SAFETY PLAN FOR THE USE OF BIOLOGICAL MATERIALS AND ASSOCIATED DEVICES

Table of Contents
1.0 Introduction
2.0 Program Administration
  2.1 Executive Management
  2.2 Institutional Biological Safety Committee
  2.3 Biological Safety Officer
  2.4 Dean or Department Head
  2.5 Principal Investigators
  2.6 Individual
  2.7 Ancillary Personnel
3.0 Biohazards and Potentially Infectious Materials
  3.1 Definitions
  3.2 Biological Agent Classification
  3.3 Risk Group Assignment
  3.4 Recombinant & Synthetic Nucleic Acids
  3.5 Other Potentially Hazardous Biological Material
  3.6 Principles of Biological Safety
  3.7 Practices and Procedures
4.0 Registration of Recombinant & Synthetic Nucleic Acid and Biohazard Research
5.0 Transfer of Biological Material
  5.1 Intramural
  5.2 Extramural
6.0 Etiologic Agent and Vector Permits
  6.1 Import
  6.2 Other
  6.3 Export licenses
7.0 Training
  7.1 Biological Safety Training
  7.2 Bloodborne Pathogen Training
8.0 Surveillance
  8.1 Record Keeping
  8.2 Laboratory Audits
  8.3 Compliance
9.0 Accidents and Incidents
10.0 Appendices
1.0 Introduction

Carnegie Mellon University uses biological materials and associated devices for teaching and research purposes. Individuals that participate in the biological safety program (BSP) use biological material for the following purposes:

- theoretical analysis, exploration, and experimentation,
- extension of investigative findings and theories of a scientific or technical nature into practical application for experimental and/or demonstration purposes, including but not limited to the experimental production, and testing of models, devices, equipment, and processes,
- demonstration, teaching, and instruction in courses offered by the university to graduate and undergraduate students.

Biological materials or associated devices covered by this plan will not be used for internal administration or external application to human beings.

Biological materials applicable to this plan are:

- all infectious organisms (bacteria, fungi, parasites, prions, rickettsia, viruses, etc.) that can cause disease in humans, or cause significant environmental or agricultural impact
- human or primate tissues, fluids, cells, or cell culture
- recombinant or synthetic nucleic acids
- transgenic plants or animals
- plasmids
- toxins (bacterial, fungal, plant, etc.)
- allergens
- infected animals and their respective tissues

The university is committed to providing a safe and healthful learning, teaching and research environment. This biosafety plan provides university-wide safety guidelines, policies, and procedures for the use and manipulation of biologicals and associated devices. The goals of the university's biological safety program are to:

- protect staff and students from exposure to infectious agents,
- prevent environmental contamination,
- comply with federal, state, and local regulations.

The university's BSP takes into consideration that the words "safe" and "safety" are ideal concepts which, while desirable, are unattainable in absolute terms. Practical planning for safety is therefore performed by evaluating risk. Recent advances in bio-printing, genetic engineering, cell fusion, immobilized cells, and enzymes, etc. have provided a new dimension to applied microbiology. Technology is advancing so rapidly that it is not possible for the university's safety specialists to anticipate each use of potentially hazardous biological or chemical systems and to effectively monitor every operation that involves these materials. Success of the university's BSP requires the researcher to have sufficient knowledge to recognize and identify the potential hazards associated with his/her work and to work with the Biological Safety Officer (BSO) to develop and institute procedures, practices, equipment and facilities to control the identified risks or reduce them to acceptable levels and conduct the activity in as safe a manner as possible. Ultimately, implementation of biosafety must be part of every laboratory activity in which biologicals and associated devices are used.

The BSP consolidates the compliance programs for the Occupational Safety and Health Administration's (OSHA) Occupational Exposure to Bloodborne Pathogens Standard (29 CFR 1910.1200), the National Institute of Health's (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (2013 NIH Guidelines) and the Center for Disease Control/NIH publication Biosafety in Microbiological and Biomedical Laboratories (5th edition), BMBL. This biosafety plan applies to all university faculty, staff, hosted visitors, students, participating guests and volunteers, and contract laborers, working at locations where the university has management control of specific biohazards.

**Biohazards at the university are defined as infectious agents or materials produced by living organisms that may cause disease in other living organisms.** This definition encompasses human pathogens and all materials that contain such pathogens (human-, nonhuman-primate, other animal, and
plant-sourced materials) and other agents (toxins, allergens, venoms, secretions, excretions, etc.) that can cause disease in humans, animals, or plants. Work with experimental animals and arthropods also constitute potential exposure to biohazards. These animals may harbor infectious agents and/or proteins in their dander, urine, saliva, serum etc., to which personnel may react or become allergic.

The BSO can address any questions or comments regarding this safety plan. Please contact the university’s Environmental Health and Safety (EHS) department at 412-268-8182 for his/her contact information.

2.0 Program Administration and Responsibilities

The university’s commitment to the safe, legal, and ethical use of biologically-derived hazardous materials is implemented through the cohesive and interdepartmental administration of the university’s BSP. Executive Management (EM), the Institutional Biological Safety Committee (IBC), the BSO and his/her staff, university Deans, Department Heads, Principal Investigators, Researchers, and employees work as a team to ensure a comprehensive and effective program. Each plays a critical role within its area of responsibility. To follow is a chart that shows the organization of those responsible for directing the BSP at the university and the roles and responsibilities of all BSP participants.

2.1 Executive Management (Director of Environmental Health and Safety)

EM is at the senior level and has the ultimate responsibility for the activities associated with the use of biological materials, associated devices, and the BSP. EM has the important role of implementing and managing the BSP.

EM or his/her delegate is a member of the IBC. He/she should attend all committee meetings. In all programs involving the use of biological materials, EM should be knowledgeable of the results or periodic audits, to ensure all activities comply with regulatory requirements and that all participants in the BSP conduct activities in a safe manner.

EM represents the highest level of management and has the authority to delegate resources including personnel for the program and appropriate funds in a timely manner. EM must be available to facilitate effective and immediate action on behalf of management, the IBC and the BSO, particularly in the event of an emergency. EM must have the authority to make prompt decisions without having to consult with higher management officials, including the authority to take whatever action is necessary to ensure that all biosafety practices are in accordance with the regulations and conditions of the BSP.

EM is involved in selecting the chairperson and members of the IBC and the BSO, and defines the role, duties and responsibilities of each. EM supports the IBC and the BSO, creating an atmosphere of cooperation and professionalism such that individuals feel comfortable raising biosafety concerns. A clear understanding that there is strong management support for and participation in the BSP enhances authority. Individuals should understand management’s expectations regarding internal enforcement of BSP requirements and the consequences for non-compliance.

2.2 Institutional Biological Safety Committee

The IBC is composed of such persons as the BSO, EM, and faculty and staff persons trained and experienced in the safe use of biological materials. Membership on the IBC should represent each area of use within the university. The primary responsibility of the IBC is to formulate policy and procedures related to the use of biohazardous agents, including: human pathogens, oncogenic viruses, other infectious agents and recombinant or synthetic nucleic acid molecules. As mandated by the NIH Guidelines, experiments involving human gene transfer, formation of transgenic animals and the generation of recombinant or synthetic nucleic acid molecules must be reviewed and approved by the IBC.

EM selects a chairperson for the committee based upon the recommendations of the presiding IBC chairperson and BSO. This individual should have knowledge of BS issues, good leadership abilities, the authority and credibility by virtue of their position within the university, and a desire to serve as chairperson, in order to facilitate the effectiveness of the IBC. The IBC establishes policies and procedures to delegate its purpose and duties. Typically, the IBC establishes a quorum for meetings, as defined in the IBC charter. An acceptable quorum consists of at least one-half of the committee’s voting members. The meeting frequency and content, as defined by the IBC charter, is sufficient to ensure that the BSP is operating in compliance with
established procedures and regulations. The IBC maintains minutes of its meetings and are available to the public upon request.

2.3 Biological Safety Officer
The BSO is responsible for biosafety and compliance with regulations for the use of biologicals and associated devices. The BSO is a member of the IBC and works closely with the IBC and EM in implementing the BSP. The BSO ensures that participants in the BSP safely perform all biosafety activities according to approved policies and procedures, and that all regulatory requirements are satisfied. The BSO and his/her staff have full access to all activities involving the use of biologicals and associated devices. The BSO has the authority to terminate any activity in which health and safety appear compromised without consulting EM or the IBC, if required.

The Biological Safety Office performs audits of all biological areas of use and individuals using biological materials to ensure that their work is in accordance with the regulations, university policies and procedures. Specific duties and responsibilities of the BSO and his/her staff include:
- monitoring compliance with university safety policies and procedures regarding potentially infectious and biohazardous materials,
- assisting Principal Investigators (PIs) and laboratory personnel in the selection of safe laboratory practices, equipment and controls,
- providing technical guidance to all personnel on matters related to biological laboratory safety,
- developing and conducting appropriate training programs to promote techniques for the safe handling and disposal of potentially infectious and biohazardous materials,
- approving the use of biohazardous materials by PIs and sets safety criteria for the handling of those agents,
- overseeing ordering and receipt of Select Agents as defined by the Department of Health and Human Services,
- investigating all reported accidents which may result in personnel or environmental exposure to biohazardous materials,
- coordinating the off-site treatment of infectious wastes,
- responding to emergencies involving biohazardous materials,
- attending all IBC meetings
- corresponding with all applicable regulatory agencies
- maintaining all required records

2.4 Dean/Department Head
A Dean or Department Head is responsible for encouraging compliance with safety, health and environmental practices and procedures in their schools or departments, respectively. Specifically, he or she shall:
- understand applicable regulations and see that requirements are met,
- enforce rules and regulations and take prompt, effective action when necessary,
- ensure compliance of principal investigators and other supervisory personnel with federal, state, and local regulations and university policies applicable to the department’s work

2.5 Principal Investigator
A PI is responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratories. This responsibility may not be shifted to inexperienced or untrained personnel. A PI is also responsible for the instruction of students and staff in the potential hazards of biologically derived materials. All protocols involving work with potentially infectious agents must be submitted to the Biological Safety Office for review and approval. For more information contact the Biological Safety Office by phone (8-8405) or email (alawson@andrew.cmu.edu). Specifically, a PI has the responsibility to know and abide by the following rules:
- ensure that any research project using recombinant and/or synthetic nucleic acids be registered with Environmental Health and Safety,
- coordinate the procurement of Select Agent and/or Toxins or biological materials requiring permits with the Biological Safety Office,
- maintain an accurate and thorough inventory for biological agents in the laboratory
- maintain an accurate and thorough Select Agent and/or Toxins and/or Controlled Substance inventory for the laboratory,
Submit protocols involving work with potentially infectious agents or Select Agents and/or Toxins to the Biological Safety Office,
- ensure that appropriate signage is used at the entrance(s) to and within the laboratory,
- create and foster a laboratory environment that encourages open discussion of biosafety issues, problems, and modification procedures,
- ensure laboratory personnel have received all applicable training, if required, before working with biological agents,
- train research personnel and students in lab specific protocols,
- ensure laboratory personnel work in accordance to the university’s BSP requirements,
- ensure that Personal Protective Equipment (PPE) appropriate to the biohazardous agent(s) is available, is in good condition, and is utilized appropriately,
- coordinate biological and infectious waste disposal with the Biological Safety Office as described in section 3.7,
- notify the BSO in the event of an exposure to biological material or if a laboratory-acquired infection is known or suspected,
- stop work posing imminent danger,
- implement corrective actions to prevent recurrence of BSP operating errors.

2.6 Individual
The health and safety of each employee and student is extremely important, and the university fosters a safe learning environment. Individuals should bring their concerns to their supervisor, department head, BSO, IBC or the Director of EHS. Each individual is expected to be conscientious in assuming personal safety responsibility. Each individual is responsible for working safely and abiding by applicable safety guidelines. All individuals who might be exposed to biohazards in the course of their activities at the university shall:
- become familiar with the university’s BSP and all laboratory procedures applicable to him/her,
- comply with safety guidelines and procedures required for the task(s) performed,
- report unsafe conditions to the PI, supervisor or the Biological Safety Office,
- seek guidance from his/her PI, supervisor or the Biological Safety Office when he/she is uncertain how to handle, store, or dispose of any hazardous or biohazardous material.

2.7 Ancillary Personnel
Ancillary personnel shall:
- ensure that he/she understands the designation of a biohazard symbol before entering a biological work or storage area,
- obtain authorization from the laboratory supervisor or PI for initial entry into a posted biohazardous area,
- notify the BSO and receive clearance prior to performing any maintenance involving plumbing, ductwork, or ventilation systems connected to biological work areas,
- emergency response personnel must ensure that any action taken is appropriate for the level of hazard and inform the BSO of the response to an emergency involving biological materials,
- shipping and receiving personnel shall place Select Agent packages in a secure location or under direct surveillance and contact the BS Office upon the arrival of a Select Agent.

3.0 Biohazards and Potentially Infectious Materials

3.1 Definitions

3.1.1 Biological material is any agent possessing or characteristic of an agent of biological origin that has the capacity to produce deleterious effects on humans or the environment, including yet not limited to, microorganisms, toxins, and allergens derived from those organisms, allergens and toxins derived from higher plants and animals.

3.1.2 Associated device is any device utilized in the storage, containment, handling or processing of a biological material. Such devices include yet are not limited to, pipettes, syringes, needles, glassware, blenders, mortal and pestle, centrifuge, biosafety cabinet, refrigerators, and freezers.

3.1.3 Pathogenicity is the ability of a microorganism to cause disease

3.1.4 Virulence is the ability of a microorganism to establish itself on or within a host and produce disease.
3.1.5 **Route of transmission** is the means by which a microorganism can enter a host (e.g. parenteral, airborne, ingestion).

3.1.6 **Agent stability** is a consideration that involves not only aerosol infectivity (e.g., from spore-forming bacteria), but also the agent's ability to survive over time in the environment outside of a host.

3.1.7 **Infectious dose** is the minimum quantity of an infectious agent that will cause disease.

3.1.8 **Concentration** is the number of infectious organisms per unit volume.

3.1.9 **Origin** in this context refers to either the geographic location, i.e. domestic or foreign, host (infected or uninfected human or animal), or nature of the source, i.e. potential zoonotic or associated with disease outbreak.

3.1.10 **Blood** refers to human and non-human primate blood, human and non-human primate blood components, and products made from human and non-human primate blood.

3.1.11 **Other Potentially Infectious Materials (OPIM)** means (1) The following human and non-human primate body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human or non-human primate (living or dead); and (3) HIV or HBV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

3.1.12 **Select Agent** means a biological agent or toxin deemed a threat to the public, animal, or plant health, or to animal or plant products and included in the Code of Federal Regulations Title 9 Part 121.3 and Title 42 Part 73.4

3.1.13 **Select Toxin** means a toxin included in the Code of Federal Regulations Title 9 Part 121.3 and Title 42 Part 73.4

3.2 **Biological Agent Classification**

Biological agent classification is possible through risk assessment. Features of microorganisms as well as host and environmental factors that influence the potential for individuals to have a biohazard exposure are identified to evaluate risk. Factors including, but not limited to, those listed below are considered when evaluating the risk of a particular agent and/or operation:

- **Pathogenicity**: The more severe the potentially acquired disease, the higher the risk.
- **Route of transmission**: Agents transmitted by respiratory exposure to aerosols have been known to cause the most laboratory-acquired infections. The greater the aerosol potential, the higher the risk of infection.
- **Host Range**: The number of species susceptible to infection with a particular agent. The wider the host range, the higher the risk.
- **Agent stability**: The greater the potential for an agent to survive in the environment, the higher the risk of infection.
- **Infectious dose**: The amount of an infectious agent needed to cause infection varies among individuals and can range from one to hundreds of organisms. The infectious dose is also influenced by the individual's immune status. The lower the infectious dose, the higher the risk.
- **Concentration**: In most cases, the risk increases as the concentration of microorganisms increases.
- **Origin**: In some cases, the geographic location or nature of the source can increase the risk of infection.
- **Availability of data from animal studies**: If human data is not available, information on the pathogenicity, infectivity, and route of exposure from animals studies may be valuable. Caution must be used when translating infectivity data from one species to another.
- **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 PPE. The availability of post-exposure prophylaxis should also be considered.
Genetic modification: Adjustments must be made for genetic modifications known or suspected to affect any of the above listed items including, but not limited to, agent pathogenicity, infectivity, virulence, transmission, stability, and availability of effective prophylaxis or treatments.

The National Institute of Health (NIH) has utilized the above mentioned risk analysis approach and defined four Risk Group categories as presented in Table 1. In the cases where the hazard is unknown, a higher risk level is assigned.

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

The NIH has further assigned various biological agents based on their relative hazard into their respective risk groups. This can be found in Appendix B of the NIH Guidelines.

3.3 Risk Group Assignment

New research or development initiatives shall be evaluated by the PI in the early planning stages for the biologic and/or toxic hazards posed by the proposed work. New or inexperienced PIs are encouraged to read this plan and appendices, to seek consultation with the BSO, as well as to review published expert opinion regarding regulatory requirements. The assessment of infection risks associated with the laboratory use of biohazardous materials requires the proper risk classification of the material, risk involved with the modification or manipulations of the biohazardous agent, the environmental risks associated with the agent, and the laboratory requirements for containment of those risks. 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. Agents not on this list may be assigned to Risk Groups by the IBC and/or BSO. If the agent is not on this appendix, the PI must contact the BSO for information concerning the Risk Group assignment of that agent. In no case, will a Risk Group assignment in this appendix be lowered without the permission of the IBC or BSO. Agents of Risk Group 2 or higher requires the submission of an Application for the use of Biological Materials to the BSO for IBC review and approval.

Principal Investigators shall at a minimum evaluate yearly his/her established, ongoing research or development initiatives to assure that risks have not changed and that the established safety program is in compliance with the current regulatory requirements. Similarly, the Biological Safety Office shall audit yearly facilities and work practices to help insure that the work is completed in a safe and environmentally sound manner. Initiatives that increase the risk profile shall be accompanied by appropriate changes in the safety applications within the work area. These include, yet are not limited to, engineering controls, PPE, training requirements, spill containment, etc.

3.4 Recombinant and Synthetic Nucleic Acid Molecules

3.4.1 Generation of recombinant and synthetic nucleic acid molecules

According to the NIH Guidelines, recombinant and synthetic nucleic acids are defined as: “(i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids; ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic
acid molecules, i.e., synthetic nucleic acids, or (iii) molecules that result from the replication of those described in (i) or (ii) above. Experiments involving the generation of recombinant or synthetic nucleic acids require registration with the university’s Regulatory Compliance Administration and may require review and approval by the IBC. The NIH Guidelines is the definitive reference for recombinant and synthetic nucleic acids research in the United States. All persons whose research involves the use or manipulation of recombinant or synthetic nucleic acids as is defined by the NIH Guidelines are also required to complete the corresponding EHS training.

3.4.2 Transgenic animals
Investigators who use or create transgenic vertebrate animals must complete an recombinant & synthetic nucleic acid registration and an Institutional Animal Care and Use (IACUC) protocol, submit them to the university’s Department of Environmental Health and Safety and Regulatory Compliance Administration for IBC and IACUC approval, respectively. Approvals must be granted by the IBC and IACUC prior to initiation of experimentation.

3.4.3 Transgenic plants
Experiments to genetically engineer plants by recombinant DNA methods require registration with the IBC. The NIH Guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants.

3.5 Other Potentially Hazardous Biological Materials

3.5.1 Human blood, blood products, body fluids, and tissues
In 1991, the OSHA promulgated the Bloodborne Pathogens Standard, 29 CFR 1910.1030, to eliminate or minimize occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV), and other bloodborne pathogens. This federal regulation, “Occupational Exposure to Bloodborne Pathogens,” mandates a combination of engineering and work practice controls, annual training, Hepatitis B vaccination, and other provisions to help control the health risk to employees resulting from occupational exposure to human blood and other potentially infectious materials (OPIM) which may contain these or other specified agents. In accordance with the federal standard, the university has established Appendix 8.1 Bloodborne Pathogen Exposure Control Plan to provide a coordinated program of education, vaccination, Standard Precautions, and exposure follow-up to minimize or eliminate workplace exposure to HBV, HIV, and OPIM.

University employees reasonably anticipating skin, eye, mucous membrane, or parenteral contact with blood or OPIM during the performance of their job duties at the university must be knowledgeable of the university’s exposure control plan and have received the required bloodborne pathogen training within 30 days of beginning their respective tasks associated with such and anticipated exposure, and annually thereafter.

Human blood, blood products, body fluids and tissues have the potential to contain infectious agents. These materials shall be handled consistent with Standard Precautions, Biosafety Level 2 practices and procedures, and require all specimens of human blood or OPIM to be treated as if they are infectious.

Additional OSHA requirements are in place for laboratory personnel working specifically with HBV or HIV. He or she must:
- attend Biological Safety Training in addition to the Bloodborne Pathogen training,
- demonstrate proficiency in standard microbiological practices and techniques and in the practices and techniques and in the practices and operations specific to the laboratory to the satisfaction of the PI before being allowed to work with HBV or HIV,
- progressively demonstrate proficiency if he/she has no prior experience in handling human pathogens. Initial work activities shall not include handling of infectious agents.

3.5.2 Tissue culture and cell lines
Cell cultures are commonly used in biomedical research, yet appropriate biosafety requirements for handling various cell lines are often subject to debate within the scientific community. The OSHA Bloodborne Pathogens Standard clearly includes human blood, most body fluids, unfixed human tissues and organs, yet is ambiguous regarding human cell lines. In 1994, OSHA issued an interpretation of the applicability of the Bloodborne Pathogens Standard toward human cell lines. According to the interpretation, human cell lines are considered to be potentially infectious and within the scope of the Bloodborne Pathogen Standard unless the
specific cell line has been characterized and documented to be free of hepatitis viruses, HIV, Epstein-Barr virus, papilloma viruses and other recognized bloodborne pathogens.\(^3\) In alignment with this interpretation, the American Type Culture Collection (ATCC) recommends that all human cell lines be accorded the same level of biosafety consideration as a line known to carry HIV.\(^4\) Moreover, the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, recommends that human and other primate cells should be handled using Biosafety Level 2 (BSL2) practices, procedures, and containment.\(^5\) In consideration of the aforementioned regulatory interpretation, consensus, and guidelines, and other factors, the university's IBC established the following in regards to the use of cell lines:

- Cell cultures known to contain an etiologic agent or oncogenic virus must be classified as the same level as that recommended for the agent.
- All clinical material (e.g. samples of human tissues and fluids obtained after surgical resection or autopsy) shall be handled at Biosafety Level 2.
- All cell and organ cultures of human origin, including well established cell lines, such as HeLa cells, shall be handled in accordance with the OSHA Bloodborne Pathogens Standard consistent with Standard Precautions, Biosafety Level 2 practices and procedures.
- Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all primate tissue, and all virus and mycoplasma-containing non-human primate cell lines are classified as Risk Group 2 and shall be handled at Biosafety Level 2.

### 3.5.3 Use of Animals

All vertebrate animal research must be coordinated through the university’s IACUC. Protocols involving the use of recombinant or synthetic nucleic acids, infectious or transmissible agents, human blood, body fluids or tissues, toxins, carcinogenic mutagenic, teratogenic chemicals, or physically hazardous chemicals (reactive, explosive, etc.) must be submitted to EHS for review and approval prior to final approval by the IACUC. PIs must notify the Biological Safety Office prior to the initiation of experimentation at Animal Biosafety Level 2 or Animal Biosafety Level 3. IACUC guidelines are available from Sponsored Research.

### 3.5.4 Select Agents

The Department of Health and Human Services (DHHS), Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) have regulations for the possession, use, storage, and transfer of biological agents and toxins that could pose a threat to human, animal, and plant health and safety. Institutions that wish to use these agents in their research programs must be registered with either the DHHS, or USDA, or both. The institution must have biosecurity and biosafety plans in place for use of the materials. The requirements for use of these agents are backed up by monetary fines and imprisonment for misuse of the materials. Principal investigators who need to procure, possess, use, store and transfer biological agents and/or toxins as defined by the United States Code of Federal Regulations (CFR), Titles 9 and 42 must contact the Biological Safety Office to register with the university’s Select Agent Program, Appendix 8.2 prior to the acquisition of these agents.

### 3.5.5 Controlled Substances

A number of substances regulated by the Drug Enforcement Administration (DEA) and other agencies are used for research or instructional purposes at the university. These substances are known as "controlled substances," and their possession and use is governed by regulations that require that procedures be established to ensure safety and prevent abuse Contact the Biosafety Office for more information.

### 3.6 Principles of Biological Safety

#### 3.6.1 Containment

The term “containment” is used to describe safe methods for managing infectious agents in the laboratory environment where they are being stored or handled. Containment is two-fold (primary and secondary) and its purpose is to reduce or eliminate exposure of laboratory personnel and other people, prevent the escape of these agents into the outside environment, and to facilitate the research efforts. Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is

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\(^3\) OSHA Letter of Interpretation

\(^4\) American Type Culture Collection Frequently Asked Questions, URL: http://www.atcc.org/TechnicalInfo/faqCellBiology.cfm#Q53

\(^5\) Biosafety in Microbiological and Biomedical Laboratories, 5th edition, URL: http://www.bmbl.od.nih.gov/
provided by good microbiological technique and the use of appropriate safety equipment. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. The three essential elements of containment include the following:

- **Laboratory practice and technique**, when reflective of standard microbiological practices and techniques, is the most important element of containment. Persons working with infectious agents or materials must be aware of potential hazards, and trained and proficient in the practices and techniques required for handling these items safely. The PI is responsible for providing or arranging the appropriate training of personnel. Each PI shall develop an operational manual which identifies specific hazards that will or may be encountered in his/her laboratory, and which specifies practices and procedures designed to minimize or eliminate risks. He/she shall advise personnel of special hazards and ensure that they have read the manual and follow the required practices and procedures.

- **Safety equipment**, or primary barrier, forms the primary barrier between personnel and the infectious material and includes personal protective equipment (i.e. personal protective clothing, respirators, face shields, safety glasses or goggles), biological safety cabinets, enclosed containers, and other engineering controls designed to minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. BSCs are designed to protect the worker, the integrity of the experiment, and the environment. There are three types of biological safety cabinets: Class I, Class II, and Class III. Refer to Section 3.7.2 Biological Safety Cabinets for more information.

- **Facility design** or secondary barrier, is commensurate with the laboratory’s function and provides a barrier to protect people working inside and outside the laboratory, and protects people or animals in the community from infectious agents which may be accidentally released from the laboratory. Secondary barriers are dictated by the risk of transmission of specific agents. At the university, the exposure risk for laboratory work is direct contact with the agents or inadvertent contact exposure through contaminated work environments. Hence secondary barriers in these laboratories include separation of the laboratory work area from public access, as well as availability of decontamination and hand washing facilities. If the risk for aerosol transmission were to increase, higher levels of primary containment and multiple secondary barriers would be necessary to prevent infectious agents from escaping the laboratory. Such design features include directing airflow, decontaminating or removing infectious agents from the exhaust air, controlling access, establishing airlocks at laboratory entrances, or physically isolating the laboratory.

Various combinations of physical containment and laboratory practice may be necessary for the safe handling of certain agents. The *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th edition provides guidance for the appropriate containment of biohazards. However, the university containment requirements and laboratory practices may be more stringent. Contact the BSO for confirmation of university requirements associated with your work.

### 3.6.2 Biosafety Level

The 5th edition of *Biosafety in Microbiological and Biomedical Laboratories* provides a complete description of all biosafety levels (BSLs). These levels are four in total, 1 through 4, and consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the risk group classifications of the infectious agents, laboratory operations, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended BSL 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 by the university’s BSO and/or IBC. To follow is a summary of recommended biosafety levels for infectious agents.
Table 2: Summary of Recommended Biosafety Levels for Infectious Agents6

<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equipment (Primary Barriers)</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause disease in healthy adults</td>
<td>Standard Microbiological Practices</td>
<td>None Required</td>
<td>Open bench top sink requirements</td>
</tr>
<tr>
<td>2</td>
<td>Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure</td>
<td>BSL-1 practice plus: • Limited access • Biohazard warning signs • “ Sharps ” precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies</td>
<td>Primary barriers + Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats, gloves, face protection as needed</td>
<td>BSL-1 plus: Autoclave available</td>
</tr>
<tr>
<td>3</td>
<td>Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences</td>
<td>BSL-2 practice plus: • Controlled access • Decontamination of all lab clothing before laundering • Decontamination of all lab clothing before exiting facility • Baseline serum</td>
<td>Primary barriers + Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing, gloves, respiratory protection as needed</td>
<td>BSL-2 plus: • Physical separation from access corridors • Self-closing, double door access • Exhausted air not recirculated • Negative airflow into laboratory</td>
</tr>
<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission</td>
<td>BSL-3 practice plus: • Clothing change before entering facility • Shower on exit • All material decontaminated on exit from facility</td>
<td>Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.</td>
<td>BSL3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in text of BMBL</td>
</tr>
</tbody>
</table>

3.6.3 Vertebrate Biosafety Level

The 5th edition of *Biosafety in Microbiological and Biomedical Laboratories* provides a complete description of all animal biosafety levels (ABSLs). The levels are designated animal biosafety level 1 through 4, for work with infectious agents in mammals. The levels are combinations of practices, safety equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. In general, the level recommended for working with an infectious agent in vivo and in vitro is comparable. To follow is a summary of recommended animal biosafety levels for infectious agents.

Table 3: Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals are Used.7

<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equipment (Primary Barriers)</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause disease in healthy adults</td>
<td>Standard animal care and management practices, including appropriate medical surveillance program.</td>
<td>As required for normal care of each species.</td>
<td>Standard animal facility • No recirculation of exhaust air • Directional air flow recommended • Handwashing sink recommended</td>
</tr>
<tr>
<td>2</td>
<td>Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure</td>
<td>ABSL-1 practice plus: • Limited access • Biohazard warning signs • “Sharps” precautions • Biosafety manual • Decontamination of all infectious waste and of animal cages prior to washing</td>
<td>ABSL-1 equipment plus primary barriers: containment equipment appropriate for animal species; PPEs: laboratory coats, gloves, face and respiratory protection as needed</td>
<td>ABSL-1 plus: • Autoclave available • Handwashing sink available in the animal room • Mechanical cage washer used</td>
</tr>
<tr>
<td>3</td>
<td>Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences</td>
<td>ABSL-2 practice plus: • Controlled access • Decontamination of all lab clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed</td>
<td>ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols; PPEs: appropriate respiratory protection</td>
<td>ABSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility</td>
</tr>
<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission</td>
<td>ABSL-3 practice plus: • Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting • All wastes are decontaminated before removal from the facility</td>
<td>ABSL-3 equipment plus: • Maximum containment equipment (i.e. Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) for all procedures/activities</td>
<td>ABSL3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in text of BMBL</td>
</tr>
</tbody>
</table>

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7. Biosafety in Microbiological and Biomedical Laboratories, 5th edition, URL: [https://www.cdc.gov/labs/BMBL.html](https://www.cdc.gov/labs/BMBL.html)
3.7 Practices and Procedures

3.7.1 Administrative controls

- **Biohazard Warning Signs**
  The OSHA regulations, Specifications for Accident Prevention Signs and Tags (29 CFR 1910.145), require that warning signs and/or symbols be used to warn personnel and visitors of the potential for hazards in the workplace. Specifically, with regard to biohazards, the universal biohazard sign must be used to: “signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals or combinations thereof, which contain or are contaminated with, viable hazardous agents”. Biohazard signs should be fluorescent orange or orange-red with the lettering and symbols a contrasting color (e.g. black or white). The university ensures that:
  1. universal biohazard symbols are used to designate the presence of biohazards, as defined in this plan.
  2. all laboratories potentially containing biohazards are provided with signs signifying the presence of biohazards, biosafety level assignment, contact person, and the necessary precautions for entry and exit.
  3. PIs are responsible for maintaining current and accurate biohazard signs.
  4. animal holding, surgery, or experimental areas are properly posted and deposted upon commencement and completion of work with a biohazard, respectively.
  5. all biohazardous waste is appropriately contained and clearly labeled. See Section 3.7.6 Infectious Waste Management.

- **Biosafety Levels**
  Table 2 summarized the essential elements of the four biosafety levels for activities involving infectious microorganisms. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. In general, Risk Groups as defined by the NIH correlate with the applicable biosafety level (i.e. Risk Group 1 agents are handled at Biosafety Level 1, Risk Group 2 agents are at Biosafety Level 2, etc.)

  There are NO Biosafety Level 3 or 4 laboratories at Carnegie Mellon. Agents requiring this level of containment may not presently be brought to the university.

- **Vertebrate Biosafety Levels**
  Table 3 summarized the four animal biosafety levels for experiments on animals infected with agents that produce or may produce human infection. As with biosafety levels, increasing levels of protection to personnel and the environment are provided as the order ascends.

  There are NO Animal Biosafety Level 3 or 4 laboratories at Carnegie Mellon. Agents requiring this level of containment may not presently be brought to the university.

- **Medical Surveillance**
  A medical surveillance program is provided through Concentra for those personnel:
  1. who are occupationally at-risk of exposure to bloodborne pathogens, and/or
  2. who have direct animal contact.

  Immunoprophylaxis often provides an additional level of protection. The university offers vaccines at no cost to clearly identified at-risk personnel for which the benefits of the vaccine (levels of antibody considered to be protective) clearly exceed the risk (local or systemic reactions). The PI is responsible for ensuring that laboratory personnel who work in BSL-2 facilities receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory. These include, yet are not limited to, hepatitis B vaccine, rabies vaccine, TB skin test, Vaccinia vaccine, etc. Periodic testing is also recommended for the agent(s) handled in the laboratory. Baseline serum samples may be collected as appropriate and additional serum specimens periodically collected, depending on the agents handled or the function of the laboratory.

  A complete description of the animal and bloodborne pathogen medical surveillance requirements is in Bloodborne Pathogen Exposure Control Plan, and Occupational Safety and Health Program for Animal Handlers, respectively.
3.7.2 Engineering controls

- **Biological Safety Cabinets**

Biological Safety Cabinets (BSCs) are designed to contain aerosols and splashes generated during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. The HEPA filter does NOT filter out volatile chemicals in the air and the biological safety cabinet should not be used in place of a chemical fume hood. BSCs are also indicated for use when working with agents that can be infectious via the inhalation route of exposure. The cabinets are designed to protect the worker, the integrity of the experiment, and the environment. Work with the potential to create infectious aerosols or splashes must be carried out in a BSC. All personnel must develop proficient technique before working with infectious material in a BSC. There are three types of biological safety cabinets used for microbiological research: Class I, Class II, Class III.

The **Class I BSC** provides personnel and environmental protection from contaminants within the cabinet, but no product protection. It is similar in air movement to a chemical fume hood, but has a High Efficiency Particulate Air (HEPA) filter in the exhaust system to protect the environment. In the Class I BSC, the unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward air movement. A minimum velocity of 75 linear feet per minute (lfpm) must be maintained through the front opening to ensure appropriate containment.

The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection. In many cases, these cabinets are used specifically to enclose equipment (e.g. centrifuges, harvesting equipment or small fermenters), or procedures, such as cage dumping, aerating cultures, or homogenizing tissues, with a potential to generate aerosols that may flow back into the room.

The **Class II BSC** provides personnel and environmental protection from the contaminants within the cabinet, and protects material being manipulated inside the cabinet (e.g. cell cultures, microbiological stocks, and formulation of nonvolatile antineoplastics or chemotherapeutic drugs). There are four types of Class II BSCs: Type A1, Type A2, Type B1, and Type B2. In a Class II BSC, air movement is drawn around the operator into the front grille of the cabinet that provides personnel protection. In addition, the downward laminar flow of the HEPA-filtered air provides protection to the product by minimizing the chance of cross-contamination along the work surface of the cabinet. Cabinet air that has been passed through the exhaust HEPA filter may be recirculated back into the laboratory (type A1 and A2) or ducted out of the building (type B1, B2, and A2 through a thimble connection to a building’s exhaust system) because it is contaminant-free. The major differences between the four types of Class II BSCs may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area. Table 4 provides a comparison of Class II Biosafety Cabinet Characteristics.

<table>
<thead>
<tr>
<th>BSC Class</th>
<th>Face Velocity (lfpm)</th>
<th>Airflow Pattern</th>
<th>Applications</th>
<th>Nonvolatile Toxic Chemicals and Radionuclides</th>
<th>Volatile Toxic Chemicals and Radionuclides</th>
</tr>
</thead>
<tbody>
<tr>
<td>II, A1</td>
<td>75</td>
<td>70% recirculated¹; 30% exhausted² into room or outside through thimble unit</td>
<td>YES</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>II, A2</td>
<td>100</td>
<td>70% recirculated¹; 30% exhausted² to outside through HEPA filtered thimble-ducted unit</td>
<td>YES*</td>
<td>YES**</td>
<td></td>
</tr>
<tr>
<td>II, B1</td>
<td>100</td>
<td>70% recirculated¹; 30% exhausted² to outside through HEPA filtered dedicated duct</td>
<td>YES</td>
<td>YES*</td>
<td></td>
</tr>
<tr>
<td>II, B2</td>
<td>100</td>
<td>100% exhausted² to outside through HEPA filtered hard-duct; No recirculation</td>
<td>YES</td>
<td>YES</td>
<td></td>
</tr>
</tbody>
</table>

¹ Air recirculated to the cabinet work area through HEPA.
² Air exhausted through the HEPA.
* In no circumstances should the chemical concentration approach the lower explosion limits of the compound.
The gas-tight Class III BSC, or glove box, provides the highest level of protection to personnel, the environment and the product. The cabinet is maintained under negative air pressure of at least 0.5 inches of water gauge. Supply air is drawn into the cabinet through HEPA filters, and the exhaust air is filtered by two HEPA filters in series before discharge to the outside. The ventilation system of the Class III BSC is often separate from the facility’s ventilation system.

The Class III BSC is the only cabinetry that provides a total physical barrier between the product and personnel. It is for use with high risk biological agents and used when absolute containment of highly infectious or hazardous material is required.

**Note:** Horizontal and vertical laminar flow “Clean Benches” are not BSCs and must not be utilized for work with biohazards and/or chemical hazards. Clean benches provide product protection by ensuring that the product is exposed only to HEPA-filtered air. These systems do not provide protection to personnel or the ambient environment and can be used for certain clean activities, such as, dust-free assembly of sterile equipment or electronic devices, and preparation of intravenous drugs.

- **Safety Equipment**

  Safety equipment includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators face shields, safety glasses, or goggles. Personal protective equipment (PPE) shall be used in combination with BSCs and other devices that contain infectious agents, animals or materials. In situations where it is impractical to work in BSCs, PPE forms the primary barrier between personnel and infectious materials. Examples include certain animal studies, animal necropsy, and activities relating to maintenance, service, or support of the laboratory facility.

  Enclosed containers used for infectious agents that can be transmitted through the aerosol route of exposure are additional safety equipment often used for processing, transporting, or storing these etiologic agents. Examples are safety centrifuge cups and safety blenders designed to prevent release of aerosols during centrifugation and blending, respectively.

  The need for this additional safety equipment must be considered when performing the risk assessment for a particular project. The BSO and/or IBC must be consulted when other containment devices are determined to be necessary.

  Refer to section 3.7.3 *Recommended Work Practices* for more information on PPE, effective use of BSCs and information on other safety equipment.

### 3.7.3 Recommended Work Practices

- **Pipettes and Pipetting Aids**

  Pipettes are used for volumetric measurements and transfer of fluids that may contain infectious, toxic, corrosive, or radioactive agents. Laboratory-associated infections have occurred from oral aspiration of infectious materials, ingestion of material via a contaminated finger, and inhalation of aerosols. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when cultures are mixed by pipetting, or when the last drop of an inoculum is blown out. A pipette may become a hazardous piece of equipment if improperly used by personnel. The safe pipetting techniques that follow are required to minimize the potential for exposure to biologically hazardous material.

  1. Never Mouth Pipette.
  2. Confine pipetting operations to a biological safety cabinet when working with biohazardous or toxic fluid.
  3. Use cotton-plugged pipettes when pipetting biohazards or toxins.
  4. Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
  5. Do not forcibly expel biohazardous material out of a pipette.
  6. Never mix biohazardous material or toxic material by suction and expulsion through a pipette.
  7. Avoid accidental release of infectious droplets. Prior to beginning work, place disinfectant soaked towel on the work surface. After use, discard and/or autoclave the towel.
  8. Use “to deliver” pipettes rather than those requiring “blowout” when working with biohazardous or toxic fluids.
Never discharge material from a pipette at a height. Allow the discharge to run down the receptacle's wall.

Always place contaminated, reusable pipettes horizontally in a pan containing sufficient liquid disinfectant to completely cover them. Autoclave the pan and pipettes as a unit before processing them as dirty glassware for reuse. Refer to Section 3.7.4 Decontamination.

Never place pipettes vertically into a cylinder.

Place horizontal pans or sharps containers for contaminated pipettes inside the biological safety cabinet to minimize movement in and out of the BSC.

Discard contaminated disposable pipettes in an appropriate sharps container. The sharps container shall be puncture resistant for the applicable sharps (i.e. in-tact glass or plastic pipettes may go into a red-bag lined biohazard box, whereas, broken or shard pipettes must go into a rigid, plastic sharps container).

**Syringes and Needles**

Syringes and hypodermic needles are dangerous instruments. The use of needles and syringes should be restricted to procedures that have no alternative (e.g. the use of blunt cannulas for procedures such as oral or intranasal animal inoculations). Needles and syringes should never be used as a substitute for pipettes. When needles and syringes must be used, adhere to the following recommendations:

1. Use disposable safety-engineered needle-locking syringe units.
2. Work in BSC when using syringes and needles with biohazardous or potentially infectious agents.
3. Wear gloves.
4. Minimize air bubbles when filling syringe.
5. Vertically expel air, liquid and bubbles from the syringe into a cotton pledget moistened with disinfectant.
6. Place cotton or gauze over the opening of a vial when withdrawing the needle to prevent aerosol production.

**Requirements, Use, and Rules of Thumb for Biological Safety Cabinets**

The cabinets are required to be tested and certified after installation and before initial use, any time they are moved, and at a minimum, annually. The PI shall provide annual certification records for each BSC under his/her control. Testing shall meet the criteria in National Sanitation Foundation Standard Number 49-2002. Contact the Biological Safety Office for assistance in scheduling an annual certification.

A BSC is required in Biosafety Level 2 laboratories whenever a laboratory procedure results in the formation of an aerosol and when the agent(s) used are infectious via inhalation or aerosols droplets. The following are a list of microbiological activities prone to aerosol formation:

1. centrifugation
2. vigorous shaking and mixing
3. pipetting
4. grinding
5. aspiration/washing
6. injection
7. sonication
8. working with materials under pressure

A BSC is required for all pathogen manipulations performed in a Biosafety Level 3 laboratory.

A BSC is only effective when operated properly by personnel, therefore:

1. Operators must understand the function and use of the BSC before working with it.
2. Operators must demonstrate proficiency in working in the BSC.
3. Operators must never modify any BSC without first contacting the BSO.

**Cryostats**

Accidental exposures can occur when working with frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent. Freezing infected tissue does not necessarily inactivate the infectious agent(s). Propellants under pressure, such as liquid nitrogen, should not be used to freeze infected tissues because the process often results in the splatter of infectious droplets. Gloves should always be worn while
preparing frozen, tissue sections. When working with biohazardous material in a cryostat, the following is recommended:

1. Consider the contents of the cryostat to be contaminated and decontaminate it frequently with a 1% iodophore solution (or appropriate alternative).
2. Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
3. Defrost and decontaminate the cryostat with a 1% iodophore solution (or appropriate alternative) and a tuberculocidal hospital disinfectant once a week and immediately after cutting tissue known to contain bloodborne pathogens, M. tuberculosis or other infectious agents.
4. Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
5. Treat solutions used for staining potentially infected frozen tissue sections as if contaminated.

### Centrifuge Equipment

Mechanical failure and the creation of aerosols are the hazards associated with operating a centrifuge. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Operators should be properly trained and principal investigators should prominently post operating instructions that include safety precautions on the unit.

Filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets often create aerosols. The greatest aerosol hazard is created if a tube breaks during centrifugation. When centrifuging biohazardous material, minimize the generation of aerosols by following the procedures below:

1. Use safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
2. Fill and open centrifuge tubes, rotors and accessories in a BSC. Do not overfill centrifuge tubes or permit closures to become wet. After tubes are filled and sealed, wipe them down with disinfectant.
3. Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
4. Always balance buckets, tubes, and rotors properly before centrifugation.
5. Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and HEPA filters.
6. When re-suspending sedimented material, use a swirling rotary motion rather than shaking and work in a BSC. If shaking is necessary, wait a few minutes to permit the aerosols to settle before opening the tube.

Small low-speed centrifuges may be placed in a BSC during operation to contain aerosols.

High-speed centrifuges pose additional hazards and manufacturer's recommendations must be followed meticulously to avoid metal fatigue, distortion, and corrosion. While operating a high-speed centrifuge, filter exhaust air from vacuum lines, do not operate with disintegrated rotors, and use proper cleaning techniques and centrifuge components.

Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable, prone to shrinkage with age, distort on boiling, and can be highly explosive in an autoclave. DO NOT autoclave celluloid tubes. If celluloid tubes must be used, decontaminate them with an appropriate chemical disinfectant.

### PPE

The Occupational Safety and Health Administration's (OSHA) 29 CFR 1910.132 mandates employers to assess potential hazards in the workplace and provide appropriate Personal Protective Equipment (PPE) for such hazards. PPE is used to protect personnel from contact with hazardous materials, infectious agents and often results in the protection of the experiment from contamination. Supervisors are responsible to perform the assessments, if applicable, provide PPE to personnel without cost, and select and train employees in the use of routine items such as lab coats, protective gloves, safety glasses, face shields, etc. In depth information specific to face protection, laboratory clothing, gloves, and respirators can
be found in the university’s Laboratory Safety Information and Hazardous Waste Generation Information Handout provided to attendees of the university’s laboratory safety training course. Contact EHS for assistance in selection of PPE.

- **Aerosol producing devices**
  The use of devices to disrupt biohazardous materials result in considerable aerosol production and should be performed in a BSC whenever possible. Prior to initiating operation of the device, review the operations manual, paying special attention to any warnings regarding those areas susceptible to contamination by your product. Special care and barrier protection (splash shields, goggles, bench napkins, gloves, etc.) are important when using ultrasonic disrupters, grinders and homogenizers not only during the agitation/disruption process, but also when handling the finished product. Allow your vessel to be at rest for a short time. The minimal wait time will permit your product to settle before opening. Decontaminate appropriately after each use, especially when working with potentially infectious materials.

**Safety blenders** are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. To ensure blender rotors are leak proof, test them with sterile saline or dye solution prior to each use with biohazardous material. Due to the potential for glass to break, the use of a glass blender is discouraged. However, if one must be used, cover the glass jar with a polypropylene jar to contain spraying of glass and contents in the event of breakage. Use safety blenders in a BSC to prevent the accidental release of aerosol during the blending process. During use, place a towel moistened with disinfectant over the top of the blender. Before opening the blender jar, allow the unit to rest for at least one minute. This allows aerosols generated during operation to settle. Decontaminate the device promptly after use.

**Lyophilizers** may be used to freeze dry biohazardous material. Lyophilizer design varies and subsequently infectious aerosol production may occur when biohazardous material is loaded or removed from the lyophilizer unit. Handle cultures as infrequently as possible and use vapor traps wherever possible. Load sample material in a BSC. Exhaust vacuum pump emissions through a HEPA-filter or, alternatively, vent emissions into a BSC to remove any hazardous agents. Disinfect all surfaces of the unit that have been exposed to the agent after lyophilization. If the lyophilizer is equipped with a removable chamber, close it off and move it to a BSC for unloading and decontamination.

**Open all glass ampoules** containing liquid or lyophilized culture material in a BSC to contain the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. For lyophilized culture material, slowly add liquid to the dried material to reconstitute its contents. Mix liquid contents without bubbling and withdraw it into a fresh container. Discard the towel and ampoule top and bottom as infectious waste.

Use polypropylene tubes to store biohazardous material in liquid nitrogen. Glass ampoules have the potential to explode when stored in liquid nitrogen and can result in eye injuries. Polypropylene tubes are available dust-free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

- **Loop sterilizers, Bunsen Burners, and Flammable Chemicals in the BSC**
  In accordance with recommendations from the CDC, World Health Organization (WHO), and the major manufacturers of BSCs, the university has taken a strong stance against the use of gas burners or alcohol flames in BSCs. The CDC reports that “open-flames are not required in the near microbe-free environment of a biological safety cabinet” and create “turbulence which disrupts the pattern of air supplied to the work surface” jeopardizing the protective airflow patterns of the cabinet. Further, the operation of an open-flame within a BSC:
  (1) disrupts the protective air flow, compromising protection of both the worker and the product;
  (2) presents a potential fire or explosion hazard due to the accumulation of flammable vapors of gases in the work area for those BSCs that recirculate a portion of the total air volume(Electrical components, such as the fan motor, lights, and electrical outlets are not designed to operate in flammable atmospheres, where a flash fire could be ignited by a spark);
  (3) causes excessive generation of sufficient heat that may damage the HEPA filter and/or melt the adhesive holding the filter together, thus compromising the cabinet’s integrity;
(4) renders the manufacturer’s warranty null and void on the cabinet: cabinet manufacturers will assume no liability in the event of fire, explosion, or worker exposure due to the use of a flammable gas in the cabinet; further, the UL approval will automatically be void.

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization and is recommended. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators are not available. The loops are semi-quantitative and can be used for counting bacteria. Instruments such as tweezers, scissors, and scalpels, can be autoclaved prior to use. Alcohol is often used to sterilize various items being used within the cabinet. Always reduce the amount of flammable chemicals in the BSC. Use only enough alcohol for one day’s work and allow it to evaporate before handling, opening, or operating any sterilized items and/or devices.

If a gas burner is deemed absolutely necessary, select a pilotless burner or touch-plate microburner with a pilot light to provide a flame on demand. An appropriate hand piping from the house gas line must be used and an easily accessible emergency shut-off valve must be placed on the outside of the biosafety cabinet. The shut-off valve must be specifically identified as such.

- **Laundry**
  The employer must clean, launder, and dispose of all personnel protective gear at no cost to employees. Apparel contaminated with blood or other potentially infectious materials should be handled as little as possible and decontaminated, preferably by autoclaving, before being sent to the laundry for cleaning. This service can be provided for a nominal cost through an outside vendor. Call EHS for details (412-268-8182). Employees who handle contaminated laundry must wear appropriate PPE and be enrolled in the university’s Exposure Control Plan.

- **Housekeeping**
  The reduction of risk and protection of the integrity of biological experiments relies significantly on good housekeeping in the laboratory. Routine housekeeping provides work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

  Laboratory personnel shall maintain laboratory benches, equipment and areas that require specialized technical knowledge. Laboratory staff is responsible to:
  1. secure biohazards at the conclusion of work.
  2. keep the laboratory neat and free of clutter. Surfaces should be clean and free of infrequently used chemicals, biologicals, glassware and equipment. Access to sinks, eyewashes, emergency showers, and fire extinguishers must not be blocked.
  3. discard infectious waste appropriately. Do not allow it to accumulate in the laboratory.
  4. free aisles and corridors of tripping hazards.
  5. pay attention to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, avoidance of overloaded electrical circuits, and avoidance of the creation of electrical hazards in wet areas.
  6. remove unnecessary items on floors, under benches or in corners.
  7. properly secure all compressed gas cylinders.
  8. never use fume hoods or BSCs for storage.

  Practical custodial concerns include:
  1. Dry sweeping, dusting, and the use of wet or dry industrial type vacuum cleaners may lead to the formation of aerosols and is prohibited in biohazard areas.
  2. Vacuum cleaners must be equipped with high efficiency particulate air (HEPA) filters to be used in the biological research laboratory posted as a biohazard area. Wet and dry units with HEPA filters on the exhaust are available form a number of manufacturers.

- **Spill Cleanup**
  Spills of biohazardous materials may constitute a significant health hazard if not handled in an appropriate manner. All personnel working with biohazardous materials must be trained in the specific cleanup and disinfectant procedures to be used for their particular laboratory.
A biological spill shall be followed by prompt action to contain and clean up the spill. When a spill occurs, stop other activities around the spill, notify others, isolate the area, and call for assistance. The degree of risk involved in a spill depends on the volume of the material spilled, the creation of infectious aerosols, the concentration of the organisms in the material spilled, the hazard of the organisms involved, the route of infection of the organisms, and the disease caused by the organisms. Contact EHS at 412-268-8182 for assistance if the spill area is too large (not easily contained, aerosols are generated, or > 1 liter). If the spill is resultant from illegal action or a person is severely injured, call Campus Police at 412-268-2323 immediately. All large spills and spills resulting in personal injury or contamination must be reported to EHS at 412-268-8182.

Spills of biological agents can contaminate areas and lead to infection of laboratory workers. Exposure prevention is the primary goal in spill containment and cleanup.

**Spill Kits in the Laboratory**

Each laboratory using biohazardous materials should have appropriate equipment and supplies on hand for managing spills and accidents involving biohazardous materials. Permanent equipment should include a safety shower, eyewash and a hand-washing sink with soap and paper towels. A biohazardous spill kit should also be kept on hand. The supplies available in a biohazard spill kit should include, but are not limited to:

1. A copy of the Spill Cleanup Protocol
2. Nitrile disposable gloves (min 8 mil thickness)
3. Lab coat(s)
4. Safety goggles
5. Disposable shoe covers (booties)
6. Absorbent material, such as absorbent paper towels, granular absorbent material, etc.
7. All-purpose disinfectant, such as normal household bleach (freshly diluted, 1:10)
8. Autoclavable bucket for diluting disinfectant (this can be used to store the kit contents when not in use)
9. Tongs and/or forceps, and/ or dustpan and hand broom or squeegee, etc. (for picking up broken glass or other contaminated sharps)
10. Sharps waste container(s)
11. Autoclavable biohazard waste bags
12. Biohazardous spill warning signs and stickers

**Small Spills in the Laboratory:**

A spill is generally considered to be small if it is easily contained, has not generated aerosols, and is not considered to be a significant threat to personnel in other areas of the laboratory. For small spills,

1. wear gloves and personal protective clothing;
2. do not handle sharps with the hands; clean up broken glass or other sharp objects with sheets of cardboard or other rigid, disposable material;
3. avoid the generation of aerosols;
4. absorb spill with an appropriate spill kit material or disposable inert absorbent material, such as, paper towels or gauze; note: most disinfectants are less effective in the presence of high concentrations of organic material;
5. place disinfectant-soaked paper towels and clean the surface with a suitable disinfectant;
6. properly dispose of all contaminated materials.

**Large Spills in the Laboratory:**

In evaluating the risks of large spill response, generation of aerosols and droplets is a primary consideration. Spills often result in aerosol formation. If there is a danger of aerosol formation and the spill is outside the BSC inside the laboratory, personnel must:

1. notify other individuals in the laboratory to leave the area immediately;
2. close doors behind you and remove any contaminated clothing and place it in an autoclave bag;
3. wash all exposed skin;
4. note the time of the spill;
5. post the area to prevent others from entering;
6. allow aerosols to settle for 30 minutes before re-entering the laboratory;
(7) assemble supplies before entering the laboratory;
(8) put on appropriate PPE (e.g. disposable gown, protective eyewear, gloves, shoe coverings, etc.);
(9) clean up spill with an appropriate disinfectant and do as follows:
  ▪ Surround spill area will disinfectant or diking material that is soaked in disinfectant.
  ▪ Place disinfectant-soaked towels over the entire spill area.
  ▪ Allow 20-minute contact time with the disinfectant to ensure adequate germicidal action.
  ▪ Wipe down non-autoclavable materials with disinfectant.
  ▪ Place items designated as contaminated in an appropriate infectious waste container (i.e. sharps container, biohazard box, autoclave bag, etc.).
  ▪ Place contaminated re-usable items in autoclavable containers, such as, biohazard bags, pans with lids. Sterilize these re-usable items via autoclaving and follow with cleaning for re-use.
  ▪ Remove protective clothing used during clean-up and place in autoclavable biohazard bag for autoclaving.

Spill Inside BSC:
If there is a danger of aerosol formation and the spill is inside the BSC, personnel must:
(1) continue to run the BSC during cleanup to contain aerosols and HEPA-filter exhaust air;
(2) don appropriate PPE before initiating clean-up,
(3) if the spill is contained on a bench diaper, remove the contaminated bench diaper and discard as infectious waste;
(4) if the spill is on the work area surface, cover spilled material with appropriate disinfectant-soaked towels;
(5) allow 20 minutes contact time, remove the contaminated towels, and discard towels as infectious waste;
(6) wipe down the interior of the cabinet and any splatter on items within the cabinet with an appropriate disinfectant-soaked towel;
(7) wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.

Spills in a Centrifuge or Other Equipment:
A biological spill in a centrifuge has the potential for producing large volumes of aerosols and for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. Perform the opening of all centrifuges slowly.
(1) If a centrifuge tube breaks while the centrifuge is running, immediately turn off motor and notify others in the laboratory, and evacuate. Note the time of the incident and do not attempt to re-enter the laboratory. Contact the BS Office to assist in clean up and decontamination. The centrifuge must be at rest for a minimum of 30 minutes prior to reentry.
(2) If breakage is discovered after the machine has stopped, re-close the lid immediately, notify others in the laboratory, and evacuate. Note the time of the incident and do not attempt to re-enter the laboratory. Contact the BS Office to assist in clean up and decontamination. The centrifuge must be at rest for a minimum of 30 minutes prior to reentry.

On becoming aware that a spill may have occurred in other types of equipment, turn off the equipment, warn others in the area, and leave the area for 30 minutes to allow time for any potential aerosols to settle. Prior to initiating clean-up and decontamination, contact the BS Office to assist.

The BS Office will initiate the following response:
(1) assemble supplies;
  ▪ disinfectant
  ▪ sharps container(s)
  ▪ towels
  ▪ tongs
  ▪ autoclave bags
  ▪ personal protective gear (disposable gown, protective eyewear, gloves, shoe coverings, respirator, etc.)
(2) don appropriate PPE and double glove;
(3) unplug equipment and slowly open centrifuge or other device to access contaminated area of equipment;
(4) if safety cup(s) or device is intact, remove unit to biological safety cabinet for further decontamination;
(5) if integrity of safety cup(s) or device is compromised, decontaminate all exposed surfaces before removing cup(s) or device to BSC for further decontamination.

Spills Outside the Laboratory:
Spills outside the laboratory are preventable during transport by ensuring that all infectious materials are placed in a rigid, securely sealed, and watertight primary container, that is subsequently contained in a second rigid, leak proof sealed container possessing absorbent in sufficient quantity to take up all contents in the event of a leakage from the primary container. The outer container must be labeled with the universal biohazard symbol. However, if a spill occurs during transport, immediately contact the BS Office for assistance, don gloves and initiate clean-up as follows:
(1) surround spill area with disinfectant or diking material that is soaked in disinfectant;
(2) place paper towels soaked in a disinfectant over the entire spill area;
(3) allow a minimum 20 minutes contact time with the appropriate disinfectant;
(4) place items designated as contaminated in an appropriate infectious waste container (i.e. sharps container, biohazard box, autoclave bag, etc.);
(5) repeat decontamination of spill area after removing contaminated materials;
(6) wash hands as soon as possible.

3.7.4 Decontamination
Decontamination is the reduction of all organisms and the destruction of pathogenic organisms in or on a material so that material is no longer considered to be capable of transmitting disease. Simply stated, it is a term used to describe the process or treatment that renders a device, instrument, or environmental surface safe to handle. A decontamination procedure can range from sterilization to simple cleaning with soap and water. Sterilization, disinfection, and antisepsis are all forms of decontamination.

- **Sterilization**: is the act or process, physical or chemical, which destroys or eliminates all forms of microbial life, including highly resistant bacterial endospores.

- **Disinfection**: is the act of destroying or irreversibly inactivating specific viruses, bacteria, or pathogenic fungi, but not necessarily their spores, on inanimate objects, such as work surfaces and equipment. Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, the object to be disinfected, and chemical exposure time, temperature, and concentration. Most disinfectants are not effective sterilizers.

- **Antisepsis**: is the application of a substance that prevents or arrests the growth or action of microorganisms by inhibiting their activity or destroying them. The term is used especially for preparations applied to living tissue. It includes swabbing an injection site on a person or animal and hand washing with germicidal solutions. Although some chemicals may be utilized as either a disinfectant or an antiseptic, adequacy for one application does not guarantee adequacy for the other. Use manufacturer’s recommendations for appropriate use of germicides.

Evaluation
The initial risk assessment for any project should include an evaluation of the processes and/or agents to be used to determine the proper method(s) of decontamination. OSHA’s Bloodborne Pathogen Standard mandates that all equipment used in the research as well as environmental and working surfaces shall be cleansed and decontaminated after contact with blood or other potentially infectious materials. The standard also requires decontamination of contaminated work surfaces after completion of procedures, immediately or as soon as feasible after any overt contamination of surfaces or any spill of potentially infectious material, and at the end of the work shift if the work surface has become contaminated. All reusable equipment shall be decontaminated immediately or as soon as feasible after visible contamination.

Methods of Decontamination
There are four main categories of physical and chemical means of decontamination. They are heat, liquid disinfection, vapors and gases, and radiation.

**Wet heat** is the most dependable method of sterilization. Steam sterilization or autoclaving generally denotes heating in an autoclave employing saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 121°C. Autoclaving rapidly achieves destruction of microorganisms, decontaminates infectious waste and sterilizes laboratory glassware, media, and reagents. The following points must be kept in mind when steam sterilization is to be used:
Materials affected by heat, e.g. denatured or melted, will be destroyed by this method.

Material to be sterilized must come in contact with steam and heat for a prescribed period of time to ensure sterilization. Containers must be open to steam penetration, or water must be placed in the container before placing in the autoclave.

Steam must flush the air out of the autoclave chamber for efficient heat transfer. Before using the autoclave, check the drain screen at the bottom of the chamber. If blocked, clean it. Additionally, the sieve shall remain clear. If a layer of air forms at the bottom of the autoclave, it can prevent efficient operation and adequate sterilization.

The use of appropriate biological indicators at locations throughout the autoclave is considered the best indicator of sterilization and should be conducted at least monthly. One indicator widely used to indicate effectiveness of wet heat is Bacillus stearothermophilus. The spores, which can survive 250°F for 5 minutes, but are killed at 250°F in 13 minutes, are more resistant to heat than most, thereby providing an adequate safety margin when validating decontamination procedures. Each type of container and load employed should be spore tested. Efficacy varies with the load, fluid volume, etc. The use of a chemical indicator, e.g. autoclave tape, is not an adequate monitor of efficacy. However, such indicators should be used with each load placed in the autoclave. This at a minimum ensures the autoclave process reached the appropriate temperature. Use extreme caution when opening the autoclave following the sterilization cycle. Live steam can cause serious injury. Additionally, malfunctioning autoclaves can fill with superheated water that will be released when the autoclave is opened. Note: Warranties and preventive maintenance plans for all autoclaves are strongly recommended by the BS Office.

For a facility to terminally treat biohazardous waste, it must be licensed by the Pennsylvania Department of Environmental Protection. Currently, there are no autoclaves at the university permitted to terminally treat hazardous waste. All terminal treatment is through the university’s infectious waste processor. Consequently, all biohazardous waste shall be placed in a biohazard box, whether previously autoclaved not, and submitted to the BS Office for appropriate disposal. According to the PA DEP, for terminal sterilization to be allowed, the sterilization process must be validated, and validation documented. Additionally, the sterilization process must also be monitored at least every 48 hours with biological indicators (spore strips, time/temperature charts, etc.) and records of monitoring kept for review. Further, any infectious and/or medical waste must be rendered unrecognizable prior to or through terminal disposal.

The following are recommended procedures for autoclaving.

1. All individuals operating an autoclave must be adequately trained by a PI or laboratory manager. An untrained person shall never operate an autoclave.
2. Ensure all containment vessels can withstand the temperature and pressure of the autoclave.
3. Never exceed the manufacturer’s recommendations for operating temperature and pressure.
4. Wear safety glasses, protective clothing, and heat resistant gloves when loading and unloading an autoclave.
5. Select the appropriate cycle. A slow exhaust shall be used for steam sterilizing fluids to prevent boiling over. The fast exhaust shall be used for glassware. The fast and dry cycle may be used for wrapped items.
6. Only use polypropylene or polyethylene autoclave bags. These bags are impermeable to steam and should not be twisted and taped shut. Secure the top of containers and bags loosely to allow steam penetration. Position bags with the neck of the bag taped loosely and leave space between items in the autoclave bag to further enhance steam penetration.
7. Autoclave bags shall always be placed in a secondary containment vessel to retain any leakage that may occur. The secondary containment must be constructed of material that will not melt, distort, or degrade during the autoclave process. Polypropylene is a plastic capable of withstanding autoclaving, but is resistant to heat transfer. Hence, materials contained in a polypropylene pan will take longer to sterilize than the same material in a stainless pan.
8. Fill liquid containers only half-full, loosen caps or use vented closures.
9. Materials with high insulating capacity, such as animal bedding or saturated absorbent pads, require increased time for the autoclave to reach sterilizing temperature, i.e. extended autoclaving cycle times.
10. Never autoclave items containing solvents, volatile or corrosive chemicals. Therefore, high concentrations of bleach solutions should not go into an autoclave.
11. Always ensure that the pressure of the autoclave chamber is at zero before opening the door. Stand behind the autoclave door and slowly open it to allow the steam to gradually escape from the autoclave chamber after cycle completion.
(12) Allow liquid materials inside the autoclave to cool down for 15-20 minutes prior to removing them.

(13) Dispose of all autoclaved waste through the infectious waste stream.

(14) Never autoclave sharps containers and other plastic items which may melt during the autoclaving process.

Dry heat is less efficient than wet heat sterilization and requires longer times and/or higher temperatures. The specific times and temperatures to be used must be determined for each type of material being sterilized. It is suitable for the destruction of viable organisms on impermeable non-organic surfaces such as glass, but it is not reliable in the presence of shallow layers of organic or inorganic materials which may act as insulation. The advantage of wet heat is an improved heat transfer to and into the cell, resulting in overall shorter exposure time and lower temperatures. Steam sterilization uses pressurized steam at 121 to 132 °C for 30 to 40 minutes. This type of heat kills all microbial cells, including spores, which are normally heat resistant. In order to produce the same effect with dry heat in an oven, the treatment temperature and time needs to be increased accordingly. Sterilization of glassware by dry heat can usually be accomplished at 160 to 170 °C for periods of 2 to 4 hours. Dry heat sterilizers should also be monitored on a monthly basis using appropriate biological indicators, such as Bacillus subtilis spore strips.

Liquid disinfection is very practical for surface decontamination and when used in sufficient concentration, as a decontaminant for liquid waste prior to final disposal in the sanitary sewer. If liquid disinfectants are used, they must have been shown to be effective against the organism(s) present. The disinfectant and the disinfection process must be validated, and the validation documented. Personnel must be trained in the appropriate use of the approved disinfectant. Personnel must also wear chemical resistant gloves and laboratory coats or gowns during the preparation and use of liquid disinfectants. BS Office personnel can assist in the development of an appropriate validation and monitoring process.

Liquid disinfectants are available under a wide variety of trade names. In general, these can be classified as: halogens, acids, alkalis, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. The more active a compound is, the more likely it is to have undesirable characteristics. No liquid disinfectant is equally useful or effective under all conditions and for all viable agents. Disinfectants must always be used in accordance with the manufacturer’s recommendations. Failure to follow the manufacturer’s recommendations can result in ineffectiveness of the disinfectant. Appendix 8.8 Properties of Common Disinfectants presents some useful information concerning commonly used disinfectants.

3.7.5 Biological Waste Management

Certain wastes are regulated and must be handled according to prescribed methods. All applicable rules and regulations of local, state, and federal agencies are to be followed in the handling, treatment, and disposal of biological waste. Biological wastes generated at the university, as defined below, are in part regulated in Pennsylvania by the Department of Environmental Protection. The university has established procedures in accordance with all applicable regulations. It is the responsibility of generators to properly adhere to these procedures and accordingly sort and dispose of all biological waste. All biological waste activities are managed by EHS and are categorized accordingly:

- **Cultures and Stocks** of infectious agents and associated biologicals, including the following:
  1. cultures from medical and pathological laboratories
  2. cultures and stocks of infectious agents from research and industrial laboratories
  3. wastes from production of biologicals
  4. discarded live and attenuated vaccines
  5. culture dishes, assemblies and devices used to conduct diagnostic tests or to transfer, inoculate and mix cultures

- **Pathological wastes** are human pathological wastes, including, tissues, organs and body parts and body fluids that are removed during medical or laboratory procedures. Hair and nails are excluded.

- **Human blood, blood products and body fluid waste** include the following:
  1. liquid waste human blood
  2. human blood products
  3. items saturated or dripping with human blood
(4) items that are caked with dried human blood, including serum, plasma, and other blood components, which are used or intended for use in patient care, specimen testing or the development of pharmaceuticals
(5) intravenous bags that contain or have contained human blood
(6) items contaminated by body fluids from persons during medical or laboratory procedures
(7) specimens of blood products or body fluids, and their containers
- **Animal wastes** include animal carcasses, body parts, blood, blood products, secretions, and excretions. Bedding of animals that were known to have been exposed to zoonotic infectious agents or non-zoonotic human pathogens during research must also be considered a biological waste.
- **Isolation wastes** are those contaminated with blood, excretion, exudates and excretion from humans who are isolated to protect others from highly virulent diseases, or isolated animals known or suspected to be infected with highly virulent diseases.
- **Contaminated Sharps** include hypodermic needles, syringes (with or without the attached needle), Pasteur pipettes, scalpels, blood vials, needles with attached tubing, culture dishes, suture needles, slides, cover slips and other broken or unbroken glass or plasticware that have been in contact with infectious or biological agents or that have been used in animal or human patient care or treatment.
- **Recombinant and synthetic nucleic acid wastes** include any items that have been in contact with recombinant and synthetic nucleic acids as defined in the context of the NIH Guidelines. Per the NIH Guidelines, recombinant and synthetic nucleic acid molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. Synthetic nucleic segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural counterparts. If the synthetic segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines. Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

At the time of generation, biological waste must be accumulated in containers compatible with the waste and separated from general waste into four classes: used sharps, fluids, other, and animal.

- **Contaminated Sharps** include needles with syringes, slides, pipettes, pipette tips, capillary tubes, scalpels, razors and any other glass or metallic object that can puncture human skin. Sharps must be disposed in a puncture resistant container appropriate for the sharp. Sharps consisting of a metal or glass material (needles, capillary tubes, etc.) must be placed in a rigid, plastic sharps container. Sharps consisting of a plastic material (i.e. pipettes and pipette tips) must be placed in a plastic-lined rigid container such as a cardboard box. Container must be labeled accordingly (i.e. Non-Infectious Sharps, Plastic Infectious Sharps, or Plastic Non-Infectious and Non-Biological Sharps).

(1) Obtain an appropriate university-approved sharps container for the collection of infectious sharps from the Biological Safety Office. A rigid plastic sharps container labeled as infectious will be issued for the collection of metal or glass sharps. A waste box labeled as infectious will be issued for the collection of plastic sharps potentially contaminated with an infectious or biological agent. Principal investigators must provide a rigid cardboard box for the collection of plastic sharps that are not contaminated with a biological agent. He/she shall ensure that these containers are labeled appropriately with Non-Infectious and Non-Biological Plastic Sharps.
(2) Place sharps container at a location in the laboratory that is accessible, preferably at the point of use.
(3) Never compromise the safety features of a sharps container or attempt to enter the sharps container for any reason.
(4) Fully depress syringes with needles into an appropriately labeled liquid waste container.*
(5) Place disposable syringe with needle, into a plastic sharps container. Never recap needles or attempt to remove needles from syringes!
(6) Place all other sharps, including syringes that are not attached to needles, into the appropriate puncture resistant container following their use.
(7) Permanently close sharps container when it becomes two-thirds (2/3) full. Close and seal the plastic sharps container or cardboard puncture resistant container with a heavy-duty tape (e.g. duct or packing tape).

(8) Dispose of all plastic sharps containers and cardboard containers used for the collection of infectious plastic sharps through the university's Biological Safety Office.

(9) Dispose of the cardboard puncture resistant containers used for the collection of non-infectious plastic sharps (i.e. Non-Infectious and Non-Biological Plastic Sharps) via the sanitary waste stream.

*If you are using acutely toxic chemicals or radioactive materials, you must call the Chemical Safety Manager or the Radiation Safety Officer, respectively, for additional guidance. Contact information may be found via the EHS website, or by calling 412-268-8182.

- **Fluids** may be decontaminated by exposure to an appropriate and previously validated disinfectant for the infectious agent(s) of interest. Refer to Appendix 8.8: Properties of Common Disinfectants to assist in the proper selection of a disinfecting agent and contact time. Only those agents and contact times indicated in Appendix 8.8 as appropriate for fluid disinfection may be employed to disinfect the respective agent of interest. Fluid wastes should be carefully poured into the appropriate disinfectant to inactivate the biohazardous agent. Following sufficient contact time, the disinfected biohazardous fluid may be poured directly down the drain if no hazardous chemical is present. If the waste contains hazardous chemicals, the liquid waste must be disposed of according to the hazardous waste guidelines provided in Carnegie Mellon University's Chemical Hygiene Plan. Drain disposal of non-hazardous liquids should be done carefully to avoid splashing and aerosol generation. Afterwards the drain should be flushed with disinfectant of sufficient quantity to at least fill the trap. The pouring of these wastes must be accompanied by large amounts of water. The empty fluid container may be autoclaved and washed if reusable or autoclaved and then discarded with other infectious waste if disposable.

Chemical disinfection of infectious waste must be monitored to assure the administrative efficacy of the treatment method. A log, Appendix 8.8, form 1 Fluid Disinfection Log, noting the agent(s) to be disinfected, date the chemical disinfectant was added to the biohazardous fluid, the chemical disinfectant, the final concentration of the disinfectant, the time the disinfectant was in contact with the biohazardous fluid, the individual performing the treatment, and method of final disposition (i.e. sanitary sewer) must be kept.

**Note:** Only those disinfectants indicated as appropriate for fluid disinfection in Appendix 8.8 may be employed in the disinfecting of fluids. If the biological or infectious agent requires an alternate disinfectant that is not indicated in Appendix 8.8 as a fluid disinfectant, contact the Biological Safety Office to assist in the proper disinfecting and/or disposal of the fluid.

- **Other** waste includes solid waste, such as pipettes and culture plates, grossly contaminated personal protective equipment, and any additional items that are not appropriate for re-use and are contaminated with a biological and/or infectious agent. Solid wastes must be discarded directly into a plastic-lined biohazard cardboard box provided by EHS. Upon becoming two-thirds (2/3) full, the container must be permanently closed by gathering and securing the plastic liner with a security tie and closing and sealing the cardboard container. These containers must not be used for any other materials or purpose.

**Note:** Lab coats, gloves, and other articles of PPE without gross contamination are not considered waste and can be stored in the biohazards area in appropriately labeled containers (autoclavable biohazard bags, at least 3 mils thick). The items can then be autoclaved at 121 degrees C for 60 - 90 minutes and returned for use.

- Research **animals or animal parts** are considered to be biological wastes and are discarded through the university's infectious waste stream. Disposal of animal carcasses in the general trash or any other manner is prohibited.

Generators must submit an on-line request through the EHS website found at [https://www.cmu.edu/ehs/Hazardous-Waste-Management/biological-waste.html](https://www.cmu.edu/ehs/Hazardous-Waste-Management/biological-waste.html) for the collection of biological waste. A request for waste pick-up must be submitted at least 48 hours in advance of scheduled
pick-up times posted on the biosafety website. Generators of biological waste should review this schedule to ensure compliance with all regulatory time frame requirements. Follow the instructions at the website for the submittal of the request, including all necessary information: Amount and type of waste, name of requestor, location, any special handling precautions, and supply requests.

EHS personnel will not remove any waste that is in a damaged, leaking, or inappropriately packaged container. A note will be attached or sent to the generator to indicate the specific error. It is the responsibility of the waste generator to correct any error.

Upon the submittal of a request for pick-up, email notices will be automatically created and sent to the Biological Safety Officer. The email will reflect the information provided on the request. This notification allows the Biological Safety Office to properly prepare the waste pick-up.

Infectious waste—waste suspected to contain pathogens (bacteria, viruses, parasites, or fungi) in sufficient concentration or quantity to cause disease in susceptible hosts—cannot be accumulated. Contaminated material should be immediately inactivated or disposed of daily or on a regular basis. If storage of contaminated material is necessary, it must be done in a manner that maintains the integrity of packing, kept away from general traffic, and labeled appropriately. Infectious waste, excluding used sharps, may be stored at room temperature in a nonputrescent state until the storage container is full. Infectious waste may not be stored at room temperature longer than 30 days from the date of generation. Infectious waste may be refrigerated for up to 30 days or frozen up to 90 days from the date of generation. All infectious waste refrigerated or frozen for the purpose of storage must be dated upon commencement of storage. All storage in refrigerators and freezers must be approved by the Biological Safety Officer.

If the infectious waste becomes rotten during storage, it must be moved off-site by the university’s infectious waste contractor within 24 hours for immediate processing and disposal.

4.0 Registration of Recombinant and Synthetic Nucleic Acid Research and Biohazard Research

Registration of recombinant and synthetic nucleic acid molecule research begins with the Carnegie Mellon University Institutional Biological Safety Committee (IBC) Recombinant or Synthetic Nucleic Acid Molecule Research Application. The university’s Department of Environmental Health and Safety provides the Carnegie Mellon University Institutional Biological Safety Committee (IBC) Recombinant or Synthetic Nucleic Acid Molecule Research Application to those principal investigators anticipating the use of recombinant or synthetic nucleic acids in their work. The purpose of the Carnegie Mellon University Institutional Biological Safety Committee (IBC) Recombinant or Synthetic Nucleic Acid Molecule Research Application is to provide the Department of Environmental Health and Safety with a list of all individuals participating in research involving the use of recombinant/ synthetic nucleic acids and determine where proposed experiments fall within the federal guidelines. The form requires the principal investigator to list the following:

- genes or type of genes to be cloned
- possible products
- species that are the source of the recombinant or synthetic nucleic acid molecules
- type of cloning vector(s)
- host (e.g. E. coli K-12) that will be used to propagate the recombinant/synthetic nucleic acid molecules
- pathogenic organisms to be used; if mammalian pathogens, the NIH risk group classification
- relevant sections of NIH Guidelines if experiments are thought to be exempt from NIH Guidelines.

Many projects are exempt from registration and full committee review under the NIH Guidelines. The university’s Department of Environmental Health and Safety will utilize the Carnegie Mellon University Institutional Biological Safety Committee (IBC) Recombinant or Synthetic Nucleic Acid Molecule Research Application to determine the need for further action on behalf of the principal investigator.

5.0 Transfer of Biological Materials

The transfer of biohazardous materials is regulated by a number of government agencies, such as the United States Department of Health and Human Services (DHHS) and Department of Transportation (DOT), and Dangerous Goods Regulations, International Air Transport Association (IATA). It is imperative that personnel are aware of applicable regulations and comply with them. The shipper (i.e. the person with direct knowledge of what is being shipped) of biohazardous material must be trained every two years and is responsible for the
proper classification, identification, packaging, labeling, and documentation of the shipped material. Failure to comply could result in the confiscation and destruction of the material and subsequent monetary fines.

5.1 Intramural Transfer

The university defines transfer as intramural or extramural receiving, shipping or movement of biohazardous materials. An intramural transfer is one that occurs within the university. Materials may be transported by foot on main campus (i.e. traveling on only university owned roadways and not on any public roadways) or between main campus and remote facilities (e.g. Student Health Services, Mellon Institute, and Carnegie Mellon Research Institute) by packaging the material in a rigid, securely sealed, water-tight primary container, contained within a second rigid, sealed, watertight container. Sufficient absorbent must be placed within the second container to take up contents of the primary container in the event of leakage and the outer container must be labeled with the universal biohazard symbol. The material is not permitted to be transferred by vehicle for transports between main campus and remote facilities. In addition, it is strongly discouraged to use vehicles to transport materials on main campus.

Note: Most personal vehicle insurance policies are void in the event that hazardous material transported

5.2 Extramural Transfer

An extramural transfer is one that occurs between the university and another institution. Most extramural transfers require vehicular, air or sea transport. The packaging and shipping of biological materials for extramural transport must comply with federal and international shipping requirements. The regulations require any biological material which may contain an infectious agent(s) to be packaged and shipped in a manner that the contents will not leak and will arrive in good condition. Contact Environmental Health and Safety (412-268-8182) for guidance in the packaging and shipping of diagnostic specimens, biological and infectious substances, and import and export of biological materials and live organisms.

Note: Although transferring material to the University of Pittsburgh or another university in close proximity to Carnegie Mellon University is an extramural transfer, due to the close proximity, the requirements of an intramural transfer may be employed for the purpose of transporting the material, if transporting by foot and not vehicle or bicycle.

6.0 Etiologic Agent or Vector Permits

Etiologic agents are those microorganisms and microbial toxins that cause disease in humans and include bacteria, bacterial toxins, fungi, rickettsiae, protozoans, and parasites. These disease-causing microorganisms may also be referred to as infectious agents. Arthropods and other organisms that transmit pathogens to animals or humans are called vectors.

6.1 Import Permits

Importation or transportation of etiologic agents is governed by federal regulation. Per United States Public Health Service (USPHS) 42 CFR-Part 71 Foreign Quarantine, (a) a person may not import into the United States, nor distribute after importation, any etiologic agent or any arthropod or other animal host or vector of human disease, or any exotic living arthropod or other animal capable of being a host or vector of human disease unless accompanied by a permit issued by the Director. (b) Any import coming within the provisions of this section will not be released from custody prior to receipt by the District Director of U.S. Customs Service of a permit issued by the Director of Centers for Disease Control and Prevention.

The following items require permits:

- **Etiologic agents** are any infectious agent known to cause disease in man.
- **Biological materials** include, unsterilized human and animal tissue specimens, such as blood, body discharges, fluids, excretions, or similar material, containing an infectious or etiologic agent.
- **Hosts and vectors** include
  
  1. Any animal known or suspected of being infected with an organism capable of causing disease in humans. Importation of turtles less than 4 inches in shell length and all nonhuman primates requires an importation permit issued by the CDC, Division of Global Migration and Quarantine.
  
  2. Any living insect or other arthropod that is known or suspected of containing an etiologic agent transmissible to man. Also, *all living fleas*, *flies*, *lice*, *mites*, *mosquitoes or ticks*, even if not suspected of being infected with an etiologic agent. This includes eggs, larvae, pupae, and nymphs, as well as adult forms.
(3) **All live bats** require an import permit from the CDC and the U.S. Department of Interior, Fish and Wildlife Services 202-358-2095.

(4) **Any snails capable of transmitting a human pathogen** (e.g. schistosomiasis). No mollusks are to be admitted without a permit from either the CDC or Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.

Importation permits are issued by the Biosafety Branch, Office of Health and Safety, CDC, after review of a completed application. Application forms are available on the CDC website or may be obtained directly from the Biological Safety Office (8-3221) or by calling the CDC at (404) 498-1600. Completed forms may be returned to CDC by mail (1600 Clifton Road NE, Mailstop E-79, Atlanta, Georgia 30333) or fax (404) 498-2275. All applications to the CDC for the importation permit should be made 10 working days in advance of the shipment date. Upon receiving the permit, principal investigators should forward a copy to the Biological Safety Office.

### 6.2 Other Permits

- **United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) permits** are required to import or transport infectious agents of livestock and biological materials containing animal, particularly livestock, material. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the United States. Applications for USDA/APHIS permits may be obtained at [https://www.aphis.usda.gov/aphis/resources/permits](https://www.aphis.usda.gov/aphis/resources/permits). Call the USDA/APHIS at (301) 734-7834 or visit [https://www.aphis.usda.gov/aphis/home](https://www.aphis.usda.gov/aphis/home) for further information.

- **United States Department of Interior (USDI) requires permits** for certain live animals and all live bats. Call (202) 358-2095 or visit [http://www.doi.gov](http://www.doi.gov) for further information.

- The **Center for Disease Control requires registration of select agents and toxins** in accordance with 42 CFR Part 73 Possession, Use, and Transfer of Select Agents and Toxins for the select agent(s) or toxin(s) listed on the import permit application. In addition, per 42 CFR Part 73.14(a) (3), Form EA-101 must be completed and submitted to the CDC Select Agent Program and granted approval prior to the shipment of the select agent(s) or toxin(s) under the import permit. Refer to Appendix 8.2 Select Agent Program and visit [http://www.cdc.gov/od/sap](http://www.cdc.gov/od/sap) for additional information.

### 6.3 Export Licenses

The export of etiologic agents of human, plant, and animal diseases may require a license from the Department of Commerce. Call the Department of Commerce Bureau of Export Administration at (202) 482-4811 or visit [https://www.bis.doc.gov/](https://www.bis.doc.gov/) for further information. Information on other United States Government Departments and Agencies with export control responsibilities can be found at [https://www.bis.doc.gov/index.php/about-bis/resource-links](https://www.bis.doc.gov/index.php/about-bis/resource-links).

### 7.0 Training

Biological safety and Bloodborne Pathogen training is provided to individuals who handle or use biological materials and have the potential for exposure to bloodborne pathogens, respectively. The scope of the training is commensurate with the hazard involved. The Biological Safety Office conducts initial training and periodically thereafter, dependent upon use and hazards associated with an individual's function at the university.

### 7.1 Biological Safety Training

Biological safety training is recommended to all faculty, staff, laboratory personnel or students who handle or work with biological materials. All faculty, staff, laboratory personnel or students who are working at Biosafety Level 2 must attend biological safety training. The training is typically 1.5 hours and provides the following instruction:

- defines biohazards: microorganisms, cells, recombinant/synthetic nucleic acids, human blood, animals, human subjects, etc.
Carnegie Mellon University  Safety Plan for the Use of Biological Materials and Associated Devices
Department of Environmental Health and Safety  Revision date: February 2020

- reviews laboratory associated illness: What are they? How they are transmitted? Who is at risk? How are they put at risk?
- provides biosafety level considerations,
- discusses personal protection and exposure control,
- reviews primary and secondary containment and considerations to help prevent exposure to potentially harmful materials,
- discusses incident response,
- discusses appropriate selection of disinfectants and actions to take in emergency and non-emergency situations.

7.2 Bloodborne Pathogen Training
The university’s Bloodborne Pathogen training course is designed to fulfill training requirements of the Occupational Safety and Health Administration’s (OSHA) Bloodborne Pathogen (BBP) standard and provides information regarding exposure control principles and practices. The training is intended for university personnel who have a reasonable, anticipated risk of occupational exposure to blood, blood products, and other potentially infectious material and is required annually. The training is typically 1 hour and provides the following instruction:
- identifies who is covered by the standard and all applicable key requirements of the standard
- reviews three specific bloodborne disease that an individual is at risk of becoming infected: hepatitis B, hepatitis C, and human immunodeficiency virus,
- describes various routes of exposure,
- discusses posting and transporting requirements,
- provides explanations of exposure control measures,
- provides various personal protective equipment options,
- discusses appropriate disinfectant selection and disposal requirements.

8.0 Surveillance

8.1 Record Keeping
All records required by the OSHA Bloodborne Pathogens Standard will be maintained by one of three entities. Carnegie Mellon University’s Department of Environmental Health and Safety will maintain the university employee training records and Sharps Injury Log. The Student Health Services will maintain inoculation and core antibody testing records. Concentra will maintain the university employee medical records regarding post-exposure evaluation, counseling, and treatment.

8.2 Laboratory Audits
EHS will conduct annual visits at a minimum to all laboratories in possession or use of biological materials. These visits are to ensure the proper use, handling, storage, and decontamination techniques employed by all personnel within the laboratory. EHS will subsequently issue a memo indicating positive reinforcement of exceptional procedures and practices, findings, and/or corrective actions applicable to the laboratory environment.

Findings and corrective actions must be addressed within a 120-day time period, unless otherwise specified. EHS will confirm that all issues have been addressed by revisiting the laboratory.

8.3 Compliance
Any willful act(s) of non-compliance on behalf of a principal investigator and/or his/her staff will be addressed immediately with the university’s BSO, Director of EHS, and/or the university’s Institutional Biological Safety Committee (IBC). Follow-up action will be dependent on the severity and/or repetitiveness of the non-compliant act.

9.0 Accident and Incidents
Rapid and accurate reporting of accidents and incidents involving exposure to biohazardous agents is important in identifying potentially hazardous operations and procedures. Furthermore, identification of exposures to infectious agents allows personnel to be treated for the agent and minimizes the potential for actually contracting a disease associated with the agent. Refer to the Bloodborne Pathogen Exposure Control Plan to learn the appropriate reporting procedures and post-exposure evaluation process.
implemented at the university for all employees having the potential for exposure or having been exposed to infectious agents and/or material.