



LAMAR UNIVERSITY

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Biological Safety Plan

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INTRODUCTION

The University is committed to providing a safe and healthy learning, teaching and research environment. The goals of the University's biological safety program are to protect the researchers, staff, and students from exposure to infectious agents, to prevent environmental contamination, to enhance the research atmosphere, and to comply with federal, state, and local regulations.

The Biological Safety Plan was developed by the Office of EHS & Risk Management and LU's Institutional Biosafety Committee (IBC). The manual provides university safety guidelines for those working with biohazards. This manual outlines general policies and procedures for using and disposing of infectious or potentially infectious materials according to applicable regulatory guidelines and LU's policy and procedures.

Laboratories must comply with the biological safety practices and procedures outlined in this manual. Principal Investigators (PIs) must contact EHS & Risk Management if they are uncertain how to categorize, handle, store, treat or discard any biologically derived material. PIs should use the information in this manual to develop site specific safety procedures for their laboratory.

EHS & Risk Management has the overall responsibility for the control of biohazards including the establishment of relevant policies and procedures. All University departments with responsibility for any aspect of biohazards or potentially infectious materials must coordinate their activities through EHS & Risk Management and Research & Sponsored Programs.

ADMINISTRATION AND RESPONSIBILITIES

EHS & Risk Management

EHS & Risk Management provides services, advice and compliance assistance to ensure employees, students, and visitors follow safe work practices when working in research laboratories. The Biological Safety Program within EHS & Risk Management monitors compliance with University safety policies and procedures regarding potentially infectious and biohazardous materials. The Biological Safety Program is designed to assist PIs and laboratory personnel in the selection of safe laboratory controls and practices that will ensure a safe working and learning environment for the University. **The Biosafety Officer (BSO) (a position currently held by the Building and Laboratory Safety Coordinator)** develops and conducts appropriate training programs to promote techniques for the safe handling and disposal of potentially infectious and biohazardous materials. The BSO works in conjunction with the Institutional Biosafety Committee to approve the use of infectious agents, biohazardous materials and recombinant DNA on campus. The BSO conducts laboratory safety audits to ensure compliance with policy and procedures and will investigate all reported accidents which may result in personnel or environmental exposure to biohazardous materials.



Deans/Department Chairs

Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their schools or departments. They should be aware of and approve all research conducted under their purview. They must ensure departmental compliance with applicable laws, regulations and guidelines covering the use of biological agents in their facility.

Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is charged by the University Provost to formulate policy and procedures related to the use of biohazardous agents, including: human pathogens, viruses, biological toxins and recombinant DNA (rDNA). As mandated by the [National Institutes of Health](#), experiments involving human gene transfer, formation of transgenic animals and the generation of rDNA or synthetic nucleic acid molecules must be reviewed and approved by the IBC. There are certain experiments that are exempted from NIH guidelines but these low risk projects must still be registered with the IBC so the committee can keep track of rDNA protocols on campus. In addition, all experiments involving infectious agents needs to be approved by the IBC before work can begin. The IBC application to register research projects is available for Research and Sponsored Programs Compliance.

Research & Sponsored Programs

Research & Sponsored Programs oversees funding for research projects that are awarded federal and state grants or use University money to conduct research at Lamar University. They coordinate the **Institutional Animal Care and Use Committee (IACUC)**, the **Institutional Biosafety Committee**, and the **Institutional Review Board (IRB)** for research projects involving animals or human subjects. They work in conjunction with EHS & Risk Management to ensure that compliance requirements are met before funding is released.

Principal Investigators

Principal Investigators (PIs) are ultimately responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratories to ensure a safe work environment for staff, visitors and students. This responsibility may not be shifted to inexperienced or untrained personnel. The PI must develop specific standard operating procedures for the research project and outline proper emergency procedures in case of accidental exposure of personnel or the environment to biological hazards. PIs must provide training to all personnel working in the lab so they have a complete understanding of the lab procedures and techniques. PIs must also document that all personnel have had said appropriate safety training. All protocols involving work with potentially infectious agents must be submitted to the IBC for review and approval.

**Employees/Students**

Employees/Students are responsible for complying with safety guidelines and procedures required for the tasks performed and reporting unsafe conditions to the PI or EHS & Risk Management. They should seek guidance from their PI or EHS & Risk Management when they are uncertain how to handle, store or dispose of any hazardous or biohazardous material. They should not begin working in the laboratory until all technical and safety training has been completed.

CLASSIFICATION OF BIOHAZARDS

Bacteria, viruses, fungi or other infectious agents are studied in the laboratory to find a cause or cure for the diseases associated with these organisms. Since many of these agents are pathogenic to humans, animals, or other forms of life, their use poses risks, which vary with each agent and the way it is used. The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled [*Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)*](#), recommends that work be done using one of four levels of containment (see Biosafety Levels). The [*NIH Guidelines for Working with rDNA or Synthetic Nucleic Acid Molecules*](#) also classifies pathogenic agents into one of four risk groups according to specific criteria (see Risk Groups). It is LU policy that all laboratories adhere to these CDC and NIH guidelines.

Microorganisms

Common microorganisms that cause disease have been classified by the CDC and NIH according to their risk associated with severity of disease. A list of those organisms can be found in Appendix A. The American Biological Safety Association and Health Canada have also grouped organisms according to their risk. New or unknown pathogens that have not been assessed by CDC/NIH must go through a risk assessment to determine their biosafety containment level. PIs must register any project involving a pathogenic agent with the Institutional Biosafety Committee (IBC) and receive their approval before work is initiated. Following receipt of the completed IBC Registration, the laboratory will be surveyed by the BSO to ascertain that it meets the containment requirements listed in BMBL for the agent being studied. The BSO will report the findings to the IBC as part of the review and approval process.

Genetically Modified Organisms

The in-vitro incorporation of segments of genetic material from one cell into another known as recombinant DNA (rDNA) technology has resulted in altered organisms which are used to manufacture products such as vaccines, hormones, interferons and enzymes. Genetically modified organisms are used for treatment of diseases, for cleaning up hazardous waste and spills, or for crops and other plants resistant to cold, disease, pests and drought.

However, rDNA technology carries with it the potential for harm. A genetically altered organism may be directly pathogenic or toxic and if released into the environment, might crowd out beneficial organisms, transfer undesirable genetic traits to wild species or mutate into a pathogenic form. The risks associated with recombinant DNA technology are to be assessed by the PI when registering their projects with the IBC.

Human Blood, Blood Products, Body Fluids and Tissues

Research projects that utilize human blood, blood products or potentially infectious body fluids are governed by the OSHA [Occupational Exposure to Bloodborne Pathogens](#) Standard. This federal regulation mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help reduce occupational exposure to human blood and other potentially infectious materials which may cause Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. PIs using human blood, blood products, body fluids or tissues must register with the IBC and complete an [Exposure Control Plan](#). The completed plan must be readily available in the laboratory for all workers. Laboratory personnel (faculty and staff) who work in HIV or HBV research laboratories must fulfill additional OSHA requirements as outlined in the LU Exposure Control Plan.

Prions

Some progressive neurological diseases are caused by unconventional proteinaceous infectious particles called prions. Prions have been associated with transmissible degenerative diseases of the central nervous system in humans (Creutzfeldt-Jacob, kuru) and animals (mad cow disease, transmissible encephalopathy of mink and scrapie in sheep and goats). These unconventional agents are resistant to destruction by chemical (10% formalin, glutaraldehyde, 70% ethanol, iodine) and physical (UV light, ionizing radiation, boiling) procedures. While there have been no documented cases of laboratory-acquired infections, extreme precautions should be taken when handling material from infected or potentially infected humans and animals. The guidelines in the [CDC](#) and [World Health Organization](#) publications should be used by the PI for the risk assessment.

Select Agents

The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules, which implement the provisions of the *USA PATRIOT Act* and *Public Health Security and Bioterrorism Preparedness and Response Act of 2002* setting forth the requirements for possession, use, and transfer of [select agents](#) and toxins. The select agents and toxins identified in the final rules have the potential to pose a severe threat to public health and safety, to animal and plant health, or to animal and plant products (see Appendix B). The final rules ([42 C.F.R. Part 73](#), [7 C.F.R. Part 331](#), and [9 C.F.R. Part 121](#)) were published in the Federal Register on March 18, 2005. The regulations state that anyone involved with the possession, use or transfer of select agents must be registered with the CDC or USDA. This registration involves extensive paperwork, background checks, security plans, record keeping and inspections. PIs must contact Research & Special Programs Compliance to register with the IBC and the appropriate federal agency prior to sending, receiving or working with any select agents.

Tissue Cultures

Cell cultures derived from humans or animals known to be infected with a pathogen, as well as cultures known or suspected to contain infectious microorganisms (e.g., herpesvirus or EBV-transformed cultures) should be assigned to the risk group appropriate for the suspected or known pathogen and handled using the relevant containment level and work practices. Repositories such as the American Type Culture Collection (ATCC) can provide information on some of the cell lines used in the lab.

In addition, mammalian cell cultures may carry unsuspected oncogenic, allergenic or infectious particles. It is impractical, if not impossible, to screen such cultures for all potentially harmful microorganisms; even well-characterized lines with a history of safe use can become contaminated by adventitious, possibly infectious, microorganisms. For this reason, it is prudent to treat all mammalian cultures as potentially infectious and to use biosafety level 2 facilities and work practices whenever working with them.

Use of Animals

The use of animals in research requires compliance with the [Public Health Service \(PHS\) Policy on Humane Care and Use of Laboratory Animals](#) and any state or local regulations covering the care or use of animals. All animal protocols involving the use of rDNA, infectious agents, human blood, and toxic chemicals must be submitted to IBC for review and approval prior to final approval by the [Institutional Animal Care and Use Committee \(IACUC\)](#). Working with animals in the wild requires different safety measures than working in the laboratory, refer to the Wildlife section for further details.

Viral Vectors

These vectors provide a broad spectrum of uses in both basic and clinical sciences in allowing both the transient and long term expression of almost any gene of interest in specific tissues either in culture or in vivo. Recent research using viral vectors has proven that viral reagents have broad potential applications for the study of disease and normal cellular processes. Special care should be given to the design, risk assessment (Section 2 of NIH Guidelines), and handling of virus vectors containing genes that make growth-regulating products, products released into the circulation, or products that may have a general effect on the host-immune system.

Adenovirus: Adenoviruses are infectious human viruses, which often cause mild respiratory illness. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices. Biosafety Level 2 (BL2) is appropriate for many constructs. Particular care should be given to vectors containing genes that make products that may be similar to products made by the deleted adenovirus genes.

Adeno-associated virus: These are infectious human viruses with no known disease association. Some AAV types are common in the general population, and these viruses have the ability to integrate into the host chromosome. [The NIH Guidelines \(Appendix B\)](#) state that "adeno-associated virus (AAV) types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a

potentially tumor gene product or a toxin molecule and are produced in the absence of a helper virus" can in most cases be handled at lower biosafety levels.

Herpesvirus: Herpesviruses include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most commonly used vector system. HSV-1 is common in the general population, but can cause encephalitis in rare cases; its utility as a vector system stems from its broad host cell range, ability to transduce neurons, and its large insert capacity.

Lentivirus: Lentiviruses are a subset of retroviruses, with the ability to integrate into host chromosomes, and to infect non-dividing cells. These viruses can cause severe immunologic and neurologic disease in their natural hosts. Lentivirus vector systems can include viruses of non-human origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human viruses (HIV). Typical lentivirus vectors take the form of virus pseudotypes bearing envelope proteins from vesicular stomatitis virus (VSV).

Poxvirus: Poxvirus vectors include avian viruses which cannot establish productive infections in humans, as well as mammalian poxviruses which can productively infect humans -- such as vaccinia virus and modified vaccinia viruses (MVA). Poxviruses are highly stable, and vaccinia virus can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus.

Retrovirus: These are infectious viruses which can integrate into transduced cells with high frequency, and which may have oncogenic potential in their natural hosts. Retrovirus vector systems are typically based on murine viruses -- most commonly, these systems include ecotropic viruses (which can infect only murine cells), amphotropic viruses (which can infect human cells) or pseudotyped viruses (which can also infect human cells). Containment for vectors with the ability to infect human cells will usually be recommended at biosafety level 2 (BL2), as per the NIH Guidelines.

LABORATORY INFECTIONS

A laboratory-acquired infection is defined as one that resulted from laboratory work, whether it occurred in a laboratory worker or in another person who happened to be exposed as a result of research or clinical work with infectious agents. If you are immune compromised, you are at a much higher risk of acquiring infections and you should meet with occupational health physician or your personal physician for a medical consultation to determine your risk of infection. Also, if you are pregnant, you should discuss the kind of work and materials that you are exposed to with your physician to determine the risk to you and/or your fetus.

Modes of Transmission

Microorganisms can enter the body through the mouth, the respiratory tract, broken or intact skin and the eyes. It should be noted that in laboratory-acquired infections, the route may not be the same as when the disease is acquired naturally.

Infectious materials and cultures of microorganisms accumulate in large amounts in clinical and microbiological laboratories and since it is necessary to transfer them from one container to another and to manipulate them in various ways, the potential hazards are increased when working with materials. That why it is so important to have documented and deliberate standard operating procedures that are used by Principal Investigators and lab workers to make certain that nobody is exposed to biohazards. Information about standard operating procedure design can be found in the chemical hygiene plan; the Biological Safety Officer can also help set up a design framework.

Infections preceded by overt personal accidents include:

1. Inoculation (resulting from pricking, jabbing or cutting the skin with contaminated instruments such as hypodermic needles, scalpels and glassware; and from animal bites or scratches).
2. Ingestion (resulting from mouth-pipetting, eating, drinking and smoking, which is why these practices are not permitted in the lab).
3. Splashing into the face and eyes.
4. Spillage and direct contact especially with skin that is cut open.

Infections not preceded by personal accidents:

1. Aerosols, droplets and fomites. Aerosols are defined as a cloud of very small liquid droplets produced whenever energy is applied to a liquid, and such liquid is allowed to escape into the environment. It has been shown that if the liquid contains infectious agents, these would be distributed in the aerosol and would remain viable for some time. The larger droplets (greater than 0.1 mm in diameter) will settle quickly and contaminate the surfaces upon which they come to rest. The smaller droplets can remain in the air for some time before being evaporated or settling on surfaces.

The infectious agents in the droplets remain in a dried state as "droplet nuclei" or fomites. The smaller the number of organisms and amount of dried material, the longer they will remain airborne, and they are moved around buildings by air currents generated by ventilation and people traffic.

It has been shown that many laboratory techniques using both simple and complex mechanical equipment, as well as laboratory accidents, produce aerosols. These include: use of microbiology loops, pipettes, syringes and needles, opening tubes and bottles, use of centrifuges and blenders, harvesting of eggs and other virological procedures, and breakage of culture tubes and vials. Because of these risks, routine disinfection and cleanup of spaces where biohazards are used is required.

Using Infectious Materials

Infectious materials must be clearly identified and stored in such a manner as to preclude accidental exposure. This normally includes double or secondary containment and labeling all samples stored in the lab and freezer/refrigerator where these samples are kept.

There are many regulations in place to forestall the problem of laboratory-acquired infections. However, the responsibility for compliance with the regulations to ensure a safe workplace lies primarily with the Principal Investigator and, secondarily, with the laboratory staff. In addition, it is crucial for the PI and laboratory staff to always bear in mind that a large number of organisms that would ordinarily be innocuous can be infectious for immunocompromised persons. Therefore, additional and more stringent measures must be established by the PI in an effort to prevent the occurrence of lab-acquired infections in such individuals.

RISK ASSESSMENT

It is the responsibility of the principal investigator to conduct a risk assessment to determine the proper work practices and containment requirements for work with biohazardous material. The risk assessment process should identify features of microorganisms as well as host and environmental factors that influence the potential for workers to have a biohazard exposure. The principal investigator should consult with a Biosafety Officer to ensure that the laboratory is in compliance with established guidelines and regulations. The following resources are used to assist in the risk assessment: [NIH Recombinant DNA Guidelines](#), [WHO Biosafety Manual](#) and the [Biosafety in Microbiological & Biomedical Laboratories, 5th ed. \(CDC/NIH\)](#). When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available. Factors to consider when evaluating risk include the following:

Pathogenicity: The more severe the potentially acquired disease, the higher the risk. Salmonella, a Risk Group 2 agent, can cause diarrhea and may progress to septicemia if ingested. Viruses such as Ebola, Marburg, and Lassa fever cause diseases with high mortality rates. There are no vaccines or treatment available. These agents belong to Risk Group 4.

Route of transmission: Agents that can be transmitted by the aerosol route have been known to cause the most laboratory-acquired infections. The greater the aerosol potential; the higher the risk of infection. Work with Mycobacterium tuberculosis is performed at Biosafety Level 3 because disease is acquired via the aerosol route.

Agent stability: The greater the potential for an agent to survive in the environment, the higher the risk. Consider factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfections when looking at the stability of an agent.

Infectious dose: Determine the amount of an infectious agent needed to cause infection in a normal person. An infectious dose can vary from one to hundreds of thousands of organisms or infectious units.

An individual's immune status can also influence the infectious dose.

Concentration: Consider whether the organisms are in solid tissue, viscous blood, sputum, etc., the volume of the material and the laboratory work planned (amplification of the material, sonication, centrifugation, etc.). In most instances, the risk increases as the concentration of microorganisms increases.

Origin: This may refer to the geographic location (domestic or foreign), host (infected or uninfected human or animal), or nature of the source (potential zoonotic or associated with a disease outbreak).

Availability of an effective prophylaxis or therapeutic intervention: Effective vaccines, if available, should be offered to laboratory personnel in advance of their handling of infectious material. However, immunization does not replace engineering controls, proper practices and procedures and the use of personal protective equipment (PPE). The availability of post-exposure prophylaxis should also be considered.

Medical surveillance: Medical surveillance programs may include monitoring employee health status, participating in post-exposure management, employee counseling prior to offering vaccination, and annual physicals.

Experience and skill level of at-risk personnel: Laboratory workers must become proficient in specific tasks prior to working with microorganisms. Laboratory workers may have to work with non-infectious materials to ensure they have the appropriate skill level prior to working with biohazardous materials. Laboratory workers must go through additional safety training before they are allowed to work with high risk materials or in a designated facility.

Risk Groups

Infectious agents are classified into risk groups based on their relative hazard. The table below presents the basis for the classification of biohazardous agents by risk group.

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)

The risk groups designation along with the risk assessment are the steps used to determine the

appropriate procedures and containment to use while working with a particular agent in the lab. Laboratories and animal facilities are classified according to their design features, construction and containment facilities to safely work with biological agents and infectious materials. These designations, called biosafety levels (BSL) and animal biosafety levels (ABSL) provide appropriate containment for the various risk group agents.

CONTAINMENT BARRIERS

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers and the outside environment to potentially hazardous agents. The three elements of containment include facility design, safety equipment and laboratory practice and techniques.

Primary containment is the protection of personnel and the immediate laboratory environment from exposure to infectious agents. It is accomplished by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials. It is accomplished by providing by a combination of facility design and operational practices. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of work practices, safety equipment and facility design to provide adequate containment.

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agent or infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop an operational manual which identifies specific hazards that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with the handling of infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular

agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.



Safety Equipment (Primary Barriers). Safety equipment includes biological safety cabinets, enclosed containers (i.e., safety centrifuge cups) and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. The BSC provides personnel, product and environment protection.

Safety equipment also may include items for personal protection such as personal protective clothing, respirators, face shields, safety glasses or goggles. Personal protective equipment is often used in combination with other safety equipment when working with biohazardous materials.

Facility Design (Secondary Barriers). The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory. Facilities must be designed to meet the requirements of the laboratory's function and the recommended biosafety level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and hand washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.



BIOSAFETY LEVELS

CDC describes four biosafety levels (BSLs) which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Biosafety Level 1 is appropriate for work done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended except for a hand washing sink. Agents can be used safely on the open bench. The standard microbiological practices are as followed:

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable state and federal regulations before removal from the facility.
8. An insect and rodent control program is in effect.



Biosafety Level 2 is applicable to work done with a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as biosafety cabinets. Primary barriers such as splash shields, face protection, gowns and gloves should be used as appropriate. Secondary barriers such as hand washing and waste decontamination facilities must be available. In addition to BSL 1 procedures, level 2 also requires the following special practices:

1. Access to the laboratory is limited or restricted by the PI when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The PI has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The PI establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by PI. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
6. The PI ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
7. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.



- b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes that re-sheath the needle, needleless systems, and other safety devices are used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.
8. Cultures, tissues, specimens of body fluids, or other potentially infectious materials are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
9. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
10. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the PI and EHS & Risk Management. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
11. Animals not involved in the work being performed are not permitted in the laboratory.

Safety Equipment (Primary Barriers)

12. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonic eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or



- centrifuge safety buckets are used, and if these rotors or safety buckets are opened only in a biological safety cabinet.
13. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
 14. Protective laboratory coats, gowns, aprons, or uniforms designated for laboratory use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; personnel should never take it home.
 15. Gloves must be worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the laboratory. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

Laboratory Facilities (Secondary Barriers)

16. Provide lockable doors for facilities that house select agents (as defined in 42 CFR 72.6).
17. Consider locating new laboratories away from public areas.
18. Each laboratory contains a sink for hand washing.
19. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
20. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
21. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
22. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
23. An eyewash station is readily available.
24. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
25. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward



flow of air without re-circulation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Biosafety Level 3 is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents (i.e., *Mycobacterium tuberculosis*, St. Louis encephalitis virus and *Coxiella burnetii*) include auto-inoculation, ingestion and exposure to infectious aerosols. Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment. Secondary barriers include controlled access to the laboratory, specialized ventilation system that minimizes the release of infectious aerosols, and a double door autoclave for decontaminated waste from the facility. In addition to BSL 2 procedures, level 3 also requires the following special practices:

1. Laboratory doors are kept closed when experiments are in progress.
2. The PI controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The PI has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.
3. The PI establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the PI or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.
5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.
6. A biosafety manual specific to the laboratory is prepared or adopted by the PI and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent



- exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.
8. The PI is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the PI or other competent scientist proficient in safe microbiological practices and techniques.
 9. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
 - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes that re-sheath the needle, needleless systems, and other safe devices are used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.
 10. All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.
 11. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
 - a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.



- b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.
12. Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
13. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
14. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided, and written records are maintained.
15. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Safety Equipment (Primary Barriers)

16. Protective laboratory clothing, such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.
17. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
18. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
19. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonic eggs, etc., are conducted in a Class II or Class III biological safety cabinet.
20. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety buckets or sealed rotors) are used.
21. Respiratory and face protections are used when in rooms containing infected animals.

Laboratory Facilities (Secondary Barriers)

22. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable. A clothes-changing room may be included in the passageway.
23. Each laboratory room contains a sink for hand washing. The sink is hands-free or automatically operated and is located near the room exit door.
24. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if



- present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of covered floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as those around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
25. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and other chemicals used to decontaminate the work surfaces and equipment.
 26. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
 27. All windows in the laboratory are closed and sealed.
 28. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfectant, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
 29. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily traveled laboratory areas.
 30. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not re-circulated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.
 31. HEPA-filtered exhaust air from a Class II biological safety cabinet can be re-circulated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.



32. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
33. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).
34. An eyewash station is readily available inside the laboratory.
35. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
36. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.
37. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

Biosafety Level 4 is applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Primary hazards to workers include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets and auto-inoculation. All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment. Isolation of aerosolized infectious materials is accomplished primarily by working in a Class III biological safety cabinet or a full-body, air-supplied positive pressure personnel suit. The facility is generally a separate building or a completely isolated zone within a complex with specialized ventilation and waste management systems to prevent release of viable agents to the environment. **At this time, Biosafety Level 4 work is not approved for Lamar University.**

Animal Biosafety Levels

There are four animal biosafety levels (ABSLs), designated Animal Biosafety Level 1 through 4, for work with infectious agents in animals. The levels are combinations of practices, safety equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. These levels are comparable to the BSL levels with additional measures taken for handling animals and zoonotic disease transmission. Further information can be found in the [CDC](#) book or through contact with the BSO.



Animal Biosafety Level 1 is suitable for work involving well characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

Animal Biosafety Level 2 is suitable for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

Animal Biosafety Level 3 is suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease.

Animal Biosafety Level 4 is suitable for addressing dangerous and exotic agents that pose high risk of like threatening disease, aerosol transmission, or related agents with unknown risk of transmission.

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) should be used to reduce employee exposure to hazards when engineering and administrative controls are not feasible or effective. PIs are required to determine all exposures to hazards in their workplace and determine if PPE should be used to protect their lab workers. The PPE must be appropriate for the task and users must be trained to understand the use and limitations of the protective gear. The PPE used in research laboratories includes: laboratory coats and gowns, eye protection, face shields and the appropriate gloves.

Coat/Gowns

The lab coat can be used to protect street clothing against biological or chemical spills as well as to provide some additional body protection. The specific hazard and the degree of protection required must be known before selecting coats for lab personnel. The CDC/NIH guidelines for bio-containment practices recommend the use of a lab coat, wrap-around gown, smock, or scrub suits while working in research labs. The lab coat can be disposable or should be washed regularly either on site or through a commercial laundry service. Contaminated lab coats should not be taken home or worn outside of the laboratory.

Eye/Face Protection Equipment

Goggles and face shields should be worn whenever procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of tissues, and manipulations of infectious materials. The PI has the responsibility to assess the potential for eye/face injuries, to train employees on the uses and limitations of PPE, to provide the type of protection required, and to ensure that the appropriate eye/face PPE is available and used by laboratory personnel.



All eye/face protection devices must meet the requirements set forth in the [ANSI Z87](#) standard. Contact EHS & Risk Management for additional information on the assessment, selection, and use of eye/face protection equipment.

Foot Protection

Safety shoes should be worn in any area where there is a significant risk of dropping heavy objects on the foot. For most biological lab use, comfortable shoes such as tennis shoes or nurses shoes should be worn. Sandals and other types of open-toed shoes are not permitted in labs using biohazards or chemicals, due to the potential exposure to infectious agents or toxic materials, as well as physical injuries associated with the work. Boots, shoe covers, or other protective footwear, and disinfectant footbath may be required for work in BSL3 labs or animal facilities.

Gloves

Skin contact is a potential source of exposure to infectious materials; it is important that the proper steps be taken to prevent such contact. Gloves should be replaced periodically, depending on frequency of use and permeability to the substance handled. Gloves should be taken off carefully to avoid contaminating hands and never reuse disposal gloves. Latex gloves are commonly used in the labs but may cause allergic reactions in certain sensitized individuals. Alternative gloves such as latex free or nitrile gloves should be available for use in the lab. Glove use should never replace the need for hand washing.

Gloves should also be worn whenever it is necessary to handle rough or sharp-edged objects, and very hot or very cold materials. The type of glove materials to be used in these situations includes, leather, aluminum-backed gloves, and other types of insulated glove materials.

Respirators

Respirators can only be used when it is not possible to minimize or eliminate exposure to a contaminant through other means. Respirators are used in biological laboratories when there is a potential for exposure through inhalation or in some cases to animal allergies. The selection and use of respirators must be done in accordance with [29 CFR§1910.134](#) and [LU's Respiratory Protection Policy](#). All individuals issued respirators must go through training, medical screening and fit testing to be approved to wear a respirator.

Cleaning and Maintenance

It is important that all PPE be kept clean and properly maintained. Cleaning is particularly important for eye and face protection where dirty or fogged lenses could impair vision. PPE should be inspected, cleaned, and maintained at regular intervals so that the PPE provides the required protection. Personal protective equipment shall not be shared between employees until it has been properly cleaned and sanitized. PPE will be distributed for individual use whenever possible.



SAFETY TRAINING

Human error and poor technique can compromise the best safeguards to protect the laboratory worker. Therefore, the key to the prevention of laboratory acquired infections and accidents, is to provide safety training to keep lab staff well informed about the recognition and control of laboratory hazards. An effective safety program begins with the Principal Investigator, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. PIs play the key role in training their immediate staff in good laboratory techniques.

Certain safety training is required by government regulations. For instance, OSHA requires annual training for those working on blood or other potentially infectious materials. Please contact EHS & Risk Management for further details.

DECONTAMINATION PROCEDURES

Sterilization, disinfection, and antisepsis are all forms of decontamination. Sterilization implies the killing of all living organisms. Disinfection refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. Antisepsis is the application of a liquid antimicrobial chemical to living tissue.

Chemical Disinfectants

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:

1. Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
2. Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.
3. Hospital Disinfectant - agent shown to be effective against *S. aureus*, *S. choleresis* and *P. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.
4. Antiseptic - agent formulated to be used on skin or tissue - not a disinfectant.

Disinfectants Commonly Used

1. Iodophors



- Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- Effective against vegetative bacteria, fungi, and viruses.
- Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- Stable in storage if kept cool and tightly covered.
- Built-in color indicator; if solution is brown or yellow, it is still active.
- Relatively harmless to humans.

2. Hypochlorites (bleach)

- Working dilution is 1:10 to 1:100 in water.
- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions should be made daily.

3. Alcohols (ethanol, isopropanol)

- The effective dilution is 70-85%.
- Effective against a broad spectrum of bacteria and many viruses.
- Fast acting.
- Leaves no residue.
- Non-corrosive.
- Not effective against bacterial spores.

Dilution of Disinfectants

1. Chlorine compounds (household bleach)

Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load or to disinfect liquid biological waste before disposal.

2. Iodophor

Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces into 5 gallons water.

3. Alcohols



Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant.



SPILL PROCEDURES

A spill kit should be kept in each laboratory where work with potentially infectious materials is conducted. Basic equipment includes: disinfectant (such as 10% chlorine bleach), a package of paper towels, gloves and goggles, biohazard bags, and forceps to pick up broken glass and possibly solidifier if necessary. Kits can be made up or bought commercially. Please contact EHS & Risk Management with questions about the use of a spill kit.

Lab Spill Cleanup Procedures

With due care, small spills can be easily cleaned up and contained with the supplies in the spill kit. Laboratory personnel should follow the procedures below for large spills and the explicit instructions outlined by the Principal Investigator.

1. Depending on the nature of the organisms or size of spill, you may need to hold your breath, leave the room immediately, and close door. Turn on ultra-violet light if these are present.
2. Warn others not to enter the contaminated area. If possible place warning signs.
3. Immediately remove contaminated clothing, place it in the biohazard bag and seal the bag. If an exposure has occurred, wash any exposed areas of the body with soap and water or take a shower if necessary. Avoid contact with other individuals as much as possible to prevent additional exposure.
4. Inform the Principal Investigator responsible for the area and if you need assistance with the clean-up contact EHS & Risk Management as soon as possible.
5. Wait at least thirty minutes to one hour for aerosols to settle and for the UV to act before re-entry into the contaminated area.
6. Put on a long-sleeved gown, mask, eye protection preferably goggles, rubber gloves, and shoe cover before re-entering the contaminated room.
7. Pour an appropriate decontaminate solution around the spill. Paper towels soaked with disinfectant may be used to cover the area. Avoid pouring the disinfectant directly onto the spill to minimize the generation of aerosols.
8. Let stand approximately thirty minutes to allow an adequate contact time.
9. Discard all materials from the spill in a biohazard bag and decontaminate any equipment that will be reused (i.e. dust pan).
10. All accidents, exposures, and potential hazards should be reported to EHS & Risk Management. In severe emergencies, telephone communication should be used to secure immediate medical care, decontaminating procedures or facility repairs.

Spills in a Biological Safety Cabinet

A spill that is confined to the interior of the Biological Safety Cabinet (not a Laminar Clean Bench) should present a little or no hazard to personnel in the area.



1. Chemical disinfectant procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinets.
2. The operator should wear safety goggles, gloves and other appropriate personal protective equipment during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant.
3. Immediately after a spill, the cabinet should be allowed to run for at least ten minutes to allow the cabinet to purge any airborne contaminants. Chemical decontamination procedures should be conducted while the cabinet continues to operate to prevent escape of contaminants from the cabinet.
4. Spray or wipe walls, work surfaces and equipment with appropriate disinfectant. Germicide should at least have a minimum contact time of ten minutes.
5. Flood the top work surface, tray, and if a BSL 1 cabinet, wipe the drain pans and catches basins below the work surface with a sponge or cloth soaked in a disinfectant. For BSL 2 cabinets, drain the tray into the cabinet base, take out the tray and remove exhaust grill work and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a disinfectant. Then replace in position and drain disinfectant from cabinet base into appropriate container and autoclave waste liquid. Gloves, cloth or sponge should be discarded in a biohazard container and autoclaved.
6. For BSL 3 or greater materials spill, decontaminate cabinet with formaldehyde and other appropriate means.
7. After contaminated gloves and clothing have been removed, wash arms, hands and face with soap and water.

LABORATORY EQUIPMENT

Autoclaves

Steam sterilization is a proven and economical process of killing microorganisms through the application of moist heat, time and pressure. Heat damages the cell's essential structures including the cytoplasmic membrane rendering the cell no longer viable. Autoclaves are classified as pressure vessels and because an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Please read the autoclave procedure section to prevent injuries or accidents when using the autoclaves on campus and to ensure that they are used properly.

Biological Safety Cabinet

The common element to all classes of biological safety cabinets is the high efficiency particulate air (HEPA) filter. This filter removes particulates of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases, but will remove particles smaller or bigger than .3 microns.



The biosafety cabinet requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least annually.

1. Class I cabinets protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or to the outside via the building exhaust.
2. Class II (Types A1, A2,B1, B2, and formally A/B3) biological safety cabinets provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environment protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).
3. Class III cabinets (sometimes called Class III glove boxes) were designed for work with infectious agents that require Biosafety Level 4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.
4. Horizontal laminar flow "clean air benches" are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a biological safety cabinet in research laboratories.

Operation of Class II Biological Safety Cabinet

1. Turn on cabinet fan 15 minutes before beginning work.
2. Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
3. Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)
4. Work as far to the back (beyond the air grill) of the BSC workspace as possible. Do not work in a BSC while a warning light or alarm is signaling.
5. Avoid using open flames inside BSCs. If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace. Flames disrupt the airflow and contribute



- to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.
6. Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a cardboard box) to prevent spilling.
 7. Wear gloves when there is potential for skin contact with infectious material.
 8. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
 9. When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.
 10. Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If a UV light is used, due to its limited penetrating ability, surfaces should be dust-free and the UV light tube should be wiped frequently with alcohol to remove dust. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior.
 11. The UV lamp should never be on while an operator is working in the cabinet.
 12. Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.

Centrifuge Containment

- Examine centrifuge tubes and bottles for cracks or stress marks before using them.
- Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.
- Centrifuge safety buckets and sealed rotors protect against release of aerosols.
- If high concentrations or large volume of infectious agents are used, open centrifuge/rotor only in a BSC.

Vacuum Systems

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, at BL2 and above, a vacuum line filter should be used.

Collection and Overflow Flasks

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows.
- If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- In BL2 and BL3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.



Vacuum Line Filter

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Gelman Vacushield are available from several scientific supply companies.

AUTOCLAVE PROCEDURES

Autoclaving Procedures

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. A combination of pressure, heat and time is needed to efficiently decontaminate waste loads so parameters must be made for each type of waste. Using an autoclave improperly can cause serious injury or contamination and can ruin the mechanical components. Before using the autoclave ensure that it is operating properly and determine the appropriate exposure time for the load. Appendix C has a SOP that can be placed near the autoclave.

Autoclave Log

Everyone using the autoclave needs to complete the Autoclave Use Log and when biological indicators are used the Biological Indicator Log needs to be completed. It is very important to complete these logs so there is a record of people using the autoclave in case it malfunctions or items are left in the unit.

Container Selection

- Polypropylene bags. Commonly called biohazard or autoclave bags, these bags are tear resistant, but can be punctured or burst in the autoclave. Therefore, place bags in a rigid container during autoclaving. Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.
- Polypropylene containers and pans. Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers, remove the lid (if applicable), turn the container on its side when possible, and select a container with the lowest sides and widest diameter possible for the autoclave.



- Stainless steel containers and pans. Stainless steel is a good conductor of heat and is less likely to increase sterilizing time, though it is more expensive than polypropylene.

Preparation and Loading of Materials

- Fill liquid containers only half full.
- Loosen caps, or use vented closures.
- Always put bags of biological waste into pans to catch spills.
- Position biohazard bags on their sides, with the bag neck taped loosely.
- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

Cycle Selection

- Use liquid cycle (slow exhaust) when autoclaving liquids, to prevent contents from boiling over.
- Select fast exhaust cycle for glassware.
- Use fast exhaust and dry cycle for wrapped items.

Time Selection

- Take into account the size (and thus surface area to volume ratio) of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- Bags of biological waste should be autoclaved for 30-50 minutes to assure decontamination.

Removing the Load

- Check that the chamber pressure is zero.
- Wear lab coat, eye and face protection, heat insulating gloves, and closed-toe shoes.
- Stand behind door when opening it, so any remaining steam does not impact you.
- Slowly open door only a crack. Beware of rush of steam.
- After the steam has been released, open autoclave door and allow liquids to cool for 20 minutes before removing.

Monitoring

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. Operators should ensure that each autoclave is routinely monitored as follows:



1. *Temperature Monitoring* - check indicating thermometers during each complete cycle to ensure the attainment of a minimum temperature of two hundred fifty degrees Fahrenheit (250°F) or (121°C) for at least one-half (1/2) hour or longer, depending on quantity and compaction of the load and in order to achieve sterilization of the entire load. Remember that greater time and/or temperatures may be necessary to effectively sterilize a load.
2. *Heat Sensitive Tape Monitoring* - use heat-sensitive tape or other device for **each load** that is processed to indicate the load has undergone the steam sterilization process. Remember this tape only indicates that the proper temperature has been reached, but does not indicate it was heated for the proper time.
3. *Biological Indicator Monitoring* - use a biological indicator such as *Bacillus stearothermophilus* placed at the center of a load processed under standard operating conditions to confirm the attainment of adequate sterilization conditions. If the autoclaves are run weekly, then the indicators should be performed monthly. If the frequency is less, than indicators are run quarterly or with each load as appropriate.

Annual Safety Inspection of Autoclaves

All autoclaves mechanical and monitoring systems should be serviced and checked annually by a service provider to ensure that the unit is running correctly. Check with the autoclave manufacture for service providers in the area.

EMERGENCY PROCEDURES

All accidents, exposures and needle sticks must be reported to EHS & Risk Management (Worker's Compensation Coordinator if employee) for correct follow-up and risk assessment. Depending on the injury and the materials used in the lab, different approaches can be taken on the type of first aid that can be utilized. When working with human material or known pathogens, seek medical attention immediately. Do not use strong disinfectants or scrub brushes that can abrade the skin as this can cause additional damage and increase the chances of pathogens penetrating the body and cause illness.

Severe Injuries

1. Call 911 for assistance and transportation to the nearest emergency room.
2. A department representative should accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.
3. Report accident to the PI and EHS & Risk Management.

Sharp Injury

1. Allow the wound to bleed under steady stream of water and wash area with soap and water.
2. Seek medical attention immediately.



3. Report the accident to the PI and EHS & Risk Management, and seek additional medical assistance if necessary.

Splashes to the Eye/Body

1. For eyes, immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. For body, immediately remove contaminated clothing and drench skin in shower for 15 minutes.
2. Seek medical attention.
3. Report the accident to the PI and EHS & Risk Management, and seek additional medical assistance if necessary.

Fires Involving Biological Materials

1. Without placing yourself in danger, put biological materials in secure location, such as incubator or freezer.
2. Activate the building fire alarm and leave the building at once.
3. Call the fire department from a safe location.
4. Meet the fire department outside and direct them to the fire location.



MEDICAL SURVEILLANCE

Medical surveillance may be necessary for laboratory personnel that use agents known to cause disease in humans or are working with animals that are susceptible to human diseases. The Occupational Health program will provide health assessments, medical test and immunizations for certain at-risk employees. Baseline serum samples may be appropriate for those working with BSL 2 or 3 agents if the agents can be monitored for serological changes to determine if an exposure has occurred in the laboratory.

Laboratory personnel that work with acute toxins or agents transmitted by aerosol route may require a respirator to perform their duties. All respirator users will require a medical evaluation before using a respirator. In addition, animal users must enroll in the Occupational Health Program and will be offered services such as a tetanus vaccination and possibility a TB tine test if working with primates.

SHIPPING BIOLOGICAL MATERIALS

Infectious agents and other dangerous goods must be transported according to the applicable regulations. The shipment of biological specimens, infectious agents and other biological materials are regulated by the International Air Transport Association (IATA), US Department of Transportation (DOT), US Postal Services (UPS) and other agencies. Carrying dangerous goods on person, in luggage, or private automobile is strictly prohibited, and those who violate the rules are subject to significant fines and criminal prosecution.

Training Requirements

Federal regulations require that people who need to ship biological materials or dry ice complete a Declaration for Dangerous Goods form must first have training. There are certain requirements for training and the course has to be repeated every two years because shipping regulations change frequently. EHS & Risk Management staff will assist PIs with shipping out materials. Do not ship dangerous good without appropriate training.

There are specific steps to take when shipping materials which include, classifying your biological agents for shipment, making sure the materials are package correctly, labeling the package, and filling out the Declaration for Dangerous Goods form.

Shipment Classification

For shipping purposes, biological materials are shipped according to the following classification; category A or B, biological product, genetically modified organisms, medical waste, and patient specimens. Category A&B are infectious substances with category A causing fatal disease in humans or animals and category B has less severe consequences. Biological products are items



manufactured and distributed for healthcare purposes and are considered a low probability of containing pathogens. Biological products are defined as materials used in the prevention, treatment or cure of disease in humans or animals, examples include; vaccines, blood products, therapeutic serums, and antitoxins. Genetically modified organisms (GMO) that are not infectious substances but are capable of altering animals or plants in a way that is not normally the result of natural reproduction can be transported as a Miscellaneous Hazard. GMO that are infectious or carried by an animal host are regulated for transportation. Patient specimens are materials for which there is minimum likelihood that pathogens are present.

Packaging Materials

Potentially hazardous materials must be packaged to withstand leakage of contents, shocks, temperature and pressure changes, and other conditions that may occur during transportation procedures. Biological materials must be packed with the triple packaging principle, with containers including a primary receptacle, a leak proof secondary container with absorbent material, and a durable outer container. The packages must comply with the package instructions from IATA such as PI 602 for infectious substances. Buying certified packages from suppliers that are specific for the materials you want to ship will ensure compliance with the packing requirements.

The proper shipping name, labels and UN markings must also be on the package before sending out for shipment. If using dry ice or liquid nitrogen with your shipment, these materials must be declared and packages properly labeled. Dry ice should never be placed in a sealed container or the package may be at risk of exploding.

IMPORTING & EXPORTING BIOLOGICAL AGENTS

Receiving and sending animals, infectious agents, and GMOs outside the United States may require the approval of federal agencies such as the CDC, USDA and the US Fish and Wildlife Services (USFWS). These regulatory agencies govern the transfer of these materials to minimize and eliminate the possible threats to public health and agriculture.

For importation of agents infectious to humans, CDC has placed the permit application and instructions on-line at <http://www.cdc.gov/od/eaipp/>. The USDA permit is required to import or domestically transfer a plant pest or livestock pathogens or any material that might contain them. Information on this process can be found at <http://www.aphis.usda.gov/ppg/permits>. For transporting fish, wildlife, or endangered species, the USFWS forms can be found at <http://forms.fws.gov/display.cfm?number1=100>.

An export license may be required from the Department of Commerce when exporting infectious agents of human, plant or animal diseases. Instructions can be found at <https://www.bis.doc.gov/index.php/licensing>. Also, the countries receiving the materials may require an import permit.



BIOLOGICAL WASTE DISPOSAL

Proper biological waste disposal will ensure safety for laboratory personnel, custodian staff, transporter, and the general public. The [Texas Administrative Code, Title 30, Part 1, Chapter 326 on Medical Waste Management](#) sets forth waste disposal regulations for biomedical and biohazardous waste generation, treatment, and disposal. These rules must be followed by all who generate biowaste on campus.

Biological waste includes infectious and non-infectious waste that may be generated in the laboratory, clinical, and campus setting. Examples of infectious or potentially infectious waste include human and animal pathogens, recombinant DNA, human blood and tissue. Examples of non-infectious waste include needles/syringes used for teaching purposes or in chemical labs, and materials that has not been contaminated with disease causing agent, such as petri dishes, media and animal carcasses.

All infectious waste must be placed in red biohazardous bags or sharps container prior to disposal. Waste containers in the lab should remain closed with a lid when not in use. Step cans will satisfy this requirement. If using a cardboard box in the lab, the bags placed in the box should be sealed and the liner must not be used as the primary bag. All infectious waste should be decontaminated by using an autoclaved or chemical inactivation before leaving the facility and must be properly labeled with PI's name and location and sealed with tape before being picked for final treatment and disposal. All bags have to be labeled unless placed in a secondary container. If a bag is placed in a secondary container, only the liner and outside container have to be labeled.

Many laboratories encounter biological waste mixed with chemicals or radionuclides, which must be managed as hazardous or radioactive waste prior to disposal. Infectious mixed waste should be rendered non-infectious before being handled as chemical or radioactive waste. The EHS & Risk Management office can assist with the best disposal method for mixed waste.

All employees who generate or handle biological waste must be trained annually on the proper procedures and policy for biological waste.



MINORS IN THE LABORATORY

Children under 18 years old must not work in laboratories that use hazardous materials such as infectious agents, chemicals and radioactive substances. However, minors may work in the laboratory if they are part of a group or individual educational program approved in advance by the department head.

The student or group must be sponsored by a member of LU's faculty. The faculty sponsor is responsible for ensuring that LU's procedures are followed and that the student's activities are supervised at all times. The student, student's parent or guardian, and PI must complete the [Minors Working in Research Laboratories or Animal Facilities form](#), and submit it to EHS & Risk Management for approval.

WILDLIFE & FIELD STUDIES

When performing field studies, it is most important to know what environmental hazards are out there (e.g. heat stroke, venomous insects or reptiles), and to understand what preventative measures and personal protection may be required. Field workers should be aware that besides the obvious risks associated with working in a field setting, there are also microbiological hazards that could be significant. Workers should consider the animals they deal with and the organisms that may be associated with these animals in evaluating the risk that certain situations may present. Most animals likely contain some disease, and some of these can be contracted by humans. For instance, turtles can harbor *Salmonella* and handling these animals can pose a risk to personnel and other turtles, so PPE and disinfectant must be used in the field to prevent transmission.

Field work should never be performed alone, and someone in the group should be CPR/First Aid certified. First aid kits should be well stocked and medicine available for those at risk of anaphylactic shock. Information about the field study, and emergency contact information, should be left with someone in the department. Emergency procedures and phone numbers must be given to the fieldworkers.



References

1. [American Biological Safety Association](#)
2. [CDC/NIH Biosafety in Microbiological and Biomedical Laboratories](#)
3. [NIH Guidelines for Research Involving Recombinant DNA Molecules or Synthetic Nucleic Acid Molecules:](#)
4. [CDC/NIH Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets](#)
5. [CDC Select Agent Program](#)
6. [Morbidity and Mortality Weekly Report](#)
7. [OSHA Bloodborne Pathogens 1910.1030](#)
8. [World Health Organization, Laboratory Biosafety Manual](#)
9. [IATA Dangerous Goods Regulation](#)

Appendix A

Risk Group List

Risk Group 1 (RG1) Agents

Bacillus subtilis

Bacillus licheniformis

Escherichia coli K-12 Host Vector Systems

* RG1 agents involve well-characterized agents not known to consistently cause disease in healthy adults. Those agents not listed in Risk Groups (RGs) 2, 3, and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

- *Giardia* including *G. lamblia*
- *Leishmania*

Risk Group 2 (RG2) Agents

* RG2 agents are associated with human disease which are rarely serious and for which preventative or therapeutic interventions are *often* available.

Bacterial Agents

- *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- *Bacillus anthracis*
- *Burkholderia* (formerly *Pseudomonas* species) except those listed in RG3
- *Campylobacter coli*, *C. fetus*, *C. jejuni*
- *Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- *Clostridium botulinum*
- *Escherichia coli* – all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- *Helicobacter pylori*
- *Legionella* including *L. pneumophila*
- *Listeria*
- *Mycobacterium*
 - o *M. avium* complex
 - o *M. bovis* BCG vaccine strain
- *Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- *Neisseria gonorrhoeae*, *N. meningitidis*
- *Salmonella*
- *Shigella*
- *Staphylococcus aureus*
- *Streptococcus* including *S. Pneumoniae*, *S. pyogenes*
- *Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
- *Yersinia enterocolitica*

Fungal Agents

- *Blastomyces dermatitidis*
- *Cryptococcus neoformans*
- *Penicillium marneffeii*

Parasitic Agents

- *Ancylostoma* human hookworms including *A. duodenale*, *A. ceylanicum*
- *Coccidia*
- *Cryptosporidium* including *C. parvum*



- *Plasmodium*
- *Schistosoma*
- *Toxoplasma* including *T. gondii*
- *Coccidioides immitis*
- *Histoplasma capsulatum*

Viruses

- Adenoviruses , human – all types
- Alpha viruses (Togaviruses) – Group A Arboviruses
 - o Eastern equine encephalomyelitis virus
 - o Venezuelan equine encephalomyelitis vaccine strain TC-83
 - o Western equine encephalomyelitis virus
- Bunyaviruses
 - o Bunyamwera virus
 - o Rift Valley fever virus vaccine strain MP-12
 - o Other viruses as listed in the reference source (see section V-C, Footnotes and References of sections 1 through 4)
- Coronaviruses
- Hepatitis A, B, C, D, and E viruses
- Herpesviruses – except Herpesvirus simiae (Monkey B virus)
 - o Cytomegalovirus
 - o Epstein Barr virus
 - o *Herpes simplex* types 1 and 2
 - o Human herpesvirus types 6 and 7
- Orthomyxoviruses
 - o Influenza viruses types A, B, and C
- Papovaviruses
 - o All human papilloma viruses
- Paramyxoviruses
 - o Newcastle disease virus
 - o Measles virus
 - o Mumps virus
- Parvoviruses
 - o Human parvovirus (B19)
- Picornaviruses
 - o Coxsackie viruses types A and B
 - o Rhinoviruses – all types

Risk Group 3 (RG3) Agents

* RG3 agents are associated with serious or lethal human disease for which preventative or therapeutic interventions *may be* available.

Bacterial Agents Including Rickettsia

- *Brucella* including *B. abortus*, *B. canis*, *B. suis*
- *Burkholderia (Pseudomonas) mallei*, *B. pseudomallei*
- *Coxiella burnetii*
- *Francisella tularensis*
- *R. rickettsii*
- *Yersinia pestis*

Fungal Agents

Viruses and Prions

- Alphaviruses
 - o St. Louis encephalitis virus
 - o Venezuelan equine encephalomyelitis
- Arenaviruses
 - o Lymphocytic choriomeningitis virus (LCM)(neurotropic strains)
- Bunyaviruses
 - o Hantaviruses including Hantaan virus
 - o Rift Valley fever virus
- Poxviruses
 - o Monkeypox virus
- Prions
 - o Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)
- Retroviruses
 - o Human immunodeficiency virus (HIV) types 1 and 2
 - o Human t cell lymphotropic virus (HTLV) types 1 and 2
 - o Simian immunodeficiency virus (SIV)
- Rhabdoviruses
 - o Vesicular stomatitis virus

- Papovaviruses
 - o Bovine papilloma virus
 - o Simian virus 40 (SV40)
- Retroviruses

Risk Group 4 (RG4) Agents

* RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Viral Agents

- Arenaviruses
 - o Lassa virus
 - o Junin virus
- Bunyaviruses (Nairovirus)
 - o Crimean-Congo hemorrhagic fever virus
- Filoviruses
 - o Ebola virus
 - o Marburg virus
- Herpesviruses (alpha)
 - o Herpesvirus simiae (Herpes B or Monkey B virus)
- Hemorrhagic fever agents and viruses as yet undefined

Animal Viral Agents in Common Use

* None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

* A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g. amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

- Baculoviruses
- Herpesviruses
 - o Murine cytomegalovirus

- Avian leucosis virus
- Bovine leukemia virus
- Feline Leukemia virus
- Murine leukemia virus
- Murine sarcoma virus

Murine Retroviral Vectors

* Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.



Appendix B

Select Agent List

HHS SELECT AGENTS AND TOXINS

- Abrin
- Botulinum neurotoxins
- Botulinum neurotoxin producing species of *Clostridium*
- Conotoxins
- Coxiella burnetii*
- Crimean-Congo haemorrhagic fever virus
- Diacetoxyscirpenol
- Eastern equine encephalitis virus Ebola viruses
- Francisella tularensis* Lassa fever virus Marburg virus
- Monkeypox virus
- Reconstructed 1918 Influenza virus Ricin
- Rickettsia prowazekii*
- SARS-associated coronavirus
- Saxitoxin
- Shigatoxin
- South American haemorrhagic fever viruses Junin Machupo Sabia
- Guarnarito

- Foot and mouth disease virus Goat pox virus
- Lumpy skin disease virus *Mycoplasma capricolum* *Mycoplasma mycoides subspecies*
- Newcastle disease virus (VVND) Peste Des Petits Ruminants virus Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus

LISTED PLANT PATHOGENS

- Peronosclerospora phillippinensis* *Phoma glycinicola*
- Ralstonia solanacearum* race 3, biovar 2
- Rathayibacter*
- Schlerophthora rayssiae var zaeae*
- Synchytrium endobioticum* *Xanthomonas oryzae*

- Staphylococcal enterotoxin
- Tetrodotoxin
- T-2 toxin
- Tick-borne encephalitis complex (flavi) viruses
- Kyasanur forest disease
- Omsk hemorrhagic fever
- Variola major virus (Smallpox virus) Variola minor virus (Alastrim) *Yersinia pestis*

SELECT AGENTS AND TOXINS (OVERLAP AGENTS)

- Bacillus anthracis* *Brucella abortus* *Brucella melitensis*
- Brucella suis* *Burkholderia mallei*
- Burkholderia pseudomallei*
- Hendra virus Nipah virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus

USDA SELECT AGENTS AND TOXINS

- African swine fever virus African horse sickness virus
- Avian influenza (highly pathogenic) Classical Swine fever virus



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Appendix C

Autoclave Standard Operating Procedures

General Information

1. Most important, wear personal protective equipment (PPE) before using the autoclave: eye or face protection, heat resistant gloves, closed toe shoes, lab coat or rubber apron.
2. Materials that are to be decontaminated/sterilized should be carried to the autoclave in closed and leak proof containers.
3. Containers: Stainless steel containers are durable and a good conductor of heat. Polypropylene containers are durable heat resistant plastic containers. (Other plastics will melt.)
4. Always clean up spills prior to running the autoclave and after the autoclave cycle is completed.

Packaging and Loading

1. Use approved autoclave bags for decontaminating biowaste.
2. Use secondary containers to protect autoclave and contain spills.
3. Prepare and load material to ensure steam penetration (add 250ml of water to bags containing solids. Open bag prior to autoclaving.).
4. Do not overfill containers (prevent spill and boil over) or use sealed containers (pressure buildup and lack of steam penetration).
5. Select appropriate cycle for the load (Determine the appropriate exposure time for the load. Consider agents or amounts of material that affect exposure time.).
6. Affix temperature sensitive tape to bags or other materials.

Operating the Autoclave

1. Before use, check log and previous readings to ensure the autoclave is operating properly.
2. Autoclave door clamps and seals should be inspected for wear and damage. Also, remove debris from the autoclave chamber floor drain.
3. Ensure the autoclave cycle attains the desired temperature (normally 121c) and pressure (minimum 20 psi) for the desired time (30 min.) and is appropriate for the material to be autoclaved.
4. Most importantly, check seam pressure valve and make sure it is on the appropriate setting. Otherwise, autoclave will not work properly, and alarm will sound.
5. Record information in the "Autoclave Use Log."

Unloading the Autoclave

1. Verify temperature (tape) and duration of exposure (print out/gauge) has been met.
2. Wait until the chamber pressure gauge reads zero before opening.
3. Open slightly to allow steam to escape (protect yourself from the steam).
4. Wait at least 10 minutes for the contents of the autoclave to cool.
5. Remove the material carefully to reduce the risk of spillage or injury (use a cart and gloves).



Verifying Autoclave Efficacy

1. Perform testing using a biological indicator (*B. stearothermophilus*)
2. Record results on “Biological Indicator Test Results” form.

Completed Autoclave Logs should be maintained by the Department



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