearothermophilus* spores are placed in the center of a typical load. A control indicator is left outside the autoclave.
- The load is processed according to standard operating procedures, usually at a temperature of 121o C for 15 minutes.
- After cycle completion, the biological indicators in the autoclave are retrieved and incubated with the control at 56o C for a minimum of 24 hours.
- A color change of the autoclaved indicator after incubation means there is growth and sterilization failure. Absence of color change indicates that sterilization of the load was complete. There should be a change in color of the control that was not autoclaved.
- Failure of sterilization may be due to overloading or improper

loading of the autoclave, insufficient sterilization time or malfunction of the autoclave.

7.5 Microwave Ovens

Microwave ovens are commonly used in laboratories for heating and dissolving materials. There are some safety issues with their use in this environment and one should be aware of them.

Safety concerns

- Do not use microwave ovens intended for food items to heat lab materials. Designated microwave ovens for food must not be in the laboratory.
- Superheated liquids can be dangerous. Be aware that all liquids can reach temperatures above their boiling point and can suddenly spill in the microwave. Always wear insulated gloves when handling these hot liquids.
- Remember to loosen lids before microwaving as explosions can occur.
- Never place metals or items containing metal components in the microwave oven.
- Allow hot liquids to cool slightly before handling or adding any components to a solution.

CHAPTER 8 – REFERENCES

U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. U.S. Government Printing Office, Washington, Biosafety in Microbiology and Biomedical Laboratories, The 6th edition, 2020.

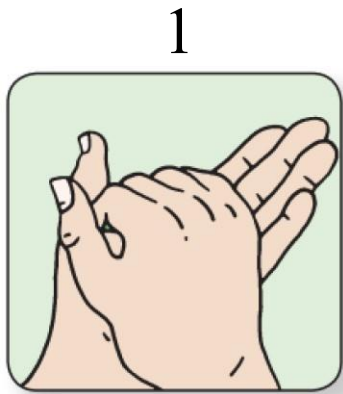
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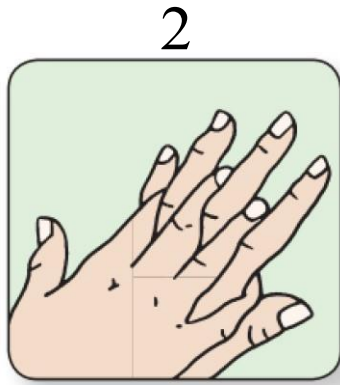
<https://www.who.int/publications/i/item/9789240011311>

APPENDIX A: HAND WASHING PROCEDURES FOR LABORATORIES

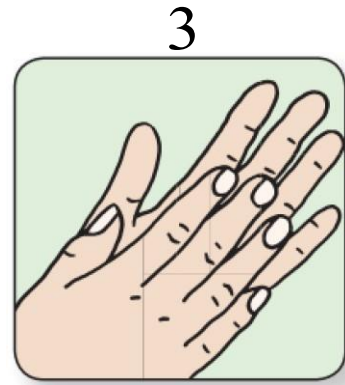
This procedure should be followed when gloves are removed and before leaving the laboratory. 90 seconds is the recommended time for washing your hands, as it has been shown to remove more pathogens. It is also recommended that a surgical scrub brush be used around the nails and when there are cracks from dry hands.



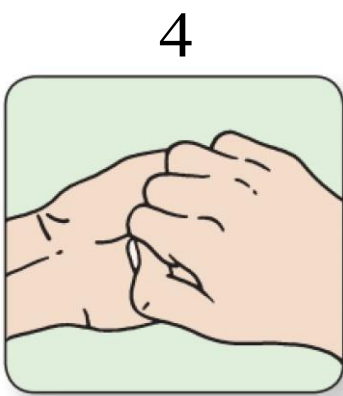
Palm to palm.



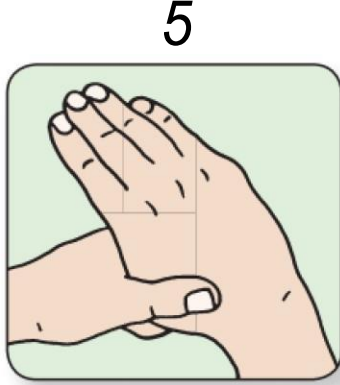
Right palm over left dorsum and left palm over right dorsum.



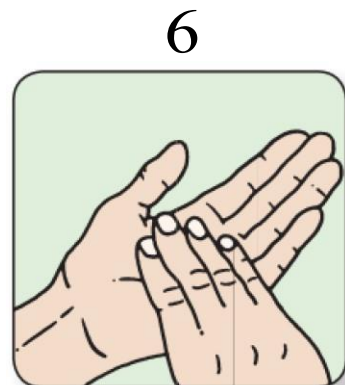
Palm to palm, fingers interlaced.



Backs of fingers to opposing palms with fingers interlocked.



Rotational rubbing of right thumb clasped in left palm, then vice versa.



Rotational rubbing, backwards and forwards with clasped fingers of hand in left palm then vice versa.

APPENDIX B: GENERAL LAB PRACTICES AND PROCEDURES

The following sections describe practices that should be performed regardless of the biological risk groups being manipulated in the lab.

PPE requirements and procedures:

- All lab members, visitors and other individuals entering the laboratory must wear the appropriate PPE in the lab. The PPE must then be removed when exiting the lab.
- Appropriate PPE must be worn when there is the known or potential risk of exposure to splashes or flying objects.
- Gloves (e.g., latex, vinyl, co-polymer) must be worn for all procedures where there may be direct skin contact with biohazardous materials. Gloves must be removed prior to leaving the laboratory and disposed of appropriately (in the biohazard container).
- Gloved hands must not touch common equipment and items such as telephones, elevator buttons or doorknobs.

Practices to minimize exposure and spread of biohazards:

- Hands must be washed when gloves are removed, prior to leaving the laboratory, where hands may come into contact with contaminated materials.
- No eating, drinking, smoking or mouth pipetting in the lab.
- Food, drinks and the storage of personal belongings are not permitted in the lab.
- Applying contact lenses and hand creams in the lab is strictly prohibited. Contact lenses can only be worn if other forms of corrective eyewear is not suitable.
- When working in the lab, long hair must be tied back or restrained so they do not get into contact with equipment, specimens or containers.
- Open wounds, cuts, scratches and grazes should be covered with waterproof dressings.
- Laboratories need to be kept clean and tidy and the storage of materials that are not pertinent to the work and cannot be easily decontaminated (e.g., journals, books, correspondence) should be minimized.
- Whenever possible, paperwork and report writing should be kept separate from biohazardous materials work areas.
- Only authorized personnel are allowed in the lab.
- Doors to the lab must remain shut.

- Leak-proof containers must be used whenever transporting items within facilities.
- Needles must never be recapped.

Decontamination and Disinfection Procedures:

- Work surfaces need to be decontaminated after each working day.
- Materials leaving the lab must be decontaminated and disinfected
- Autoclaves must undergo regular efficacy testing. These tests need to be documented and kept on file. Cycle logs (for temperature and pressure) must also be maintained.
- Appropriate disinfectants must be accessible in areas where the biohazardous materials are being handled.
- Spills, accidents, exposure and loss of containment must be reported to the supervisor and documented.
- Known or suspected contaminated clothing must be decontaminated prior to laundering.

APPENDIX C: working with human blood and body fluids

As human blood and body fluids could potentially contain pathogens that could infect the user, it should be worked with under containment level 2 requirements and practices. In addition, the following specific practices should be followed when handling human blood.

General information:

- Wear appropriate PPE (at a minimum, this should include: lab coats, long pants/skirts, appropriate footwear and gloves).
- Work in containment level 2 facilities with containment level 2 practices.
- Use the appropriate biohazardous waste bags.
- Decontaminate all waste appropriately either through autoclaving or chemical disinfection.
- Wash your hands after removing gloves and handling contaminated or potentially contaminated materials.
- Obtain Hepatitis B vaccination and other medical surveillance as deemed appropriate.
- Always treat all needles and sharp as if they have been contaminated.
- Never recap or purposely bend, shear, or break needles.
- Always dispose of needles and sharps in a secure, leak-proof, puncture-resistant container

Acceptable sources of blood:

When doing experiments with human blood, the samples can only be obtained from the following sources:

- Commercial sources

Note: When using volunteers, the appropriate ethics approval must be completed.

First Aid measures when working with blood: Needle stick/Sharp injury:

- Apply first aid.
- Wash thoroughly with soap and water (do not use any other caustic solutions like bleach).
- Obtain medical attention immediately (Emergency Care).
- Obtain the appropriate medical screening.
- Report the incident to the supervisor and fill out the appropriate accident/incident forms.

- Eye splash: Flush the eye out at an eye wash station for 15-20 minutes.
- Obtain medical attention immediately (Emergency Care)
- Obtain the appropriate medical screening.
- Report the incident to the supervisor and fill out the appropriate accident/incident forms. Skin exposure:
 - Wash thoroughly with soap and water (do not use any other caustic solutions like bleach).
 - Obtain the appropriate medical screening.
 - Report the incident to the supervisor and fill out the appropriate accident/incident forms.

Standard Operation Procedure (SOP)

Transporting biohazardous waste across ASU campus for autoclaving

Prior to preparing to transport biohazardous waste from your facility to the Life Science Building (LSB), you must first make arrangements to secure a time slot for autoclaving. This ensures that the waste will go directly into the autoclave upon arrival. To arrange an autoclaving time slot, please contact the C-STEM laboratory manager at 334-604-9324.

- **Packaging the biohazardous waste:** The biohazardous waste must be collected in a certified biosafety bag, which should be closed when it is filled to no more than 2/3 of its volume.
- **Closing the Biosafety bag:** Gather top of the bag, twist it, and close with an overhand knot to minimize the risk of leaks and spills (Do not tie the bag by crossing tabs).
 - Double-bag the biosafety bag and close the second bag as indicated above.
 - *Biohazardous waste bags awaiting transfer should always be stored in pans or secondary containment to prevent spills!*
- **Transporting the Biosafety bag:** Place the biohazardous waste bag into a break-resistant secondary container with a proper lid and close the lid tightly.
 - The volume of the secondary container should be at least 10% larger than the volume of the biohazardous content.
 - If the biohazard waste contains fluid, line the secondary container with an absorbent tissue to anticipate potential leaks or spills.
 - Before leaving the lab, wipe the outside of the secondary container with a

disinfecting solution, preferably 70% alcohol.

- Guidelines for transporting secondary waste container into the LSB for autoclaving.
 - Proceed to the backdoor of the LSB building.
 - Bring the waste container down the ramp behind the LSB and leave it at the back door.
 - Enter the LSB through the main entrance. (NOTE: Remove gloves before touching the door and have a clean set to use inside the building).
 - Once inside, go to the main office and ask for the key to the back door (Note: Inform office that you have an appointment to use the autoclave).
 - Retrieve the autoclave keys from the C-STEM lab manager.
 - Go to the basement and open the back door where the waste container is located.
 - Walk down the hallway until you reach a door that says Autoclave/Glass Equipment.
 - Get the cart located inside the room and bring it outside to load the biohazard waste container.
 - Go back inside the corridor and await further instructions from the C-STEM lab manager.

Procedure for Handling Biosafety Incidents or Violations

Internal Reporting

Internal reporting must occur as soon as a violation or incident is discovered.

- **Principal Investigator (PI)/Supervisor:** Any lab personnel (including students) who have a biosafety concern or have witnessed a biosafety violation must immediately inform their PI/supervisor. The PI must address the concerns or investigate and address the violation. In the case of a serious biosafety violation, the PI must recommend the violator for Biosafety retraining and forward a copy of the retraining certificate to the Institutional Biosafety Committee (IBC).
- **Anonymous Reporting**
Anyone who witnesses a biosafety violation on the ASU campus can directly notify the institutional Biosafety Committee (IBC, ibc@alasu.edu) in good faith without fear of retaliation.
- **IBC:** Violations that the PI cannot address must be reported to the IBC. The IBC will review the violation and determine the severity of the non-compliance.
- **Institutional Official:** In case of serious harm or recurring trends, the Director of the office of research and sponsored programs (ORSP) will be notified by the IBC.

Investigation and Resolution Process

When a violation is reported to the IBC, the following steps will be taken:

- **Initial investigation:** The IBC Chair will conduct a preliminary review to assess the severity and nature of the non-compliance.
- **Committee Review:** The full IBC will investigate the event, which may include interviewing personnel and inspecting the laboratory/facility.
- **Determination:** The IBC will determine if the violation was "serious" or "continuing" and outline the required Corrective Action Plan.
- **Sanctions:** In case of recurring severe violations, the IBC may suspend or terminate the biosafety approval for the project or advise the University officials to restrict laboratory access until compliance is re-established.

External Reporting Requirements

ASU is legally required to report specific types of violations to the NIH Office of Science Policy (OSP).

- **Immediate Reports:** Spills or accidents in BSL-2 labs involving overt exposure must be immediately reported.
- **30-Day Reports:** Any significant problems, violations of the NIH Guidelines, or research-related accidents/illnesses constitute a trigger for a mandatory 30-day formal report. These include:

a. Administrative & Regulatory Violations

- ***Lapse in IBC Approval:*** Conducting research with biohazards without prior IBC approval.
- ***Unauthorized Changes:*** Significant modifications to a research project's risk profile or scope without prior notification and approval from the IBC.
- ***Training Failures:*** Failure of the PI to ensure that all laboratory personnel are adequately trained in required biosafety practices and techniques.

b. Containment Breaches

- ***Environmental Release:*** Any accidental release of a Risk Group 2 or 3 agent outside of primary containment (e.g., a spill outside of a Biosafety Cabinet).
- ***Equipment Failure:*** Malfunctions in critical safety equipment, such as a failed centrifuge or a biosafety cabinet that was not certified annually as required.
- ***Improper Disposal:*** Failure to follow prescribed sterilization or disposal procedures for biohazardous waste, including rDNA materials.

c. Animal & Plant Incidents

- ***Transgenic Escapes:*** The escape or improper disposition of a transgenic animal (e.g., a mouse) or the unauthorized release of transgenic plants, seeds, or pollen.
- ***Animal Bites/Scratches:*** Injuries sustained from research animals that are infected or contain rDNA molecules, which are often classified as overt exposures.

d. Personnel Exposures (Standard & Potential)

- ***Potential Exposure in BSL-2:*** A potential exposure in a BSL-2 lab (e.g., a splash to the face that did not result in an

infection) must still be documented and reported within 30 days.

- ***Research-Related Illness:*** Any illness in lab personnel that can be reasonably linked to the biological agents used in the research.