Charles R. Drew University of Medicine and Science

BIOSAFETY MANUAL

Version 1.1

IBC Approval Date: March 9, 2021

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TABLE OF CONTENTS

Emergency Phone Numbers and Resources

Emergency Response – CALL 911

Name	Phone Number	
Environmental Health & Safety (EHS) Specialist	(323) 357-3659	
Hazardous Materials Specialist, MLK	(310) 668-4057	
CDU Security	(323) 563-4918 (323) 326-4859 (*after hours*)	
Bayside Medical Center 2301 West El Segundo Blvd. Hawthorne, CA 90250	(323) 757-2118	• Work-related injury for employees (faculty and staff only) during regular **business hours**
Bayside Medical Center 2301 West El Segundo Blvd. Hawthorne, CA 90250	(323) 757-2118	• Student related injuries (all types) • Work-related injury for employees (faculty and staff) **after hours** only • Immunizations • Medical Surveillance
St. Francis Medical Center Emergency, 3630 East Imperial Hwy, Lynwood, CA 90262	(310) 900-4525	
St. Francis Industrial Medicine	(310) 900-2790	
LA County Health Department, Communicable Disease Control	(213) 240-7941	
LA County Health Department, Emergency Office	911	

Web Resources

Resource	Website
Centers for Disease Control and Prevention	http://www.cdc.gov/

National Institutes of Health	**http://www.nih.gov/**
National Institute for Occupational Safety and Health	**http://www.cdc.gov/niosh/homepage.html**

Reference

Boston University Biosafety Manual

Chapter 1

Biological Safety Program

Purpose

The purpose of this Biosafety Manual (Manual) is to define policies and procedures pertaining to the use of biological materials in research and teaching at Charles R. Drew University of Medicine and Science (CDU). These policies and procedures are designed to safeguard personnel and the environment from biologically hazardous materials without unduly limiting academic research. This manual also offers guidelines to comply with federal and state regulatory requirements.

The work practices, procedures, and policies specified in this manual are based on current regulatory requirements and accepted best biosafety practices. Implementation of these measures should reduce the likelihood that an incident involving a biological agent will occur and will fulfill regulatory biosafety expectations. Laboratory microbiological work usually involves potential exposure to biological hazards, as well as to chemical and radiological hazards. Consequently, this manual should be used in conjunction with the CDU Chemical Hygiene Plans and Radiation Safety Manual, respectively.

For information about specific Biological Safety Programs for operations not covered in this Manual, contact the Institutional Biosafety Committee (IBC) at (323) 563-4966.

Scope

This Manual applies to all CDU research activities involving biological agents. All faculty, staff, students, and visitors who work on projects, whether externally or internally sponsored, or funded or not, at any CDU facilities are included in the scope of this manual. The Manual is also applicable to any laboratory teaching activities that use recombinant or synthetic nucleic acid molecules and/or biological agents that have a potential for causing harm.

Biological agents include all infectious microorganisms (*e.g.*, bacteria, fungi, parasites, prions, rickettsias, viruses, etc.) that can cause disease in humans or pose significant environmental or agricultural impact, as well as the toxins derived from such organisms. Additionally, recombinant or synthetic nucleic acid molecules; human or non-human primate tissues, fluids, cells, or cell cultures; transgenic animals; and any work with animals and their tissues, which are known to be reservoirs of zoonotic diseases, are wholly or partly covered by the procedures and policies in this Manual.

Note: In this document, the term “Institution” is used to refer to CDU.

Biological Safety Program Goals

The goals of the Biological Safety Program, referenced in this Manual as the Biosafety Program, are to protect laboratory workers, the public, and the environment from potentially hazardous biological agents. The IBC advocates the use of biosafety precautions that effectively reduce or eliminate the risk of exposure to potentially hazardous agents used in research. In developing its guidelines, the IBC is ensuring that all policies and procedures are in accordance with both the regulatory frameworks governing the use of biological materials and the best practices adopted nationally.

Chapter 3 contains a listing and summary of the regulations and guidelines that govern the use of biological materials in research.

Roles and Responsibilities

Success of the Biosafety Program, like any other safety program, requires a team effort involving the IBC, Principal Investigators, laboratory workers, Occupational Health and Safety Committee (OHSC) and the Office of Research Integrity and Compliance (ORIC). The Biological Safety Program is also an integral part of the Occupational Health and Safety Program (OHSP), Hazardous Chemical Safety Program (HCSP), and Radiation Safety Program (RSP).

Principal Investigators are responsible for the health and safety of personnel who work under their supervision and occupy their laboratory space. CDU administration, IBC, DRO, OHSC, and ORIC endorse this manual and encourage active participation in maintaining high standards at CDU.

Institutional Official

The Institutional Official (IO) is the university President and has overall responsibility for

- Oversight for the control of hazards in the research laboratories and for ensuring that comprehensive, enterprise-wide programs are in place for the safe handling of all hazardous materials (*e.g*., biological, chemical, radiological, etc.).

- All non-financial research compliance at CDU.

- Direct functional responsibility for the Institutional Biosafety Committee (IBC) and Biosafety Program.

- In consultation with the provost and deans of College of Medicine (COM), College of Health and Science (COSH) and School of Nursing (SON), as well the CDU leadership, IO appoints various committee members. The IBC have been charged with planning and implementing the Biosafety Program, the purpose of which is to ensure the health and safety of all personnel working with biohazardous or infectious agents and recombinant or synthetic nucleic acid molecules.

Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is responsible for the overall oversight of the Biosafety Program at CDU (See Appendix P for IBC Oversight Program). The IBC carries out these functions pursuant to requirements set forth by the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), Occupational Safety and Health Administration (OSHA), Los Angeles County Department of Public Health, California Department of Public Health (DPH), and CDU.

The IBC's responsibilities include:

- Overall oversight of the Institutional Biosafety Program at CDU, including development of new, and review of existing, policies and procedures designed to enhance the biosafety program. Reviews and approves training programs and establishes qualifications for individuals working with biological materials.

- Coordinates the biosafety requirements with other campus-wide committees (*e.g*., IACUC, IRB) or programs (*e.g*., Occupational Health).

- Reviews and approves new research proposals involving recombinant or synthetic nucleic acid molecules and biohazardous material in accordance with guidelines established by NIH, CDC, Cal/OSHA, and CDU, as well as maintains project approval and reviews amendments.

- Sets required containment levels for research projects. Generally, biosafety levels (BSL) established by the CDC and NIH will be used as the level of containment; however, the IBC has the authority to increase or decrease the level of containment according to the project's specific circumstances. However, the IBC also has the authority to upgrade laboratory containment levels if the protocol review identifies specific hazards associated with the proposed operations.

- Approves design specifications and criteria for containment facilities. Develops comprehensive inspection programs, as well as receives and reviews the findings of such inspections. Investigates violations of biosafety procedures or policies and significant accidents or illnesses involving biological agents.

- Develop policies and procedures for proper disposal of recombinant or synthetic nucleic acids and biohazardous wastes.

- If appropriate, recommends disciplinary action to the proper CDU officials.

- Other activities as delegated by the Institutional Official.

Biosafety Officer

The Biosafety Officer (BSO) is responsible for providing guidance on the safe handling of biological agents and overall management of the Biosafety Program. The BSO is a voting member of the IBC.

The BSO's specific responsibilities include, but are not necessarily limited to, the following:

- Provides technical advice to the IBC, and researchers on laboratory containment, security, and safety procedures. Oversees periodic and unscheduled inspections to ensure that laboratory standards are rigorously maintained.

- Develops emergency plans for handling spills, personnel contamination and other research-related injuries and accidents.

- Develops and implements laboratory safety practices.

- Develops design specifications and criteria for containment facilities.

- Coordinates the preparation of IBC reports submitted to regulatory agencies.

- Acts as a liaison during regulatory inspections and works with the IBC, IO and compliance officer to coordinate responses to any regulatory findings.

- Provides training programs as approved by the IBC.

The BSO regularly reports on the Biosafety Program to the IBC. The BSO's report should include routine operational updates and any significant problems or violations of the regulatory mandates or IBC requirements on any research-related accidents or illnesses that have occurred.

Environmental Health and Safety (EHS) Specialist

The Environmental Health and Safety (EHS) Specialist ensures that operations are conducted in accordance with the criteria and guidelines. These include:

- Disposal of medical waste.

- Selection of appropriate protective clothing for individuals working with hazardous, including biohazardous, materials.

- Development and implementation of emergency response and preparedness.

- Monitoring work areas, including the presence of allergens.

Office of Research Integrity and Compliance (ORIC)

ORIC works closely with the IBC and Environmental Health and Safety (EHS) Specialist to ensure smooth functioning of the IBC and that the researchers conducting recombinant or synthetic nucleic acid molecules and/or biohazardous materials are properly trained, qualified and adhere to the applicable regulations and policies.

Principal Investigators

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

- Ensures that specific laboratory hazards are effectively communicated to laboratory personnel; personnel have received appropriate training and are competent to perform procedures used in the laboratory; and controls are in place to minimize risks associated with these hazards.

- Develops laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory.

- Ensures that engineering controls are available, in good working order, and are used appropriately to minimize exposure to biohazardous agents.

- Ensures that appropriate personal protective equipment (PPE) is available and used by laboratory personnel.

- Ensures that all laboratory personnel receive general biosafety training that is conducted as part of the Biosafety Program, as well as specific training on the hazards, procedures, and practices relevant to the laboratory in which they are working. **All training must be documented and records maintained.**

- Notifies the IBC and obtains prior IBC approval for work involving recombinant DNA and/or biohazardous material and conforms to all terms and conditions of IBC approval.

- Ensures that laboratory workers are provided immunizations and medical surveillance prior to, and in the event of, exposure to biohazardous agents as appropriate (based on current CDC and IBC recommendations). Immunizations are available at Bayside Medical Center for employees.

- Notifies the Environmental Health and Safety (EHS) Specialist or in their absence, IBC Chair or the Director of Research Integrity and Compliance of any spills, accidents, illness or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment.

- Ensures that biological agents are disposed of according to regulations, as outlined in this Manual.

- Ensures that biohazardous materials to be transported are packaged and shipped in accordance with regulations.

- Ensures that periodic inspections of the laboratory are conducted.

- Designates a safety officer for the lab. The PI automatically becomes the safety officer for the lab if no one has been designated.

Laboratory Workers

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. An incident caused by one laboratory worker can have a widespread effect on others. Laboratory workers are expected to

- Follow procedures and practices established by CDU, the IBC, and the laboratory.

- Use best biosafety laboratory practices to minimize exposures to biological agents and to avoid other incidents (such as personal injuries, chemical and radiation spills, laboratory fires, explosion, etc.).

- Attend biosafety and other laboratory safety training as required.

- Report spills, accidents, and unsafe laboratory conditions to the PI, Environmental Health and Safety (EHS) Specialist, IBC and other responsible parties.

- Utilize control measures, such as biological safety cabinets and personal protective equipment, to prevent exposure to biological agents and contamination of personnel and facilities.

Chapter 2

Approval of Research Projects

Who Needs Approval?

Principal Investigators (PIs) at CDU planning to carry out research using recombinant or synthetic nucleic acid molecules and/or biologically hazardous materials that pose a potential risk to the health of human or animals, either directly through infection or indirectly through damage to the environment, require review and approval by the IBC **before** they can start their work.

All investigators using recombinant DNA and/or biologically hazardous materials must register with the IBC regardless of whether the study requires full committee review.

Investigators shall submit "Project and Material Registration for Research Involving the Use of Recombinant or Synthetic Nucleic Acid Molecules and/or Biological Materials" (IBC Registration Form) and "Inventory of Microorganisms, Cell Lines, Primary Cell Culture, Human/Animal Materials" (Inventory), if they will be using the following:

1. Recombinant or synthetic nucleic acid molecules
2. Biohazardous Agents
3. Infectious Agents
4. Human blood, body fluids, unfixed tissue, or human cell cultures
5. Animal blood, body fluids, unfixed tissue, or animal cell cultures
6. Tissues, organ, or cell lines, cultures of human or non-human primate origin
7. Creating transgenic animals

Currently, CDU does not support research involving transgenic plants or select agents and toxins. Human gene therapy studies will be considered on a case-by-case basis.

When working with potentially infectious agents and experimental animals, IBC review is necessary in addition to review by the Institutional Animal Care and Use Committee (IACUC).

PIs whose research will involve biohazardous materials, infectious agents, human tissues (*e.g.*, blood, cells, fluids, etc.) or whose research comes under the governance of applicable federal regulations, state and local laws, CDU policies and procedures, are required to complete a "Project and Material Registration for Research Involving the Use of Recombinant or Synthetic Nucleic Acid Molecules and/or Biological Materials" and obtain IBC approval prior to receiving such materials or commencing such research.

Relevant federal and state regulations and institutional policies, include but not limited to the following:

- *NIH Guidelines* for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
- California OSHA Bloodborne Pathogen Standard
- California Department of Public Health Medical Waste Management Act
- International, federal, and state transport regulations (DOT, IATA)

Note: If a Principal Investigator is performing this type of work without IBC approval, he or she is out of compliance with current NIH and local regulations and has placed the institution in that position as well.

New Applications

A new "Project and Material Registration for Research Involving the Use of Recombinant or Synthetic Nucleic Acid Molecules and/or Biological Materials" must be submitted and reviewed by the IBC for any research using rDNA and/or biologically hazardous materials. PIs seeking IBC approval for the first time also need to submit updated curriculum vitae (or NIH biosketch) with their application. One application for both recombinant and synthetic nucleic acid molecules and biohazardous work may be downloaded from the IBC website at http://www.cdrewu.edu/IBC/Forms.

IBC approval of recombinant DNA and biohazardous research projects is effective for three years. PIs must complete a renewal form annually to continue work for up to three years after the initial approval. After three years, the application must be resubmitted and reviewed by the IBC.

Renewals

A renewal notice is sent to the PI listed on the original approval in the first and second year after initial approval of a protocol. The PI is asked to list all proposed deviations from the protocol as initially approved (or since the last renewal notice); changes in laboratory location; changes in laboratory staff working on the project; and any project titles to be added.

If there are significant deviations from the protocol, especially deviations that affect the containment level (*i.e.*, new study organisms, a new host-vector-donor system, or any other modifications that may affect the containment level), the IBC may ask the PI to seek an additional approval to cover the additional experiments.

When a project is renewed, all new laboratory staff should submit a Medical Surveillance form to the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 and complete lab safety training. Online Biosafety/Biosecurity training is available through the Collaborative Institutional Training Initiative (CITI) at http://www.citiprogram.org.

For changes in PI, the new PI must attach his or her updated curriculum vitae (or NIH biosketch).

Amendments

Amendments must be submitted in electronic or hard copy form for changes within an approved project. All changes should be detailed in the section on "Protocol Amendment", which the IBC must review and approve. The IBC may conduct expedited review on the following changes:

1. Addition of similar projects funded by different funding source (all grant titles must be registered with the IBC)
2. Addition of laboratory space (only to work performed in registered lab space)
3. Changes to non-PI personnel (individuals must be trained in lab techniques and have complied with necessary trainings or approval procedures, such as medical surveillance and lab safety training)

If technical changes are extensive, the IBC may require the PI to submit a completely new application. A change in PI also requires full committee review. The new PI must attach his or her updated vitae (or NIH biosketch) to the amendment.

Biohazardous and Potentially Infectious Materials

Categories

Biohazards are infectious agents or biologically derived infectious materials that present a risk or potential risk to the health of humans or animals, either directly through infection or indirectly through damage to the environment. Infectious agents have the ability to replicate and give rise to potentially large populations in nature when small numbers are released from a controlled situation.

The following is a listing of the potentially hazardous biological materials and agents. PIs should follow the instructions in the IBC Registration Form carefully to ensure that all appropriate sections of the application are completed. If a PI intends to use biological agents that are not listed in this section, they should contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 or IBC for advice regarding proper completion of the IBC Registration Form.

1. Human and animal pathogens
2. Viruses, including oncogenic and defective viruses
3. Bacteria, including those with drug-resistant plasmids
4. Fungi
5. Parasites
6. All human and animal blood, blood products, tissues, and certain body fluids
7. Cultured cells (all human or certain animal, including non-human primates) and the potentially infectious agents these cells may contain
8. Allergens
9. Certain recombinant nucleic acid products

10. Clinical and diagnostic specimens
11. Infected animals and animal tissues
12. Non-human primates and any tissues derived from them (can transmit Herpes B virus)

Recombinant or Synthetic Nucleic Acid Molecules

Definition of Recombinant or Synthetic Nucleic Acid Molecules

In the context of the NIH Guidelines, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA 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 DNA counterpart. If the synthetic DNA 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.

Generation or Use of rDNA

The NIH's Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is the definitive regulatory reference for recombinant DNA (rDNA) research in the United States. http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf

There may be experiments not covered by the guidelines, which would require review and approval by outside agencies before initiation. If the experimental protocol is not covered by the *NIH Guidelines*, contact the IBC at (323) 563-4966 to determine further review requirements.

Note: rDNA work with BSL-3 or BSL-4 classification is not permitted at CDU.

Human Gene Therapy

All protocols involving human gene therapy must be submitted to the NIH Office of Biotechnology Activities to undergo initial review by the Recombinant Advisory Committee (RAC). After RAC review, the protocol must be approved by the IBC and IRB.

Currently, CDU will consider human gene therapy studies on a case-by-case basis. For more details about IBC approval of human gene therapy protocols, call (323) 563-4966. For information about IRB submissions, call (323) 563-5902.

Use of Animals

The use of animals in research requires compliance with the "Animal Welfare Act", issued by the USDA's Animal and Plant Health Inspection Service (APHIS); the "Public Health Service Policy on Humane Care and Use of Laboratory Animals', administered by NIH's Office of Laboratory Animal Welfare (OLAW); and all applicable state or local regulations covering the care and use of animals. All protocols involving the use of animals must be reviewed and approved by the IACUC before their implementation.

All PIs planning to use recombinant and synthetic nucleic acid molecules and/or biohazardous materials must receive IBC approval before initiating the experiments.

Transgenic Animals

PIs that use transgenic animals at ABSL-2 or at ABSL-1, if not considered exempt under the NIH Guidelines (Section III-E, Appendix C-VIII) must complete an IBC application and submit it to the IBC for approval prior to the start of the experiment. In addition, the IACUC must approve the protocol.

Tissue Culture Cell Lines

For the classification of biohazardous agents by Risk Groups, please refer to NIH's Guidelines for Research Involving Recombinant DNA Molecules; Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, U.S. Department of Health and Human Services.

Risk Group	Characteristics of the Agents	Biosafety Containment Levels
1	Agents that are not associated with disease in healthy human adults	**Biosafety Level 1** • Non-primate • Normal, non-human primate origin (monkeys) • Do not harbor primate virus • Not contaminated with bacteria, mycoplasma, or fungi • Well established cell lines that do not cause infection in humans
2	Associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available	**Biosafety Level 2** • Primate cell line derived from lymphoid or tumor tissue • Exposed to or transformed by a primate oncogenic virus

	Note: All human and non-human primate cell lines must be used at BSL-2 as they may harbor previously undefined pathogens. ***Note:*** When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same level as that recommended for the agent or virus.	• All clinical material (*e.g.*, samples of human tissues and fluids obtained after surgical resection or autopsy for use in organ culture or establishment of primary cell cultures) • All primate tissue • All cell lines new to the laboratory (until proven to be free of adventitious agents) • All virus and mycoplasma-containing primate cell lines
3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk) ***Note:*** Not allowed at CDU	**Biosafety Level 3** • High titer HIV work • St. Louis encephalitis virus • Venezuelan equine encephalomyelitis virus • *M. Tuberculosis* • Concentrated lentivirus or lentiviral vectors with high likelihood of aerosol formation • *Francisella tularensis*
4	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) ***Note:*** Not allowed at CDU	**Biosafety Level 4** • Marburg • Ebola • Junin • Machupo • Guanaito

Human Tissue and Cell Culture

Working with Human Tissues and Cells

All unfixed human tissue and cells are to be assumed to be infectious (the concept of "universal precautions") and must be handled using BSL-2 practices and procedures. Persons who are exposed to these materials in the laboratory are considered to have potential exposure to bloodborne pathogens, such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV), and must be included in the Bloodborne Pathogens program. These persons must be offered the hepatitis B vaccination (they do not have to accept) and receive annual bloodborne pathogens training.

Cell Culture

Human or animal pathogens may be associated with cell or organ cultures. Cell cultures known (or suspected) to contain an etiologic agent or an oncogenic virus are classified at the same biosafety level as that recommended for the agent.

The following cell cultures and tissues require BSL-2 or higher containment and procedures:

- All cultured cells derived from human sources, including immortalized and “well established” cell lines.
- All cultured cells derived from non-human primates, primate lymphoid, or tumor tissue.
- All cultured cells exposed to or transformed by a primate oncogenic virus.
- All clinical materials, such as samples of human tissue obtained from surgery, biopsy, or autopsy.
- All primate and sheep tissue.
- All uncharacterized cultured cells new to the laboratory until proven to be free of infectious agents.
- All virus-containing primate cultured cells.
- All mycoplasma-containing cultured cells.

Chapter 3

Regulations and Guidelines

Introduction

Generally, the regulatory process provides the guidance and direction for development of a governance process, an oversight mechanism for the biosafety operational program and the management and administrative systems associated with the implementation of the essential elements of the biosafety program.

Understanding the regulatory requirements provide the general framework of laws and statutes generated at the federal, state and local levels which are subsequently transformed into institutional policies and procedures allowing for establishment of goals, objectives, responsibilities and accountability standards that all faculty, staff, student or employee must adhere to when conducting research. These principles form the basis of the institutional compliance management program for biosafety practices in the basic science and clinical research laboratories in the University.

An effective biosafety management program requires at its very core a thorough understanding in regulatory affairs, an interpretation of these regulatory requirements and a management implementation program for these regulatory principles.

Given the importance of the regulatory compliance in the biosafety program, it is essential that the institution chronicles all applicable federal, state and local regulations applicable to the effective implementation of the Biological Safety Program.

The following is a summary of federal, state, and local agencies regulations guidelines that either regulate or provide guidelines and standards covering the use of biological agents.

Management of Regulations

Scope of Regulatory Guidelines – Federal, State and Local Regulations

Regulations are developed by federal, state and local agencies, which apply to employers and their respective staff to comply with in the execution of their duties and responsibilities.

Institutional Biosafety Committee (IBC) at this institution has oversight over the biosafety program. The committee develops and approves policies and procedures for implementation in its operational programs and provides a basis of conducting periodical monitoring and evaluation of its biosafety program by the implementation of biosafety compliance management procedures and inspection oversight of its governance, operational and management structures of its program.

In addition, the committee reviews, analyzes and evaluates the effectiveness of its program based on the federal, state and local regulatory requirements and accreditation agencies standards and principles.

Federal Guidelines

1. **Centers for Disease Controls and Prevention and the National Institutes of Health:** *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th Edition, 2007.

   This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety and is the basis for this Manual.

2. **National Institutes of Health:** *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)

   This document provides guidelines for constructing and handling recombinant DNA molecules (rDNA) and organisms containing rDNA. Institutions that receive any NIH funding for rDNA research are required to comply with these guidelines as a condition for funding.

   This document requires that each institution establish an Institutional Biosafety Committee (IBC) with oversight and the authority to approve proposed rDNA research utilizing the NIH guidelines as the minimum standard for implementing an effective biosafety program.

   Summary of Experiments Covered by the NIH Guidelines for Research Involving Recombinant DNA Molecules (Section III)

   This section describes six categories of experiments involving recombinant or synthetic nucleic acid molecules.

   1. those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see Section III-A),
   2. those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see Section III-B),
   3. those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see Section III-C),
   4. those that require Institutional Biosafety Committee approval before initiation (see Section III-D),
   5. those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-E), and
   6. those that are exempt from the *NIH Guidelines* (see Section III-F).

Note: *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed*. If an experiment falls into

Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.

Any change in containment level, which is different from those specified in the *NIH Guidelines*, may not be initiated without the express approval of NIH/OBA (see Section IV-C-1-b-(2) and its subsections, *Minor Actions*).

State Regulations

In addition to the federal agencies administering their regulatory requirements, State agencies also develop and administer regulatory requirements that require compliance by employers or institution. State agencies that are involve in the administration of biosafety requirements are as follows:

1. **Bloodborne Pathogens (Cal/OSHA, Title 8, Section 5193)**

   This regulation covers occupational exposure to human blood and other potentially infectious materials, including human tissue and cells. Cal/OSHA specifies the methods of control, combination of engineering controls, safe work practices, and training to reduce the risk of infection. Personnel potentially exposed to human blood and other potentially infectious material must be offered immunization against hepatitis B and receive annual training. Personnel who work with HIV or hepatitis B in a research laboratory must receive additional training and demonstrate competence and proficiency when working with human pathogens.

2. **Tuberculosis (TB) CONTROL AND MANAGEMENT (Cal/OSHA, Title 8)**

   This state department of health administers the program for the management and containment and control of TB in a health care or research environment

3. **Aerosol Transmissible Diseases (Cal/OSHA, Title 8, Section 5199)**

   This State agency administers the regulations that require laboratories that perform procedures with materials that contain pathogens or aerosol transmittable pathogens or aerosol generating procedures.

4. **Cal/OSHA- STATE Public Health Department**

   Cal State Health Department*: Recombinant DNA :* Public Health and *Biological Laboratory Regulation*

   These regulations require that all institutions in the State of California that work with recombinant DNA molecules or that operates BSL-3 or BSL-4 laboratories must be licensed by the State of California.

These regulations require strict adherence to the CDC/NIH guidelines, as well as other regulations that the State Health Department and the Public Health Department may imposed.

5. California State Health Department

Medical Surveillance and Disease Surveillance and Reporting Regulation

This regulation requires all institutions in the City of Los Angeles that engage in research with select agents, Risk Group 4 agents, and other agents named by CDU IBC as high-risk agents must be registered and maintain disease surveillance and reporting programs in effect to minimize potential exposures to these high-risk agents.

6. State Department of Public Health: California: Environmental Health and Safety:

The State and local Department of Public Health regulates the sterilization monitoring, storage and disposal of potentially infectious material. It also includes requirements for labeling, records management, program evaluation and compliance management.

Other Federal Regulations

1. Select Agents and Toxin

Department of Health and Human Services: *42 CFR Parts 42 and 43 Possession, Use, and Transfer of Select Agents and Toxin; Final Rule;* and the Department of Agriculture's Animal and Plant Health Inspection Service: 7 CFR Parts 331 and 9 CFR Parts 121, *Agricultural Bioterrorism Protection Act of 2002: Possession, Use, and Transfer of Biological Agents and Toxin; Final Rule*. These regulations require institutions that possess, use, or transfer certain biological agents and toxins ("select agents") to be registered and approved by DHHS and/or APHIS.

CDU does not support research involving the use of select agents and toxins.

2. U.S. Department of Transportation (US DOT) and the International Air Transportation Authority (IATA)

These organizations have strict requirements governing the shipment and transportation of hazardous materials, including biological agents and other "Dangerous Goods".

Chapter 11 provides information on shipping regulations.

3. Centers for Disease Control and Prevention

The CDC has established specific regulatory requirements for importation or transportation of etiologic agents, which include a permit application that must be submitted and approved *prior* to any such importations.

The federal regulation governing the importation of etiologic agents is USPHS 42 CFR - Part 71 Foreign Quarantine. Part 71.54, Etiologic agents, hosts, and vectors.

4. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Veterinary Services

USDA, APHIS, and VS regulate the importation of animals and animal-derived materials to ensure that exotic animal and poultry diseases are not introduced into the United States. Generally, a USDA veterinary permit is needed for materials derived from animals or exposed to animal-source materials.

Materials that require a permit include animal tissues, blood, cells or cell lines of livestock or poultry origin, RNA/DNA extracts, hormones, enzymes, monoclonal antibodies for *in vivo* use in non-human species, certain polyclonal antibodies, anti-sera, bulk shipments of test kit reagents, and microorganisms, including bacteria, viruses, protozoa, and fungi. Exceptions to this requirement are human and nonhuman primate tissues, serum, and blood.

5. U.S. Department of Commerce (DOC)

The DOC has specific regulatory requirements for exportation of biological materials. These regulations are both agent and country specific and must be followed strictly.

6. U.S. Homeland Security

This Department develops principles and standards governing the requirements for the utilization of biological materials for biosecurity and bioterrorism surveillance.

7. NIH Office of Laboratory Animal Welfare (OLAW)

This office administers the program and ensures compliance with the principles of care and use of animal in research program.

Accreditation Agency

1. Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)

This is an independent accredited body, which determines whether an institution meets the standards for accreditation of its animal care program.

CDU Institutional Regulatory Committees and Administration

1. Institutional Biosafety Committee (IBC)

The IBC has oversight over the biosafety program and administers the governance and the operational program based on the charge of the committee. It develops and reviews policies and procedures and approves and disapproves research protocols requiring review. Specific policies and procedures developed are implemented in the dispensation of its operational program.

2. Occupational Health and Safety Committee (OHSC)

This committee is responsible for performing risk assessment/risk management and for reviewing procedures that would minimize or prevent risk associated with occupational health and safety.

3. The Occupational Health Office

This office is responsible for the management and implementation of the operational aspects of the program and ensures that the staff associated with this office has the experience, regulatory understanding, qualifications and knowledge to ensure effective implementation of the essential program elements.

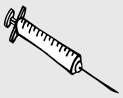
4. Office of Research Integrity and Compliance

This office is responsible for providing administrative support for IBC and IRB and ensuring compliance through education, providing consultation, assisting in the communication between the committee/board and the investigators.

Chapter 4

Biosafety Principles

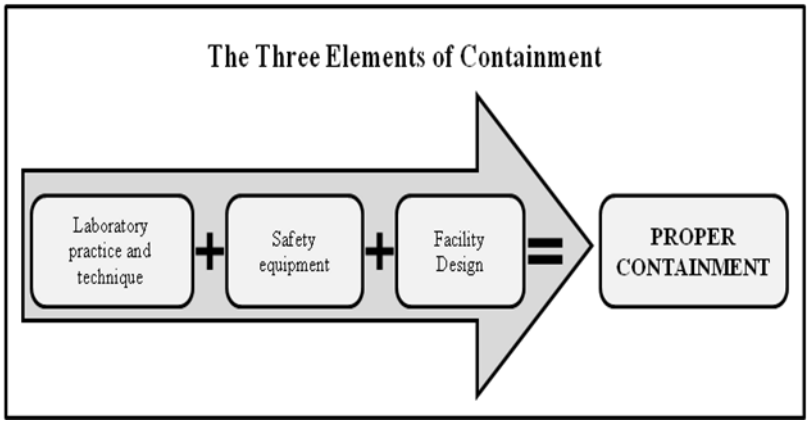
The risk of exposure to biological agents in a research environment depends on a number of parameters (*e.g*., the agent, its virulence, subject's susceptibility, route of transmission, etc.). In general, the biosafety procedures used are designed to prevent such exposures by containing the agents. To properly design the containment, it is important to recognize the potential routes of transmission for the given agent.

Routes of Transmission			
	Type		**Description**
1	**Skin and Mucous Membrane Contact**		Low-energy procedures, such as decanting of liquids, pipetting, removal of screw caps, vortex mixing, streaking agar plates, and inoculation of animals, can result in the generation of infectious droplets, as well as direct contact with infectious material. Eye contact is also considered a route of exposure.
2	**Ingestion**		Mouth pipetting presents the highest risk for ingestion of infectious material. Splashing of material into the mouth and indirect oral exposure through touching the mouth with contaminated hands can also result in the ingestion of infectious material. Storage of food or drinks in laboratories working with biological agents, as well as storage of utensils and eating and drinking in the lab, applying makeup to the face, etc, can also result in ingestion of infectious material.
3	**Percutaneous Inoculation**		Use of syringes and needles are considered the greatest risk of exposure through inoculation. Inoculation can also occur as a result of cuts and scratches from contaminated items, as well as animal bites. Attention should be paid during procedures involving contaminated materials so that inadvertent "poking" does not occur as a result of negligence. Proper disposal procedures should also be followed.
4	**Inhalation**		Many procedures have the potential for generating respirable aerosols, including sonication, centrifugation, "blowing out" of pipettes, heating inoculating loops, lyophilizing, and changing litter in animal cages. Proper protocols for performing these procedures should always be followed, such as *always* balancing components of the centrifuge items prior to usages. The use of broken or faulty equipment, cracked or outdated containers, tubes, etc, should be avoided to prevent the risk of breakage during procedures and release of aerosols. Preparation and mixture of chemical reagents can also generate fumes that can be hazardous when inhaled. Proper precautions such as use of fume hoods should be used, **and MSDS sheets consulted prior to performing the reagent preparation**.

Containment

The term "containment" is used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents.

The three elements of containment include laboratory practice and technique, safety equipment, and facility design.



Primary Containment

The protection of personnel and the immediate laboratory environment from exposure to infectious agents is provided by good microbiological technique and the use of appropriate safety equipment, such as biological safety cabinets.

Secondary Containment

Protecting the laboratory's external environment from exposure to infectious materials is accomplished by a combination of facility design and operational practices. The risk evaluation of the work to be done with a specific agent will determine the appropriate combination of these elements.

Safety Equipment

Safety equipment includes biological safety equipment, enclosed containers, safety centrifuge cups, and other engineered controls designed to minimize exposure to biological agents. Safety equipment is most effective at minimizing exposure when workers are trained on the proper use of such equipment and the equipment is regularly inspected and maintained.

New staff and students should always undergo proper training and certification testifying their knowledge of the proper use of safety equipment prior to performing any laboratory procedures. For equipment with complex sequence of usage instructions, a simple summary

checklist of steps for usage can be printed and displayed nearby so that users can consistently follow through and minimize accidentally "skipping" steps.

Biological Safety Cabinets (BSC)

Biological safety cabinets are the most important safety equipment for protection of personnel and the laboratory environment, and protection of the research product from contamination.

Proper use of a biological safety cabinet (BSC) provides a high level of containment that protects the operator from exposure while providing some protection from contamination of the material being handled within the work environment. Because of its importance in providing containment and safety protections in the laboratory, a BSC is considered one of the most critical pieces of safety equipment in biological laboratories. BSCs are designed to contain aerosols generated during work with biological material through the use of laminar air flow and high efficiency particulate air (HEPA) filtration.

It is important to note that among laboratory staff, BSC's are sometimes referred to as "hoods." A BSC is distinct from a chemical fume hood. Chemical fume hoods are engineered to protect against exposure to fumes, noxious gases, and chemicals. They do not have HEPA filters and cannot be used to work with biohazardous materials. It is important that this distinction is noted among new staff and new students.

Three types of BSCs (Class I, II, and III) are used in laboratories.

Types of Biosafety Cabinets (Class I, II, III)

CLASS I and CLASS II	CLASS III
Open-fronted Class I and Class II BSCs are partial containment devices that provide a primary barrier offering significant levels of protection to laboratory personnel and to the environment when used in combination with good laboratory technique.	Class III Biosafety Cabinets are meant to be complete containment devices for use while working with highly dangerous materials.
CLASS I	**CLASS II**	**CLASS III**
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. It provides protection to personnel and the environment from contaminants within the cabinet but does not protect the work within the cabinet from "contaminated" room air.	The Class II BSC protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination. It meets requirements to protect personnel, the environment, and the product. The two basic types of Class II BSCs are Type A and Type B. The major differences between the two types 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.	The gas-tight Class III BSC, or glove box, provides the highest attainable level of protection to personnel, the environment, and the product. It is the only unit that provides a total physical barrier between the product and personnel. It is used with high-risk biological agents and when absolute containment of highly infectious or hazardous material is required.

It is important to note that horizontal laminar flow benches *must not* be utilized for work with biohazardous or chemically hazardous agents. These units provide *product protection* by ensuring the product is exposed only to HEPA-filtered air. They do not provide protection to personnel or the ambient environment.

The following is a general example of a protocol that should be followed when using a biosafety cabinet for work with biological materials:

- Turn the cabinet on at least 5-10 minutes prior to use, if the cabinet is not left running. If a UV bulb is used, make sure to turn off the UV before placing hands and arms into the cabinet. Verify the cabinet is operating properly and there is airflow. At least daily, or each time the cabinet is operated, the operator or user should observe the magnehelic gauge and note its relative position. Magnehelic gauges measure the pressure drop across the outlet HEPA filter and are important indicators of filter integrity. The gauge will typically indicate the same measurement over a long period of time. A significant change in the reading over a short period of time may indicate clogging or a leaking filter. In such cases, the hood should not be used until the problem is identified and resolved. If the BSC located within a laboratory does not have a magnehelic gauge, users must understand the operation of the airflow monitor, controls, and alarm settings.

- Disinfect work surface with 70% alcohol or other suitable disinfectant. Make sure to wipe down not only the bottom of the cabinet but the adjacent sides and back as well since undetected splashing of reagents or materials may have occurred during prior use.

- Sterilize/disinfect items prior to placing them into the cabinet so that they can be worked with efficiently without unnecessary disruption of the airflow. Sterile unopened individual packages (such as syringes, or pipette tips) should be opened in the cabinet. Sterile tubes and flasks should be uncapped only when in the cabinet. It is recommended to work with materials from the “clean” side to the “dirty” side.

- Wear appropriate personal protective equipment (PPE). This includes a buttoned laboratory coat and gloves. Long hair should be tied back.

- Adjust the working height of the stool or stand so that the worker's face is above the front opening.

- Delay manipulation of materials for approximately 1 minute after placing the hands/arms inside the cabinet.

- Minimize the frequency of moving hands in and out of the cabinet. Do not touch items such as the lab coat, face, clothing, hair, etc. with hands (even gloved hands) if they will go back into the cabinet.

- Do not disturb the airflow by covering any of the grill or slots with materials.

- Work at a moderate pace to prevent airflow disruption that occurs with rapid movements.

- Wipe the bottom and sides of the cabinet surfaces with disinfectant when work is completed. Remove vacuum tubes, receptacles, beakers, and other items that were used during the procedure. Minimize items left in the cabinet.

Proper operation and maintenance of a BSC requires knowledge of how the system operates, as well as training and experience in effective techniques for working within the cabinet space without compromising its functions.

BSCs used as primary barriers must be certified annually by a qualified vendor. Contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 for information about vendors or other BSC-related information.

The certification process is quick and relatively inexpensive and ensures that the hood is meeting its operating specifications and providing maximum protection. In addition, certifiers provide service and preventive maintenance for cabinets and can often forecast expensive requirements like HEPA filter replacements, allowing PIs to budget for the event. PIs who fail to have their hoods recertified annually are sometimes faced with large, unanticipated expenses that could have been prevented.

If BSC recertification is required, the recertification must be completed before the current certification expires. If the certification lapses, the BSC **may not** be used for BSL-2 or higher procedures until recertified and may be labeled "Not Certified for Use as a Biosafety Cabinet" by the Environmental Health and Safety (EHS) Specialist. Unless a good reason exists for more frequent certification, a one-year certificate life is appropriate. The certificate will generally expire on the last day of the month in which the certification was performed, one year later (for example, a certificate issued on June 2, 2013 will expire on June 30, 2014).

Additional details concerning the design and use of BSCs are provided in Appendix C.

Personal Protective Equipment (PPE)

Personal protective equipment (PPE) includes safety eyewear, face shields, gloves, appropriate respiratory protection, and lab coats. This equipment is used to supplement the containment provided by laboratory safety equipment in case its failure results in direct exposure of personnel to the biological agents (see more in detail in Chapter 5).

Note: PPE is designed to protect laboratory workers from serious exposure to biohazardous materials and should be used in conjunction with appropriate engineering and administrative

controls. At a minimum, staff must use lab coats, safety glasses, and gloves whenever there is a potential for skin contact, splash, or aerosols. Covered shoes are strongly advised.

Facility Design

The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, as well as to protect people or animals in the community from infectious agents that may be accidentally released from the laboratory. Facility design must be commensurate with the laboratory's function and the recommended biosafety level for the agent being used or stored. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access; availability of decontamination equipment (*e.g.*, autoclave*); and hand washing facilities. CDU currently does not have BSL-3 or BSL-4 facilities.

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

***Note:** It is IBC policy that autoclaves used to sterilize biohazardous materials be validated monthly using a sporulation test and that validation records be kept (see Appendix D). Biohazardous materials can also be disposed of in a red bag as medical waste without autoclaving. Waste bag picked up by Occupational Hygiene Technologist for autoclaving.

Biosafety Levels

Four biosafety levels (BSLs) represent combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed and the documented or suspected routes of transmission of the infectious agents, as well as for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely.

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules classifies “human etiologic agents” on the basis of their relative pathogenicity. Agents are categorized into four risk groups (RG).

As a general rule, a biosafety level should be used that matches the highest RG classification of the organisms involved. For example, work with vaccinia virus, a Risk Group 2 (RG2) agent,

should be conducted at BSL-2 or higher; simultaneous work with *Escherichia coli* (RG1), Epstein-Barr virus (RG2), and *Mycobacterium tuberculosis* (RG3) should be conducted at BSL-3.

Descriptions of biosafety levels, as well as assigned biosafety levels for specific organisms, are contained in the CDC/NIH document, Biosafety in Microbiological and Biomedical Laboratories (BMBL). The BMBL outlines four biosafety levels, summarized below:

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	▪ No primary barriers required. ▪ PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required
2	▪ Agents associated with human disease ▪ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: ▪ Limited access ▪ Biohazard warning signs ▪ "Sharps" precautions ▪ Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: ▪ BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ▪ PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: ▪ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practice plus: ▪ Controlled access ▪ Decontamination of all waste ▪ Decontamination of laboratory clothing before laundering	Primary barriers: ▪ BSCs or other physical containment devices used for all open manipulations of agents ▪ PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: ▪ Physical separation from access corridors ▪ Self-closing, double-door access ▪ Exhausted air not recirculated ▪ Negative airflow into laboratory ▪ Entry through airlock or anteroom ▪ Hand washing sink near laboratory exit
4	▪ Dangerous/exotic agents which post high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ▪ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ▪ Related agents with unknown risk of transmission	BSL-3 practices plus: ▪ Clothing change before entering ▪ Shower on exit ▪ All material decontaminated on exit from facility	Primary barriers: ▪ All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	BSL-3 plus: ▪ Separate building or isolated zone ▪ Dedicated supply and exhaust, vacuum, and decontamination systems ▪ Other requirements outlined in the text

Note: Consult the BMBL for a more complete description of the four biosafety levels, as well as recommended biosafety levels for specific organisms.

In addition to the four biosafety levels described above, there are also four biosafety levels for work with infectious agents in vertebrate animals, sometimes referred to as the Animal Biosafety Level (ABSL).

"A"BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy human adults; RG1	Standard animal care and management practices, including appropriate medical surveillance programs.	As required for normal care of each species.	▪ Standard animal facility ▪ No recirculation of exhaust air ▪ Directional air flow recommended ▪ Handwashing sink recommended
2	Associated with human disease; RG2	ABSL-1 practices plus: ▪ Limited access ▪ Biohazard warning signs ▪ Sharps precautions ▪ Biosafety manual ▪ Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus primary barriers: ▪containment equipment appropriate for animal species. ▪PPE: laboratory ▪coats, gloves, face, and ▪respiratory protection as needed	ABSL-1 facility plus: ▪ Autoclave available ▪ Handwashing sink available in the animal room. ▪ Mechanical cage washer used
3	Indigenous or exotic agents with potential for serious health effects; RG3	ABSL-2 practices plus: ▪Controlled access ▪Decontamination of clothing before laundering ▪Cages decontaminated before bedding removed ▪Disinfectant foot bath as needed	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. ▪ *PPE: appropriate respiratory Protection*	ABSL-2 facility plus: ▪ Physical separation from access corridors ▪ Self-closing, double door access ▪ Sealed penetrations ▪ Sealed windows ▪ Autoclave available in facility
4	Dangerous/exotic agents that pose high risk of life-threatening disease; RG4	ABSL-3 practices plus: ▪Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower upon exiting ▪All wastes are decontaminated before removal from the facility	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) used for all procedures and activities	ABSL-3 facility plus: ▪ Separate building or isolated zone ▪ Dedicated supply and exhaust, vacuum, and decontamination systems ▪ Other requirements outlined in the text

References

1. Boston University Medical Center Biosafety Manual
2. Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards: Updated Version. National Research Council (US) Committee on Prudent Practices in the Laboratory. Washington (DC): National Academies Press (US); 2011
3. UC Irvine Biosafety Manual
4. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, CDC/NIH

Chapter 5

Laboratory Biosafety Practices

Introduction

Human safety is the first priority when conducting research using biological materials. Knowledge of the materials, agents, equipment, and best safety practices is necessary to ensure safety in the laboratory.

The foundations of protective practices in a laboratory lie in an individual's laboratory experience, technical knowledge, personal work habits, and attitude toward laboratory safety. Unlike administrative controls, which are behaviors dictated by regulation or laboratory policy, the term "protective behavior" is used to define an innate part of each individual worker's personal approach to the laboratory environment. As such, "protective behaviors" form the first and most important line of defense against injury or exposure in the biomedical workplace.

Objective

To outline specific biosafety practices that must be followed when working in a laboratory. Following these best practices should help prevent injury, illness, and hazardous exposure to the individual as well as to others in the laboratory.

Scope

This policy applies to departmental chairs, managers, project directors, laboratory supervisors, Principle Investigators, laboratory workers, students, interns, visitors, and all other individuals participating in laboratory and research work at CDU.

Procedures

Basic Biosafety Laboratory Practices

Prudent practices and good techniques are of primary importance in laboratory safety. Both are based on sound technical knowledge, experience, common sense, and an attitude of courtesy and consideration for others.

Techniques and practices are written in detail in the following references:

1. "Standard Microbiological Practices" in the CDC/NIH's *Biosafety in Microbiological and Biomedical Laboratories*;
2. *NIH Guidelines* for *Research Involving Recombinant or Synthetic Nucleic Acid Molecules*;

3. National Research Council's *Biosafety in the Laboratory – Prudent Practices for the Handling and Disposal of Infectious Materials* (National Academy Press, Washington, D.C., 1989); and
4. Other laboratory safety text and reference books.

The seven basic rules of biosafety, based on the National Research Council's *Prudent Practices* document, should be the basis of any personal laboratory work ethic.

Table 1: Biosafety practices and blocked routes exposure

	Biosafety Practice	Routes of Exposure Blocked
1	Do not mouth pipette.	Inhalation, ingestion, skin, and mucous membrane contact
2	Manipulate infectious fluids carefully to avoid spills and the production of aerosols	Inhalation, skin, and mucous membrane contact
3	Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives; dispose of sharps in leak- and puncture-proof containers.	Percutaneous, inhalation
4	Use lab coats, gloves, safety eyewear, and other personal protective equipment.	Skin and mucous membrane contact
5	Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents.	Skin and mucous membrane contact
6	Decontaminate work surface before and after use, and immediately after spills.	Skin and mucous membrane contact
7	Do not eat, drink, store foods, apply cosmetics or smoke in the laboratory.	Ingestion, skin, and mucous membrane contact

Basic Microbiological Laboratory Practices and Techniques

Containment of infectious agents is essential and is achieved by strict adherence to standard microbiological practices and techniques.

Any individual working with infectious agents or infected materials must be aware of potential hazards, be trained and demonstrate proficiency in safety procedures. The PI is responsible for ensuring that laboratory personnel are adequately trained; the PI may delegate a laboratory supervisor to provide training, but the responsibility remains with the PI.

Each laboratory should have a written document that lists specific hazards that will or maybe encountered in the laboratory and outlines procedures to minimize or eliminate these risks. Laboratory personnel must be made aware of special hazards and should be required to read and to follow the safety procedures dealing with those hazardous materials as well as the standard operating procedures associated with the assigned lab. It is highly recommended that the lab have written documentation that each member has read and understands the safety procedures. Each lab must have a scientist available who is trained and knowledgeable in relevant techniques, safety procedures, and hazards related to the infectious agents to direct and advise the laboratory workers.

In some cases, additional safety measures are needed when working with certain infectious agents or performing certain procedures. The PI is responsible for identifying and enforcing these additional safety practices.

Appropriate facility design, engineering features, safety equipment, and management practices may be necessary to achieve a safe lab environment.

Each laboratory will designate at least one individual to serve as the Safety Officer. In the absence of the designation, the Principal Investigator of the laboratory will serve as the Safety Officer.

Note: Although each individual is responsible for his or her own safety, the PI will be held ultimately responsible for ensuring that persons working in the laboratory are adequately trained, provided with proper equipment and materials to conduct experiments safely and that they follow proper and prescribed safety procedures.

Organization of the Laboratory and Cleanliness

It is extremely important to maintain an organized and clean laboratory. Injuries and exposures are more likely to occur in labs that are poorly maintained, unclean, and disorganized.

It becomes even more critical to maintain an organized, clean space when the workspace is shared. Coworkers must rely on one another to maximize efficiency and safety. Personal materials should be labeled, waste properly disposed, and the shared space disinfected or cleaned prior to leaving it for the next user.

Follow these guidelines when working in the laboratory:

1. Perform routine housekeeping of work areas to minimize and eliminate significant sources of contamination and hazards.

2. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

3. Laboratory personnel are responsible for cleaning laboratory benches, equipment, and areas that require specialized technical knowledge.

4. Access to exits, sinks, eyewashes, emergency showers, and fire extinguishers must not be blocked.

5. The work place should be free of physical hazards.

6. Electrical safety is a priority, especially as it relates to the use of extension cords. Equipment should be properly grounded. Overloaded electrical circuits and the creation of electrical hazards in wet areas are to be avoided.

7. Surfaces should be clean and free of infrequently used chemicals, glassware, and equipment.

8. Unnecessary items on floors, under benches, or in corners should be removed.

9. All compressed gas cylinders should be properly secured with chains, straps, or a rack that is bolted to the floor or wall.

Personal hygiene, including proper hand washing, is also an important aspect of cleanliness in the lab. Hands should be washed immediately after removing gloves to remove contamination that may have occurred by glove micropuncture or during prior exposure. Such practice also ensures that cross-contamination does not occur to themselves, other individuals and areas.

Cosmetic tasks, such as applying makeup, trimming fingernails, or brushing hair are not to be done in the lab. These activities provide new opportunities for exposure and contribute to retrograde contamination of the laboratory environment.

Universal Precautions

“Universal precautions” refers to the practice of avoiding contact with all human bodily fluids. Universal precautions should be followed by all laboratory personnel and stipulate that all human blood, bodily fluids, and tissues be handled as though they are infectious. Universal precautions apply to all applicable laboratory specimens.

Refer to OSHA’s Bloodborne Pathogens (BBP) Standard (Title 29 of the Code of Federal Regulations at 29 CFR 1910.1030) for more specific information.

Administrative Controls

Administrative controls are an important aspect of biosafety and should be set in place to assist in the maintenance of proper safety practices. Policies and procedures are useful for standardizing the safe handling of potentially hazardous biological materials. These may include but are not limited to training, medical surveillance, vaccinations, laboratory inspections, and access control.

Biological Hazard Information

Laboratory workers must be knowledgeable of the hazards associated with the biological agents being used in the laboratory and have access to hazard information on the specific agents. No individual should ever work with a biological agent without proper knowledge of the risks and hazards of that agent. The following are useful sources of hazard information for biological agents.

Microbial Agents

The CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories (BMBL) has descriptions of biosafety levels and recommended biosafety practices for specific biological agents.

Toxins

Isolated biological toxins are chemical hazards, although many such toxins produce adverse effects at doses significantly below that of "traditional" laboratory chemicals. Material Safety Data Sheets (MSDSs) must be maintained and readily available at all times for any biological toxin present in the lab.

MSDSs for a specific toxin should be obtained from the vendor upon receipt of the toxin. Never begin working with a toxin without first reading and understanding the MSDS.

Toxicology textbooks, such as Casarett & Doull's Toxicology, also provide hazard information for toxins.

Written Standard Operating Procedures

For general information on proper practices, it is essential and required for the lab to maintain this Manual and the CDC/NIH publications *Biosafety in Microbiological and Biomedical Laboratories* and *Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules*. Since these references only provide general topics, each laboratory must develop their laboratory-specific SOPs that cover the biosafety concerns and laboratory practices for their own laboratory.

For example, laboratory-specific SOPs should address safe manipulation of specific organisms, specific exposure control methods, and specific decontamination and waste-handling requirements. Appendix O provides a recommended standard format for SOPs, and laboratories are encouraged to use this format (including use of pictures and illustrations). The laboratory-specific SOPs need not duplicate the more general SOPs in this Manual or the CDC/NIH documents, but should serve as supplements with detailed information. The laboratory-specific SOPS must be included with the initial IBC registration form and inventory.

Security and Inventory of Biological Agents

The potential for bioterrorism and concern for public health and safety necessitates regulatory oversight and proper security of biological agents. Although many of the agents used in research laboratories do not pose a real risk to health and safety of the workers or the public, the perception of such risks is of great importance.

Each PI must develop site-specific protocols to secure all biological materials from unauthorized removal. It is the PI's responsibility to ensure that his or her laboratory implements sufficient security measures and procedures to prevent unauthorized access to biological agents.

In many instances, during the application review process, the IBC will review the proposed security measures and either approve or recommend enhancements to the proposed plans.

Prevention of Aerosols and Droplets

When working with liquids or dry powders it is likely for aerosols or droplets to be generated. High-energy procedures, such as centrifuging, vortexing and mixing, tend to produce aerosols that stay airborne for extended periods and are small enough to be inhaled, while low-energy procedures, including opening containers and streaking plates, produce droplets that settle quickly on surfaces, skin, and mucous membranes. It is important to understand how generation of these aerosols and droplets can be minimized or prevented and how one can protect himself or herself from exposure.

Utilization of Biological Safety Cabinets

In general, the following guidelines are recommended when using biological safety cabinets (BSCs):

- The BSC must be certified when it is installed, after it is moved, and annually.
- The magnehelic gauge should be checked regularly. This gauge will normally run at a relatively fixed value. When it deviates significantly, the cabinet should not be used until the cause of the deviation has been identified and fixed.
- Lab personnel should understand how the BSC works and how to use it effectively.

- Lab personnel should be familiar with the safe and effective use of any UV lamps inside the BSC and use appropriate precautions to avoid UV-related injuries. UV lamps require special maintenance.

- The BSC's protective airflow pattern should not be disrupted. Rapid arm movement, nearby workers, items placed on the air outflow and inflow areas, improper placement of the sash (lifting the sash too high), and open laboratory doors may disrupt the airflow pattern and reduce the cabinet's effectiveness.

- Work should be planned to minimize the need to exit and reenter the room.

- Accumulation of materials in the BSC work volume should be minimized to reduce turbulence and ensure proper laminar airflow.

- The BSC should be left running whenever the cabinet is in use.

- Proper disinfectants that avoid damaging the cabinet's interior should be used.

- The work surface should be wiped with an appropriate disinfectant before use. Each item needed for the planned procedures should be wiped off and placed in the cabinet. Be sure to choose the disinfectant with effectiveness against the agent(s) you are using. The BSC should run for at least 5 minutes before starting work to allow for stabilization of airflow.

- If a piece of equipment, such as a centrifuge or blender, will create air turbulence in the BSC, it should be placed in the back one-third of the cabinet. All other work should be stopped while this equipment is operating.

- Open flames should be avoided in the work volume because they create airflow turbulence that may compromise sterility. In addition, the heat build-up may damage the HEPA filters. If a flame is necessary, a burner with a pilot light should be used. Electric devices, such as loop sterilizers, are often satisfactory alternatives to open flames.

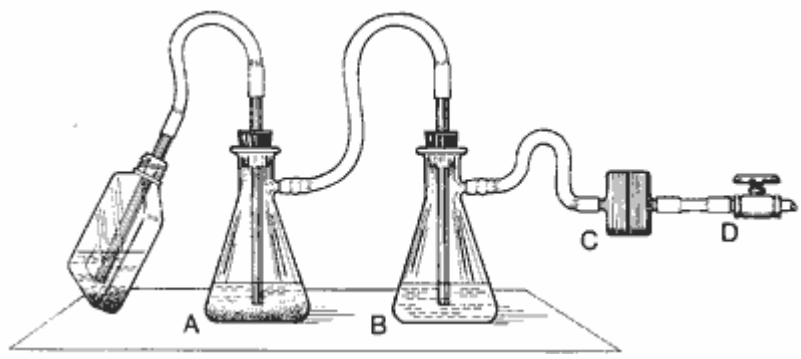
- A pan with disinfectant and/or a sharps container should be placed inside the BSC for pipette/sharps disposal. Vertical pipette discard canisters on the floor outside the cabinet should be avoided.

- Contaminated and clean items should be segregated, and personnel should work from "clean to dirty." The biohazardous waste collection bag should be in a rigid container. Do not block airflow into the front and rear exhaust grilles.

- Move arms slowly when removing items from or introducing items into the cabinet work volume.

- Protect the facility vacuum system from biohazards by using dual aspirator flasks in series (A and B) and placing an in-line hydrophobic HEPA filter (C) between the vacuum trap and the source valve (D) in the cabinet:

Note: The flasks should be placed in a secondary container, such as a plastic tub.



- All spills in the cabinet should be cleaned immediately. Work should not resume for 10 minutes.

- When work is complete, all materials should be removed from the BSC and all interior surfaces should be wiped with an appropriate disinfectant.

- Gloves must be removed before exiting the BSC, after touching or handling contaminated materials.

- Laboratory coats must be removed and hands thoroughly washed before leaving the laboratory.

Utilization of Pipettes

Pipettes are used for volumetric measurements and the transfer of fluids that may contain infectious, toxic, corrosive, or radioactive agents. Infections can occur and have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger, touching the face (eyes, nose, etc.), 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.

The following are guidelines for safe pipetting to minimize the potential for exposure to hazardous materials:

- Never mouth pipette. Always use a pipetting instrument.
- If working with biohazardous or toxic fluid, confine pipetting operations to a biological safety cabinet.
- Always use cotton-plugged pipettes when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.
- Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- Do not forcibly expel biohazardous material out of a pipette.
- Never mix biohazardous or toxic material by suction and expulsion through a pipette.
- When pipetting, avoid accidental release of infectious droplets.
- Use "to deliver" pipettes rather than "to contain" pipettes, which require "blowout." Be careful not to dislodge the residual liquid.
- Whenever possible, allow the solution to run down the container wall when releasing it from the pipette. Do not release the solution from a height into the container as this can cause splashing and aerosols.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated, broken, or intact Pasteur pipettes and broken glass in a sharps container. Dispose of the container properly when it is, at most, three-fourths full.
- Pans or sharps containers for contaminated pipettes should be placed inside the BSC, if possible.
- Proper procedures for disposal of plastic pipettes are presented in Chapter 9.

Utilization of Centrifugation

Use of a centrifuge can present hazards such as mechanical failure and the creation of aerosols. To help avoid mechanical failure of the centrifuge, be sure to follow the manufacturer's instructions for use and maintenance. Users should be properly trained in how to operate the

machine. Operating instructions and safety instructions should be posted in a conspicuous location on or near the unit.

The following can result in aerosols: filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets. A significant aerosol hazard can be created if a tube breaks during centrifugation.

To minimize the generation of aerosols when centrifuging biohazardous material, the following guidelines are provided:

- Use sealed tubes and 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.

- Fill and open centrifuge tubes, rotors, and accessories in a biological safety cabinet. Avoid over filling centrifuge tubes to prevent closures and seals from becoming wet. After tubes are filled and sealed, spray or wipe them with disinfectant.

- In the event of breakage during centrifugation, decontaminate the unit prior to reuse.

- Always balance buckets, tubes, and rotors properly before centrifugation to avoid an imbalanced centrifuge.

- Avoid decanting or pouring off supernatant; unless the supernatant must be retained. Instead, use a vacuum aspirator with appropriate in-line reservoirs and filters.

- Work in a biological safety cabinet when resuspending sedimented material. Use as whirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.

- Small, low-speed centrifuges maybe placed in a biological safety cabinet during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatigue resulting in disintegration of rotors. Use proper cleaning techniques for the centrifuge components. Manufacturers' recommendations must be meticulously followed to avoid metal fatigue, distortion, and corrosion.

- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used.

Utilization of Cryostats

The sharp cutting edges and extremely cold temperatures of a cryostat pose risks to the users. It is imperative to be aware of these risks and how to minimize them.

The following guidelines should be followed when using cryostats:

- Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent may pose a risk since freezing tissue does not necessarily inactivate infectious agents. Use of freezing propellants under pressure is not recommended with frozen sections because they may cause spattering of droplets of potentially infectious material.

- Appropriate gloves should be worn while preparing frozen sections.

- When working with human or infected animal tissue, consider that the contents of the cryostat may become contaminated. Be sure to decontaminate it frequently with 70% alcohol or an appropriate disinfectant.

- Consider pieces of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.

- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after using tissue known to contain bloodborne pathogens, *M. tuberculosis*, or other infectious agents.

- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.

- Solutions used for staining potentially infected frozen sections should be considered contaminated.

Utilization of Inoculating Loops

Use of flaming inoculating loops can result in spattering, release of aerosols, and droplets. Use of disposable plastic loops is preferred since it minimizes these risks.

Use of Absorbent Materials

Work surfaces should be covered with absorbent paper or sheets to collect splashes and drips in order to minimize the spread of contamination. The absorbent paper should be changed at the end of the laboratory procedure as part of the final clean up, or at least daily during use.

Utilization of Miscellaneous Aerosol-Producing Devices and Activities

Use of any of the devices listed below results in considerable aerosol production. Blending, cell-disrupting, and grinding equipment should be used in a Biosafety Cabinet when working with biohazardous materials.

Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar. They have a cooling element to help prevent biological inactivation and can tolerate sterilization by autoclaving.

- If blender rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material.

- The use of glass blender jars is not recommended because of the potential for breakage. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. The blender must be operated with in a secondary container.

- A towel moistened with disinfectant should be placed over the top of the blender during use.

- When opening blenders, be aware of potential contamination hazards such as droplets that might become airborne or fall onto surfaces; liquid residue on the cap; and expansion of the volume due to aeration.

- Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle.

- Placing the blender in a BSC will provide protection against airborne hazards and placement of a tray lined with absorbent pads will assist with contamination control.

- The device should be decontaminated promptly after use.

Lyophilizers

Depending on lyophilizer design, aerosol production may occur when material is loaded into or removed from the lyophilizer unit.

- If possible, sample material should be loaded in a BSC.

- The vacuum pump exhaust should be filtered to remove any hazardous agents or the pump can be vented into a BSC.

- After lyophilization is complete, all surfaces of the unit that have been exposed to the agent should be disinfected.

- If the lyophilizer is equipped with a removable chamber it should be closed off and moved to a BSC for unloading and decontamination.

- Handling of cultures should be minimized and vapor traps should be used wherever possible.

Sonicators

Sonication is the use of sound-wave energy for dispersion, disruption, or inactivation of biological materials, such as viruses. Sonicators generate sound waves at very high frequencies (~20,000+Hz range) which is outside normal hearing range. The following are hazards associated with sonicators:

- **Noise:** Although the 20,000-Hz frequency is outside normal hearing range, there are other sources of noise, such as vibration from any loose equipment or other items on the bench or the liquid itself. If the noise levels are high, normal hearing protection devices should be worn.

- **Aerosols:** Aerosols present a more serious potential hazard and must be taken into consideration. Precautions listed for blenders and lyophilizers should be observed.

Ampoules

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to control any aerosol produced. Sealed-glass ampoules used to store biohazardous material in liquid nitrogen have exploded and caused eye injuries. Using polypropylene tubes (cryovials) avoids this hazard. These tubes are available dust-free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat-sealable polypropylene tubes are also available. The following are safety guidelines when using ampoules:

- Gloves must be worn when opening ampoules or cryovials.

- To open a sealed-glass ampoule, nick the neck of the ampoule with a file (if it does not already have a nick), wrap it in a disinfectant- soaked disposable towel, hold the ampoule upright, direct the nick away from yourself, and snap it open at the nick. Do this entire process inside a BSC.

- Reconstitute the contents of the ampoule by adding liquids slowly to avoid aerosolization of the dried material.

- Mix the contents slowly without generating bubbles and withdraw the solution into a fresh container. Discard the disposable towel and the ampoule's top and bottom as medical waste into a sharp's container

Loop Sterilizers and Bunsen Burners

Use of loop sterilizers and Bunsen burners poses a risk of spreading infectious organisms and causing inadvertent fires. The following are safety guidelines for the use of inoculating loops or needles and Bunsen burners:

- **Innoculating loops or needles:** Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols, which may contain viable infectious microorganisms. A way to avoid this is by heating the shaft of the loop until the sample has been heat-dried before flaming the loop itself.

    - Using a side-arm burner or electronic micro-incinerator can control spatter.
    - Flaming can be avoided by using sterile, disposable plastic loops.

- **Open flames**

    - Open flames inside the cabinet create airflow turbulence and heat buildup may damage the HEPA filters. Open flames are extremely dangerous around flammable materials, such as ethanol.
    - Avoiding fires in biosafety cabinets.
    - Use disposable pre-sterilized loops and spreaders.
    - Position the alcohol container so that it cannot be tipped over.
    - Reduce the amount of flammable chemicals and supplies in the cabinet; use only enough alcohol for one experiment.
    - Have a snuffing lid available in case the alcohol in the container catches fire.
    - Do not use any low flash point chemicals in a BSC.
    - Continuous flame gas burners should not be used in a BSC. These burners can produce turbulence that disturbs the cabinet's protective airflow patterns. Additionally, the heat produced by the continuous flame may damage the HEPA filter. If a gas burner must be used, one with a pilot light should be selected. Electric loop sterilizers should also be considered.

Personal Protective Equipment (PPE)

Personal protective equipment (PPE) must be provided at no cost to personnel. Although not a substitute for the use of BSCs and good laboratory practices, PPE is considered a primary barrier to infectious agents and other hazardous materials and proper use will reduce the

likelihood of infection and or injury. PPE is the least-desirable exposure control method because its failure results in direct exposure to the agent.

PPE is most effective when used to supplement primary control methods such as biological safety cabinets, safety centrifuge cups, and other containment devices. Appropriate clothing may also protect the experiment from contamination.

The following are PPE to be worn in the lab as appropriate:

Face Protection

Goggles or safety glasses with solid-side shields in combination with masks, or chin-length face shields or other splatter guards, are required for anticipated splashes, sprays, or splatters of infectious or other hazardous materials to the face. Wearing contact lenses is not appropriate in the laboratory setting.

Laboratory Clothing

Laboratory coats, smocks, scrub suits, and gowns are considered laboratory clothing.

- Long-sleeved garments (to cover arms and legs) should be used to minimize the contamination of skin or street clothes and to reduce shedding of microorganisms from the arms.

- In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination.

- If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated.

- Additional criteria for selecting clothing include comfort, appearance, closure types and location, antistatic properties, and durability.

- Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas.

- Disposable clothing should be available for visitors, maintenance, and service workers in the event it is required. All protective clothing should be discarded in the laboratory, disinfected, or laundered by the facility.

- Personnel must not launder laboratory clothing at home.

- Open-toed shoes, shorts, and short skirts should never be worn in the lab

Gloves

Gloves must be selected on the basis of the hazards involved and the activities to be conducted.

- Gloves must be worn when working with biohazardous and/or toxic materials and physically hazardous agents.

- Temperature-resistant gloves must be worn when handling hot materials, dry ice, or materials being removed from cryogenic storage devices.

- Delicate work requiring a high degree of precision dictates the use of thin-walled gloves.

- When working with hazardous materials, the glove should overlap the lower sleeve and cuff of the laboratory garment. A long-sleeved glove or disposable arm-shield may be worn for further protection of the garment.

- In some instances, double-gloving may be appropriate. If a spill occurs, hands will be protected after the contaminated outer gloves are removed.

- Gloves must be disposed of when contaminated, removed when work with infectious materials is completed, and never worn outside the laboratory.

- Disposable gloves must not be washed or reused.

- Protection from contact with toxic or corrosive chemicals may also be required. For assistance in glove selection, contact the Occupational Health Committee.

Respirators

Respirators are selected based on the hazard involved and the protection factor required. Certain laboratory and clinical situations require respiratory protection to prevent inhalation of infectious agents. Regulations, as well as good safety practice, require that personnel be medically evaluated, specifically trained, and fit tested prior to wearing respiratory protective equipment.

Contact the Biosafety Officer or Occupational Health Committee if respiratory protective equipment is required or if there are questions about the respiratory protection program.

Note: Use of respirators requires completion of the Respiratory Questionnaire for medical clearance from the Occupational Health Program and fit testing.

Footwear and Miscellaneous Clothing Guidelines

Open-toed shoes or sandals are not allowed in the lab. In addition, wearing shorts or other clothing that exposes the lower legs is generally considered unsuitable in laboratories because it increases the potential for skin contamination and absorption of contaminants.

Further guidance on the use of PPE can be found in the Chemical Hygiene Plan.

Storage and Labeling of Biological Agents

Biological agents must be stored using leak-proof and sealed containers. Containers must be clearly labeled with the identity of the agent and should include the universal biohazard symbol (see below) as physical space on the container permits. At a minimum, secondary (or outside) containers must include the universal biohazard symbol (identity of contents is also desirable).

Freezers, refrigerators, and other storage areas must also be labeled with the biohazard symbol; exceptions to this policy will be considered on an individual basis by the IBC. Waste and contaminated equipment or other objects to be decontaminated must also be labeled with the biohazard symbol.

Universal Biohazard Symbol

The Cal/OSHA Bloodborne Pathogen Standard specifically requires that containers of human blood or other potentially infectious material (OPIM), contaminated waste, and refrigerators, freezers, and other storage containers used to store or transport blood or OPIM be labeled with the universal biohazard symbol (fluorescent orange or orange-red).

Biohazard Labels and Signs

Each laboratory must have a sign at the entrance that provides safety information to visitors and service personnel. Room signs must contain designations for all laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, reproductive hazards, biohazards, radioactive materials, lasers, and magnetic fields). Please see Appendix M for OSHA Hazard Communication Standard Pictogram on labels to alert users of the chemical hazards.

Biohazard signs will be posted at the following:

- Entrances to laboratories and animal rooms that use agents classified as BSL-2 or BSL-3.
- Cages or animal rooms used for housing animals infected with BSL-2 or BSL-3 agents.

For a sample of door signage, see Appendix M.

Certain other areas and pieces of equipment within a laboratory may also require signs. Refrigerators, freezers, cabinets, and other storage facilities require the biohazard symbol whenever they are used to store infectious agents of Risk Group 2 or higher; human blood or blood products; unfixed tissues; cell or organ cultures; body fluids; or excreta. Large pieces of equipment for handling such materials (*e.g.*, centrifuges, biological safety cabinets) must be similarly labeled.

Regulatory References

The following contain extensive information on techniques and practices and should be consulted for more detail:

1. "Standard Microbiological Practices" in the CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories
2. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
3. National Research Council's Biosafety in the Laboratory - Prudent Practices for the Handling and Disposal of Infectious Materials (National Academy Press, Washington, D.C., 1989)
4. OSHA's Bloodborne Pathogens (BBP) Standard (Title 29 of the Code of Federal Regulations at 29 CFR 1910.1030)
5. Cal/OSHA, California Code of Regulations, Title 8
6. Canadian Laboratory Centre for Disease Control – Material Safety Data Sheets for Microbial Agents (www.phac-aspc.gc.ca/msds-ftss/index.html). https://web3.unt.edu/riskman/index.2.php?section=onlinetraining&group=biosafety&module7

Chapter 6

Laboratory Training

Training is a critical component of any integrated Biological Safety Program. Training is intended to provide the understanding, technical knowledge, and tools that the trainee can use to improve his or her daily laboratory safety practices.

At a minimum, all personnel working with biological materials at CDU must have training in the following areas prior to the start of their experiments:

- Completion of the CITI Biosafety Training for the respective learner group at http://www.citiprogram.org. Online training includes basic courses on biosafety, recombinant DNA, and direct shipping.
- Knowledge of the CDU Biosafety Manual.
- Experimental procedures to be used.
- Decontamination and spill clean-up procedures.
- Safe handling methods for any infectious agent and/or recombinant and synthetic nucleic acid molecules they might be handling.
- Proper methods for transporting infectious agents and other biohazardous materials.
- Bloodborne Pathogens Standard (if you work with human blood or blood products, unfixed tissue, body fluids, organ, or primary tissue and/or samples contaminated with bloodborne pathogens).
- Other specialized training as deemed appropriate by the IBC or the Environmental Health and Safety (EHS) Specialist.
- Reporting accidents, spills, and illnesses.

The PI is responsible for ensuring that his or her employees receive proper training in the biohazards and controls specific to his or her laboratory and the safe conduct of the experimental procedures to be used. The Biosafety Program provides different types of training associated with safe laboratory practices, including BSL-2 work, bloodborne pathogens, use of personal protective equipment, medical waste management, packing and shipping potentially infectious materials/dangerous goods, hazard communication, risk assessment, etc.

Mandated General Biosafety Training

This training is required by law and/or policy and must be obtained through the Environmental Health and Safety (EHS) Specialist because of the regulatory aspects that must be included. An example of mandated general biosafety training is initial bloodborne pathogens training and annual retraining (see Appendix H Bloodborne Pathogen Standard). Mandated general biosafety training is required for all laboratory workers (faculty, staff, students, and visiting scientists) at CDU. The exact training required for a particular person will depend on the hazards to which he or she is exposed.

New employees, faculty, and staff must attend this training program immediately following their hiring or as soon as practical and before beginning laboratory work. Attendance at new employee orientation does not fulfill this requirement. Training includes, but is not limited to, laboratory safety practices, biosafety, chemical safety, bloodborne pathogens, and hazardous waste operations.

Laboratory Safety Training

Laboratory safety training satisfies the basic competency regulatory requirements for those working in labs. It does not satisfy the need for department-specific training, shipment of infectious agents, or other specialized training.

Mandated Specific Training

Mandated specific training is also required by law and/or policy. In some cases, it is administered and tracked by the Environmental Health and Safety (EHS) Specialist, who maintains the record files.

Training laboratory personnel in the unique hazards, equipment, and procedures for a given laboratory is the responsibility of the PI or designated laboratory manager to administer, document, and track. This training is mandated and must be provided by the PI or laboratory manager on a periodic basis to all laboratory personnel.

Documentation is also required and must include at least the date and duration of training, name and position of the trainer, topics covered, and names of the trainees.

HIV/HBV Laboratory Training

Personnel who work in research laboratories that culture, produce, or otherwise perform microbiological manipulation of human immunodeficiency virus (HIV) or hepatitis B virus (HBV) must receive additional training beyond the standard bloodborne pathogen training. Prior to working with HIV or HBV, laboratory workers must demonstrate proficiency in standard microbiological techniques, and in the practices and techniques specific to the laboratory. Additionally, workers must have prior experience in handling human pathogens before working with HIV or HBV.

Personnel who do not have experience with human pathogens must be trained in the laboratory before working with HIV or HBV. Initial training should not immediately start with the handling of infectious agents; rather, training and work activities should be progressive as proper techniques are demonstrated. Workers are permitted to handle infectious agents only after demonstrating proficiency to the laboratory supervisor's satisfaction. Although this specialized, laboratory-specific training is the laboratory supervisor's responsibility, the training should be coordinated with the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 to ensure proper documentation and recordkeeping.

Packaging and Shipping of Infectious Agents Training

Personnel who package and ship Dangerous Goods, such as infectious agents (including recombinant viral vectors), infectious human material (blood and clinical samples for pathological testing), genetically modified organisms (including E. coli clones), biological toxins, dry ice, liquid nitrogen, an formaldehyde (≥10% solution) are required by federal and international regulations to receive training every two or three years.

Select Agents Training

CDU does not support research involving select agents and/or toxins at this time. Contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 or Office of Research Integrity and Compliance for information.

Biosafety Level 3 Training

CDU does not support research involving BSL-3 biocontainment. Contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 or Office of Research Integrity and Compliance for information.

Laboratory-Specific Training

Individual laboratories are required to develop specific training for the particular agents and procedures that personnel will perform in that laboratory. This training should be specific to the hazards in the laboratory and to each person's laboratory duties. Each person in the laboratory must understand the hazards associated with laboratory operations, how to prevent exposures to biological and chemical agents, and exposure evaluation procedures. Laboratory-specific training should not duplicate the general biosafety training, but instead should supplement it.

Each laboratory must maintain training records. The records should include the names and signatures of the instructor(s) and laboratory personnel, signature of the PI (if not the instructor), topic of training, and date that training was conducted. The information should be recorded on a documentation form and maintained by the laboratory. Ongoing training is

required as new hazards and procedures are introduced into the laboratory. The occurrence of spills, spread of contamination, near misses, etc., also indicate the need for refresher training.

Other Safety Training

Personnel who utilize hazardous chemicals, radioisotopes, or X-ray generating devices must attend additional laboratory safety trainings.

Refresher Training

All laboratory workers and certain categories of building occupants will be subject to periodic mandatory refresher training. The scope and details of these refresher trainings will be determined by the IBC and will range from annually (for high-risk operations, or those required by regulatory mandates, such as Bloodborne Pathogen Standard) to every three years (for low-risk operations, such as BSL-1). The occurrence of spills, spread of contamination, near misses, or any type of misconduct indicate the need for refresher training.

Chapter 7

Decontamination and Sterilization

Decontamination is the removal of a contaminating substance from a device, instrument, work surface, or living organism. Decontamination procedures can range from steam sterilization under pressure by utilizing an autoclave or utilizing ethylene oxide, to simple cleaning with soap and water. Sterilization, disinfection, and antiseptics are all forms of decontamination.

Sterilization is the use of a physical or chemical procedure to destroy all microbial life, including viruses, fungi, bacteria, and highly resistant bacterial endospores.

Disinfection reduces most pathogenic, non-spore-forming microorganisms but not necessarily all microbial forms on inanimate objects (work surfaces, equipment, etc.). Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, the object to be disinfected, the chemical composition of the disinfectant and the chemical exposure time, temperature, and concentration.

Antisepsis is the application of an antimicrobial substance to skin or living tissue to inhibit or destroy microorganisms. It includes using germicidal solutions (bactericidal, viricidal, and fungicidal solutions) for swabbing an injection site on a person or animal and for hand washing. Although some chemicals may be utilized as either a disinfectant or an antiseptic, potency, efficacy, and adequacy for one application does not guarantee adequacy for another. Manufacturers' recommendations for appropriate use of germicides should always be followed.

General Procedures

Decontamination of cultures and objects contaminated by biological agents is routinely performed in microbiological laboratories. Decontamination is a vital component of microbiological safety practice and serves to protect laboratory personnel (as well as others) from infection and the release of infectious organisms to the outside environment (primarily through person-to-person transmission). Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of cultured organisms.

- Infectious wastes such as liquid and solid will be handled, treated and disposed according to biological hazardous waste policies and procedures.
    - Liquid wastes such as bacterial or viral culture media from BSL2 labs shall be treated by autoclave decontamination using the liquid cycle.
    - Solid wastes from the BSL2 laboratories will be segregated and placed in biohazard containers lined with biohazardous waste bags and disposed as biological wastes. This waste is sealed by the laboratory and decontaminated by the Occupational Hygiene Technologist prior to disposal by a contracted vendor to a legal disposal site.

- Autoclaving is the preferred method for treating biological wastes.
- A disinfectant should be chosen based on its potency, efficacy and chemical composition that is appropriate for the organism in use.
- All liquid biological cultures shall be deactivated with appropriate disinfectant.
- All solid biological waste shall be disposed of in the biohazard waste containers.

Methods of Decontamination

The three main categories of physical and chemical decontamination are heat, liquid disinfection, and vapors and gases.

- **Heat**: Wet heat is the most dependable method of sterilization. Autoclaving (saturated steam under pressure of approximately 25 psi to achieve a chamber temperature of at least 250° F for a prescribed time – usually 30 minutes) is the best method of rapidly achieving destruction of all forms of microbial life.
    - In addition to proper temperature and time, prevention of entrapped air is critical to achieving sterility because of air's poor heat transfer properties.
    - Material to be sterilized must come into contact with steam and heat. Indicators of proper autoclave operation (*e.g*., autoclave tape or autoclave-sensitive labels) shall be used with each load to visually indicate successful processing.
    - Use of autoclave tape alone is not an adequate monitor of the sterilization's success.
        - The tape will indicate that the autoclave reached an appropriate temperature but does not indicate the length of time that the autoclave maintained this temperature. Therefore, at the completion of each autoclave cycle, an autoclave print report shall be automatically printed to indicate the temperature, pressure and drying time achieved for each autoclave cycle.
        - Monthly testing shall be performed with ampoules containing heat-resistant spores (see appendix C).
- **Liquid disinfection**: A liquid disinfectant (*e.g*., 1:10 solution of household bleach yielding a final hypochlorite concentration of 0.5%) is used to wipe or soak potentially contaminated materials for a period of time to kill all pathogenic agents present. Quaternary ammonium compounds and phenolic solutions can also be used effectively,

but each disinfectant requires varying amounts of contact time and an effective concentration.

- **Gas and vapor:** Potentially contaminated articles are exposed to a sterilizing gas (*e.g*., ethylene oxide or ETO) or vapors from a chemical (*e.g*., formaldehyde). Because of the hazardous nature of the gases and vapors used, this requires specially designed equipment and facilities.

Autoclaving

Autoclaving uses saturated steam under pressure (approximately 25 psi) to achieve a temperature in the autoclave of at least 125°C (250°F). Autoclaving can be used to destroy vegetative bacteria, bacterial spores, and viruses. When decontaminating biohazardous waste, it is recommended that the temperature **in the waste** reach a minimum of 125°C for a minimum of 30 minutes. The total processing time required to meet these conditions depends on several loading factors (see below); however, it is recommended that a minimum autoclave cycle of one hour be used when decontaminating waste.

When using an autoclave, the following guidelines shall be taken into consideration:

- Biohazardous materials shall not be placed in autoclaves overnight in anticipation of autoclaving the next day.
- Autoclaves shall not be operated by untrained personnel.
- Special precautions shall be taken to prevent accidental removal of material from an autoclave before it has been sterilized or the simultaneous opening of both doors on a double door autoclave.
- Dry hypochlorite, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth, or oil.
- I In the event of an emergency when using the autoclave, press the “abort” button to terminate the autoclave cycle.

WARNING!
OXIDIZER + ORGANIC MATERIAL + HEAT = POSSIBLE EXPLOSION

Do not place lab chemicals in an autoclave for sterilization

Three factors in combination determine the effectiveness of autoclaving:

Temperature: An autoclave uses steam under a pressure of approximately 25 psi to achieve a chamber temperature of at least 125°C. Although the autoclave chamber may reach 125°C, this does not necessarily mean that the interior of the load will reach this temperature.

Time: A minimum autoclave cycle time of 30 minutes at a chamber temperature of 125°C (time does not begin as soon as the autoclave cycle is initiated) is commonly recommended for sterilization of clean items. However, the total processing time required to achieve decontamination depends on several loading factors, including the load container (heat transfer properties); the amount of water added to the load; liquid cycle, dry cycle and the weight of the load. For increased loads, an increased cycle time will be required to ensure effective decontamination.

Contact: Steam saturation is essential for maximum heat transfer. Steam must contact all areas of the load. Autoclave bags and other containers shall be left partially open (or otherwise permit entry of steam) to ensure adequate contact. Studies have shown that adding water to the interior of the bag improves the time-temperature profile of the autoclave cycle, thereby increasing the autoclave's sterilization efficiency.

Dry Heat

Because it requires higher temperature and longer contact time, dry heat is less effective than moist heat (autoclaving). Nevertheless, dry heat is preferable to moist heat for decontamination of anhydrous materials and closed containers because the moisture component of the steam used in an autoclave will not effectively penetrate anhydrous materials and closed containers.

The highest dry heat equivalent temperature that these materials will reach in an autoclave is 125°C. The highest temperature that material will reach in a dry heat oven will be the actual temperature inside the oven.

Chemical Disinfection

Disinfection is the decontamination of work surfaces, equipment, biological safety cabinets, and other inanimate objects using antimicrobial agents. Several chemical agents are used as disinfectants. Laboratory workers shall remember that there are hazards associated with all of these chemical disinfectants.

- Inhalation and skin contact shall be minimized, and eye contact avoided.

- Appropriate heat resistant gloves and safety eyewear shall always be worn when handling these chemicals.

Pertinent information for some of the common chemical disinfectants is summarized in table format at the end of this chapter.

Summary of Chemical Disinfectants

		Effective Against[a]						
Disinfectant	Use Parameters	Vegetative cells	Lipophilic viruses	Tubercle bacilli	Hydrophilic viruses	Bacterial spores	Important Characteristics	Potential Application
Alcohol (ethyl, isopropyl)	Conc.: 70-85% Contact time: 10-30 min	+	+	+	±		Eye irritant, toxic, flammable, inactivated by organic matter.	Surfaces: work and equipment
Chlorine Compounds (*e.g.* bleach and chlorine dioxide)	Conc.: varies; 0.5% (bleach) Contact time; 10-30 min (bleach) Contact time: 5 mins (Clidox®)	+	+	+	+	±	May leave residue; corrosive; skin, eye and respiratory irritant; inactivated by organic matter; make up at least weekly.	Spills, equipment surfaces, instruments, glassware, water baths
Quaternary Ammonium Compounds	Conc.: 0.1-2% Contact time: 10-30 min.	+	+				Toxic, inactivated by organic matter.	Surfaces (work and equipment), BSCs, floor maintenance, glassware, instruments
Phenolic Compounds	Conc.: 0.2-3% Contact time: 10-30 min	+	+	+	±		Leaves residue; corrosive; skin, eye and respiratory irritant; toxic; inactivated by organic matter.	Surfaces (work and equipment), BSC, floors, spills, glassware, instruments, water baths
Iodophor Compounds	Conc.: 4-8% Contact time: 10-30 min	+	+	+	±		Leaves residue; corrosive; skin and eye irritant; toxic; inactivated by organic matter.	Surfaces (work and equipment), BSCs, glassware, water baths
Formaldehyde[b] (Formalin)	Conc.: 4-8% Contact time: 10-30 min	+	+	+	+	±	Leaves residue; skin, eye and respiratory irritant; toxic (carcinogen). California warning of potential carcinogenic properties	Not recommended for regular disinfectant use due to its irritant properties and potential to be carcinogenic
Glutaraldehyde	Conc.: 2% Contact time: 10-60 min	+	+	+	+	+	Leaves residue; skin, eye and respiratory irritant; toxic.	Equipment surfaces glassware, instruments

a: + = very positive response, ± = less positive response. A blank denotes a negative response or not applicable.
b: due to its irritating characteristics and status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation system.

References:

1. http://www.cdc.gov/hicpac/Disinfection_Sterilization/7_0formaldehyde.html
2. CDC Guidelines for Disinfection and Sterilization in Healthcare Facilities, 2008
3. *Laboratory Safety: Principles and Practices*, 2nd edition, Diane O. Fleming, John H. Richardson, Jerry J. Tulis, and Donald Vesley, eds., American Society for Microbiology, Washington, D.C.

Chapter 8

Biohazardous Spill Response

Even with the most careful planning and implementation of a research project, the possibility of an incident or spill involving biological materials exists. The following procedures are intended to provide a planned response to such rare events.

In any spill scenario, the priority of actions is determined by the "PEP" rule – **P**eople, **E**nvironment and **P**roperty.

The highest priority is to provide aid to injured personnel and prevent other personnel from access to the spill area.

Note: The following are the general requirements and guidelines for Biohazardous Spill Response.

Pre-planning for Biohazardous Spill Cleanup

All biohazardous material spills do not represent the same level of risk to personnel and the environment, making each spill somewhat unique.

The volume of a spill is not necessarily a valid measure of the risks involved.

For example, dropping a glass vial containing 1.0 ml of lyophilized anthrax spores poses much greater risk to laboratory staff than dropping a 10 liter glass bottle of *Escherichia coli* K-12 culture.

Factors other than volume that must be considered in spill risk assessment include:

- Location (*e.g*., biohazard cabinet, countertop, floor, equipment)
- Nature (*e.g*., tip-over, aerosolizing (spray/splash), drop from a height)
- Toxicity/infectivity of spilled material
- Volatility and viscosity of spilled material
- Other properties of material (*e.g*., pH, molarlty/normality, temperature)
- Nature of affected surfaces (*e.g*., absorbent, pitted, smooth)

- Complicating materials (*e.g.*, broken glass, clothing, mixing with other materials)
- Susceptibility of spilled material to neutralization/disinfection
- Mode of transmission

Nevertheless, pre-planning of spill response will lower the risk of cleaning up a spill and will increase the likelihood that the spill is handled appropriately.

Laboratory supervisors shall prepare their laboratory for typical spill scenarios expected in the laboratory. Laboratory workers shall be informed by the Principal Investigator of the following:

- The hazards of the biological agents used in the laboratory,
- The risk associated with these agents during spill scenarios,
- Methods to safely clean up the agents, and
- Laboratory procedures to properly dispose of cleanup materials.

Spill Cleanup Materials

Each laboratory area shall have spill cleanup materials available to respond to the largest spill anticipated for that area.

At a minimum, the following spill cleanup materials shall be available in the laboratory:

- Gloves (thick, chemical-resistant gloves or double pair of thin, nitrile gloves are recommended)
- Safety goggles (a face shield is strongly recommended to avoid splashes to the nose and mouth)
- Lab coat or smock to protect clothing and body
- Absorbent pads
- Disinfectant appropriate for the agents used in the laboratory
- Forceps or other devices to pick up contaminated material (especially sharps)
- Sharps disposal container

- Autoclavable biohazard bags
- The chemical spill kits distributed throughout Charles R. Drew University laboratory facilities by Occupational Health Office (OHO) are not usually suitable for biological spills
- Bloodborne Pathogen spill kits can be used for biological spills
- Additional items needed for the cleanup of biohazardous agents can be maintain in the laboratory.

Biohazardous Spill Cleanup Risk Assessment

Several factors must be considered when assessing the risk that a spill represents:

- Consideration as to whether the spill is classified as major or minor
- Volume and concentration of the spilled material
- The infectious dose of the spilled material and routes of exposure
- Transmissibility
- Location of the spill
- Degree of aerosolization of the agent resulting from the spill
- Susceptibility of the spilled material to disinfection
- Nature of the affected surface(s) and its ability to “hide” organisms from disinfection
- Immune status of immediate personnel

As with any spill scenario (biological, chemical, or radiological), **the safety of personnel is the most important consideration**.

Cleanup is to begin only after it is determined that the personnel who will decontaminate the spill have appropriate knowledge, training, and equipment and has reached the level of competency and proficiency to manage the spill.

Biohazardous Spill Cleanup Procedures

The following are general biohazardous spill cleanup procedures that are appropriate for most spill scenarios; however, the appropriate response to any spill is based on an assessment of the risk associated with that particular situation.

If in doubt, immediately call the Environmental Health & Safety (EHS) Specialist at 323-357-3659.

Emergency telephone numbers are attached on the first page and as an appendix to this manual. Refer to attached list of emergency numbers.

Biohazardous Spills Inside Biological Safety Cabinets

- Wear a laboratory coat (disposable recommended), safety glasses, and gloves (appropriate for the biological agent and the chemical disinfectant) during cleanup.
- Allow the BSC to run continually during cleanup.
- Do not store unnecessary materials in the BSC. This can affect air flow and present a further risk in cleaning up the spill.
- Surround the affected spill area with absorbent material to prevent spread of the spill.
- Apply disinfectant appropriate for the biological agent and allow a minimum of 20 minutes contact time (or as directed by manufacturer's instructions). Alcohol or other flammable liquids are not recommended.
- Wipe up the spill with a disposable cloth or a towel soaked with disinfectant.
- Wipe the BSC's walls and work surface, as well as any equipment in the cabinet, with a disinfectant soaked cloth.
- Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
- Allow non-autoclavable items to have a minimum of 20 minutes contact time with the disinfectant (or as directed by manufacturer's instructions) before removing them from the BSC.
- Remove protective clothing and place in a biohazard waste bag for autoclaving.
- Thoroughly wash hands, forearms, and face with soap and water.

- Allow BSC to run for a minimum of 10 minutes before resuming work in the cabinet or shutting off the cabinet.

Biohazardous Spills in the Laboratory, Outside the Biological Safety Cabinet

If a **BSL-1 agent** or **less than 100 ml of a BSL-2** agent is spilled, the following procedures shall be followed:

- Remove any contaminated clothing and place in a biohazard waste bag for autoclaving, and wash all areas affected by skin contact with soap and water.

- Wear a long-sleeved gown or lab coat (disposable recommended), shoe covers, safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant).

- Place absorbent pads over the spill (to absorb liquid), then place a second layer of disinfectant-soaked absorbent pads over the spill.

- Pour additional disinfectant around the spill, being careful to minimize aerosolization, and work from the periphery toward the center, ensuring thorough contact between the spill and the disinfectant. Disinfect all items in the spill area.

- Allow a minimum of 20 minutes contact time (or as directed by Manufacturer's directions) with the disinfectant.

- Wipe down all equipment, tools, etc., with disinfectant.

- Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.

- Remove protective clothing and place in a biohazard waste bag for autoclaving.

- Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.

If the spill involves a **BSL-3 agent**, or **greater than 100 ml of a BSL-2 agent**, immediately evacuate all personnel from the affected area. Wait for aerosol to settle (usually a minimum of 30 minutes) before entering the spill area.

Exception: If the laboratory is not under negative pressure, cleanup shall begin as soon as possible to minimize the spread of aerosols.

In addition, the following procedures shall be followed:

- Notify Occupational Health Office, as soon as possible, for assistance with the cleanup.
- The Hazardous Material Specialist shall provide direction in managing this process.
- Remove any contaminated clothing and place in a biohazard waste bag for autoclaving and wash all areas affected by skin contact with soap and water.
- Wear a long-sleeved gown or lab coat (disposable recommended), shoe covers, safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant).

For cleanup of a BSL-3 agent, a HEPA-filtered respirator may be required.

- Place absorbent pads over the spill (to absorb liquid), then place a second layer of disinfectant-soaked absorbent pads over the spill.
- Pour additional disinfectant around the spill, being careful to minimize aerosolization, and work from the periphery toward the center, ensuring thorough contact between the spill and the disinfectant.

Disinfect all items in the spill area.

- Allow a minimum of 20 minutes contact time (or as directed by manufacturer's directions) with the disinfectant.
- Wipe down all equipment, tools, etc., with disinfectant.
- Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
- Remove protective clothing and place in a biohazard waste bag for autoclaving.
- Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.

Biohazardous Spills Inside a Centrifuge

- Clear the area of all personnel and allow aerosol to settle (usually a minimum of 30 minutes) before re-entering the area.
- Wear a laboratory coat (disposable recommended), safety glasses, and gloves during cleanup.

- Prepare and submit a report of the spill to the Occupational Health Office and Institutional Biosafety Committee.

Usage and Fit Test shall be required when HEPA-filtered respirators are required.

- Transfer the rotor and buckets to a BSC for cleanup using appropriate PPE.
- Using an appropriate disinfectant, thoroughly disinfect the inside of the centrifuge, the rotor, and buckets.
- Discard cleanup materials and protective clothing as bio-hazardous waste.
- Thoroughly wash hands, forearms, and face with soap and water.

Biohazardous Spills Outside the Laboratory During Transport

All biological agents are to be transported from the laboratory inside an unbreakable, well-sealed, primary container containing absorbent material that is contained inside a second unbreakable, well-sealed leak-proof container (Refer to Chapter 11 for Transportation guidelines).

Both the primary and secondary containers must be labeled with the universal biohazard symbol and the identity of the agent. In the event a transport container drops and its contents are spilled, the following procedures shall be followed:

- Immediately clear the area of all personnel and secure the area.
- Cleanup shall be initiated as soon as possible to prevent spread of aerosol.
- Attempt cleanup **only** if appropriate cleanup materials and protective clothing is available.
- Notify the Environmental Health and Safety (EHS) Specialist at 323-357-3659.

Note: *Employees should become familiar with other non-spill emergencies, such as fire and medical. Please refer to attached emergency numbers.*

Site-Specific Spill Procedures

Spill Response

When responding to a spill, the following rules shall be followed:

- **Attend to the injured**: Ensure receipt of immediate medical care and do not attempt to move the injured individual(s) unless ambient conditions become life-threatening. Individuals splashed, sprayed with, or otherwise exposed to human blood or other body fluids or tissues during a spill shall need to remove contaminated clothing and utilize basic first aid, washing any wounds immediately.

- **Await assistance**: Unless laboratory personnel are trained and properly supplied with personal protective equipment, **DO NOT** attempt to clean up the spill. Personnel shall immediately call the Hazardous Materials Specialist located in the Occupational Health Office at (323) 563-5990 or 323-563-4817.

- **Isolate the spill**: Evacuate the immediate spill area or the entire room in the case of an aerosolizing (splashing or spraying) spill or a spill of volatile material. Prevent others from entering the spill area with barricades, signs or, if necessary, a sentry.

- **Contain the spill**: Place absorbent material around, on, or in the flow path of the spilled material *only if it can be done safely*.

- **Provide information**: Provide the information requested by the Occupational Health personnel and await arrival of the emergency provider.

- **Clean up**: Clean up shall take place **ONLY** if laboratory personnel are trained, properly supplied with personal protective equipment, and otherwise able to clean up and disinfect the spill safely.

Chapter 9

Biohazardous and Medical Waste Disposal

Regulation

In the State of California, biohazardous waste is governed by the Department of Public Health, Medical Waste management Act (MWMA), under the California Health and Safety Code, Sections 117600 – 118360.

Definitions (MWMA January 2012)

Biohazardous Waste (CHSC Section 117635)

(a) Laboratory waste, including, but not limited to, all of the following:

  (1) Human or animal specimen cultures from medical and pathology laboratories.
  (2) Cultures and stocks of infectious agents from research and industrial laboratories.
  (3) Wastes from the production of bacteria, viruses, spores, discarded live and attenuated vaccines in human health care or research, discarded animal vaccines, including Brucellosis and Contagious Ecthyma, as identified by the department, and culture dishes and devices used to transfer, inoculate, and mix cultures.

(b) Human surgery specimens or tissues removed at surgery or autopsy, which are suspected by the attending physician and surgeon or dentist of being contaminated with infectious agents known to be contagious to humans.

(c) Animal parts, tissues, fluids, or carcasses suspected by the attending veterinarian of being contaminated with infectious agents known to be contagious to humans.

(d) Waste, which at the point of transport from the generator's site, at the point of disposal, or thereafter, contains recognizable fluid blood, fluid blood products, containers or equipment containing blood that is fluid, or blood from animals known to be infected with diseases which are highly communicable to humans.

(e) Waste containing discarded materials contaminated with excretion, exudates, or secretions from humans or animals that are required to be isolated by the infection control staff, the attending physician and surgeon, the attending veterinarian, or the local health officer, to protect others from highly communicable diseases or diseases of animals that are highly communicable to humans.

(f)

(1) Waste which is hazardous only because it is comprised of human surgery specimens or tissues which have been fixed in formaldehyde or other fixatives, or only because the waste is contaminated through contact with, or having previously contained, chemotherapeutic agents, including, but not limited to, gloves, disposable gowns, towels, and intravenous solution bags and attached tubing which are empty. A biohazardous waste which meets the conditions of this paragraph is not subject to Chapter 6.5 (commencing with Section 25100) of Division 20.
(2) For purpose of this subdivision, "chemotherapeutic agent" means an agent that kills or prevents the reproduction of malignant cells.
(3) For purposes of this subdivision, a container, or inner liner removed from a container, which previously contained a chemotherapeutic agent, is empty if the container or inner liner removed from the container has been emptied by the generator as much as possible, using methods commonly employed to remove waste or material from containers or liners, so that the following conditions are met:
    (A) If the material which the container or inner liner held is pourable, no material can be poured or drained from the container or inner liner when held in any orientation, including, but not limited to, when tilted or inverted.
    (B) If the material which the container or inner liner held is not pourable, no material or waste remains in the container or inner liner that can feasibly be removed by scraping.

(g) Waste that is hazardous only because it is comprised of pharmaceuticals, as defined in Section 117747. Notwithstanding subdivision (a) of Section 117690, medical waste includes biohazardous waste that meets the conditions of this subdivision. Biohazardous waste that meets the conditions of this subdivision is not subject to Chapter 6.5 (commencing with Section 25100) of division 20.

Infectious Agent (CHSC Section 117675)

Infectious agent means a type of microorganism, bacteria, mold, parasite, or virus, including, but not limited to, organisms managed as Biosafety Level II, III, or IV by the federal Centers for Disease Control and Prevention, that normally causes, or significantly contributes to the cause of, increased morbidity or mortality of human beings.

Medical Waste (CHSC Section 117690)

"Medical waste" means waste which meets both of the following requirements:

1. The waste is composed of waste which is generated or produced as a result of any of the following actions:
    a. Diagnosis, treatment, or immunization of human beings or animals.

   b. Research pertaining to the activities specified in subparagraph (i).
   c. The production or testing of biologicals.
   d. The accumulation of properly contained home-generated sharps waste that is brought by a patient, a member of the patient's family, or by a person authorized by the enforcement agency, to a point of consolidation approved by the enforcement agency pursuant to Section 117904 or authorized pursuant to Section 118147.
   e. Removal of a regulated waste, as defined in Section 5193 of Title 8 of the California Code of Regulations, from a trauma scene by a trauma scene waste management practitioner.

2. The waste is either of the following:
   a. Biohazardous waste
   b. Sharps waste

For purposes of this section, "biological" means medicinal preparations made from living organisms and their products, including, but not limited to, serums, vaccines, antigens, and anti-toxins.

Mixed Waste (CHSC Section 117730)

Mixed waste means mixtures of medical and non-medical waste. Mixed waste is medical waste, except for all of the following:

1. Medical waste and hazardous waste is hazardous waste and is subject to regulation as specified in the statues and regulations applicable to hazardous waste.
2. Medical waste and radioactive waste is radioactive waste and is subject to regulation as specified in the statutes and regulations applicable to radioactive waste.
3. Medical waste, hazardous waste, and radioactive waste is radioactive mixed waste and is subject to regulation as specified in the statutes and regulations applicable to hazardous waste and radioactive waste.

Sharps Waste (CHSC Section 117755)

Sharps waste means any device having acute rigid corners, edges, or protuberances capable of cutting or piercing, including, but not limited to, all of the following:

1. Hypodermic needles, hypodermic needles with syringes, blades, needles with attached tubing, syringes contaminated with biohazardous waste, acupuncture needles, and root canal files.
2. Broken glass items, such as Pasteur pipettes and blood vials contaminated with biohazardous waste.
3. Any item capable of cutting or piercing that is contaminated with trauma scene waste.

Biohazardous Waste

Biohazardous waste includes waste materials derived from cultures and stocks of infectious agents, human pathological wastes, contaminated animal carcasses and body parts, all sharps, and human blood and blood products.

Proper handling and disposal of biohazardous waste is necessary to prevent infection of personnel (laboratory workers, custodians, laboratory visitors, etc.) and release to the environment. OSHA and State of California regulations require that biohazardous waste be properly labeled, stored, and disposed.

Labeling Biohazardous Waste

At a minimum, all biohazardous waste must be labeled with the universal biohazard symbol. Additional information, such as the type of waste (such as “sharps” or “liquid waste”) and origin of the waste, is recommended.

Handling and Disposal of Biohazardous Waste

Sharps

Sharps include all syringes, lancets, scalpels, and other similar medical instruments (whether or not contaminated), as well as contaminated Pasteur pipettes, broken glass, and other instruments or materials that can cut or puncture personnel.

- Sharps must be collected in rigid containers that are leak-proof and resistant to puncture from the sharps. Sharps containers must be designed so that sharps can be safely introduced into the container but not easily retrieved.

- Containers shall be red in color and labeled with the universal biohazard symbol visible in all directions. When the sharps container is approximately ¾ full, CDU personnel shall seal the waste container and be picked up by the Occupational Hygiene Technologist located in the Occupational Health Office.

- Personnel shall seal the waste container and complete the waste log notification for “pick up” of waste . Waste shall be picked up and be prepared for final disposition by the Biohazard Technician. A contracted vendor shall retrieve the sharp waste and transport it to the regulated waste disposal site.

Uncontaminated Laboratory Glassware and Broken Glass

Collect uncontaminated laboratory glassware and broken glass in rigid containers (separate from other waste) that will prevent cuts and punctures to personnel. Containers shall be

labeled “broken glass”. Broken glass is to be disposed of as ordinary trash by placing them in a specified container on campus for later retrieval by a contracted vendor.

Solid Biohazardous Waste

Solid biohazardous waste includes microbial agents, tissue culture, and contaminated material (such as petri dishes, pipettes, contaminated glass, etc.). These materials are collected in red biohazard bags that are double-lined and placed in labelled biohazardous containers.

- Personnel shall seal the waste container for pick up by the Occupational Hygiene Technologist located in the Occupational Health Office.
- Personnel shall seal the waste container and fill out a biohazard waste log with notification of pick-up.
- The Occupational Hygiene Technologist shall process the waste in accordance with regulatory requirements.

A licensed contracted vendor retrieves the waste from the University at predetermined intervals after the waste is processed.

Liquid Biohazardous Waste

Liquid biohazardous waste includes all blood and liquid waste from humans or animals, and all other liquid biohazardous waste (such as microbial cultures). Collect liquid waste in closeable, rigid, plastic, leak-proof containers labeled with the universal biohazard symbol.

- Human and animal blood and body fluids shall not be disposed of by flushing directly into the sanitary sewer, but shall be handled as biohazardous liquid waste (wear laboratory coat, safety glasses and face shield, and gloves, and be careful to minimize splashing).

- Human, animal blood and body fluids shall be processed by Occupational Hygiene Technologist using autoclavable regulated procedures.

- All other liquid waste shall be autoclaved or treated with a disinfectant prior to disposal.

- Liquid waste treated with small quantities of bleach [1:10 (vol/vol)] shall be disposed of by flushing directly to the sink. Liquid waste treated with other chemical disinfectants must be disposed of as hazardous chemical waste.

- The autoclavable standard for liquid Biohazard waste shall involve the use of the liquid cycle.

Animal Carcasses, Body Parts, and Tissue

All carcasses are to be placed in a red biohazard bag secured with a plastic tie.

- All non-preserved carcasses shall be stored in a freezer or cold storage area prior to disposal. **Secure limbs and sharp protrusions** so they do not puncture the bag.

- Only carcasses obtained by IACUC approved euthanized methods can be stored in a designated freezer.

A licensed vendor retrieves the waste from the designated area within the animal care facility.

Chapter 10

CDC/USDA Select Agents

Definition of Select Agent

Specific biological agents and toxins considered to be a severe threat to public health and safety because of their potential use as bioterrorism agents (*e.g.*, *Bacillus anthracis*, *Botulinum* neurotoxins, Ebola virus, etc.).

No laboratories are currently authorized or approved to use select agents in any laboratory at the University.

Chapter 11

Transportation of Biological Materials

Introduction

The packaging and transportation of biological materials are guided by regulatory guidelines under the jurisdiction of local, state, federal, and international agencies.

This is particularly important when classified hazardous materials are transported through the public transportation system that involves the use of public roadways, airways, and sea lanes accessible to the public and placing members of the public at risk.

Regulatory Requirements

Regulatory requirements from several agencies govern the packaging, labeling, and handling and general transportation mechanism of biohazardous materials. Adherence to these standard guidelines is imperative to avoid deficiencies of the standards.

The intent of the packaging and transportation regulations is to prevent accidental exposure of personnel who may handle the material during its shipment. Therefore, certain general criteria apply to all possible transportation scenarios.

Policy

1. Charles R. Drew University of Medicine and Science shall develop and implement effective packaging, labeling, handling and general transportation requirements for shipping “Dangerous Goods” to different locations.

2. Principal Investigators shall ensure that all shipping controls, standards, labeling, markings with the correct classifications for shipping “Dangerous Goods” are utilized in accordance with federal, state and local agencies standard requirements.

Scope

This policy applies to all Principal Investigator, faculty, staff, student and any employee engaged in research activities that are responsible for shipping dangerous materials.

Procedures

Prior to transporting any biological materials, the following controls shall be implemented and utilized:

Transportation/Managerial Controls and Standards

- Emergency procedures (*e.g*., contact names and information, spill cleanup, disinfection protocols, etc.) shall be known to the person carrying the materials.
- Container shall be appropriate for the material being transported.
- Material shall be packed so that it shall stay upright during transportation.
- The containers shall be properly labeled.
- Proper protective clothing shall be worn during the packaging of the material.
- Hands shall be washed after handling materials.
- Open cuts or other wounds shall be covered before handling the materials.
- Aerosol generation shall be avoided when handling and packing the materials.
- The person packaging the material shall ensure that the exterior surfaces of each package are free of any potential contamination by the packed material.

Transportation within a Campus: Inter-laboratory

The following requirements shall be observed during the transportation of biological materials within a campus (*e.g*., between two laboratories):

- All laboratory materials shall be transported in a secondary container that is gasket-sealed, puncture resistant, and leak-resistant. Materials shall never be carried in hands or pockets.
- The secondary container shall be closeable and easy to decontaminate; an absorbent pad (or similar material) shall be placed inside the secondary container to absorb any spills.
- Before leaving the laboratory, the container must be properly prepared for transport.
- Label information shall include the identity of the biological material or agent, the universal biohazard symbol (if the material or agent is in, or above, Risk Group 2), and the sending and receiving laboratory identification (*e.g*., PI name and room number).

- Each individual container shall have enough label information to identify its contents. Other information shall be on the outside of the package.

- The container shall be carried directly to the intended laboratory and not taken to offices, cafeterias, or other public or inappropriate locations.

- Upon delivery, the receiving laboratory personnel shall be informed and the material properly stored.

- The package shall be carefully inspected for signs of leakage or other contamination and, if necessary, decontaminated before opening.

Transportation between Campuses: Between Institutions

Transportation of biological samples between institutions is subject to the general conditions described above. In addition, because the transportation takes place through the public roads and highway system, the following conditions also apply:

- All biological samples shall be packed according to Department of Transportation/International (DOT)/International Air Transport Association (IATA) regulations; this includes triple-packaging all samples, even if exempt materials.

- The specimen shall be placed inside a primary container with a tight-fitting, leak-resistant top (*e.g*., full round-threaded screw cap with seal or stopper).

- The primary receptacle or secondary container shall be labeled with the universal biohazard symbol if it contains bloodborne pathogen materials, as per required under the Cal/OSHA and Federal OSHA Bloodborne Pathogen standards.

- The primary receptacle is placed within a secondary (outermost) container that must meet the following specifications:

    - Shatter-/puncture-resistant and leak-resistant.
    - Enough extra space to hold absorbent and cushioning materials around the primary receptacle.

- Label information shall include the category of the infectious biological material or agent, (*i.e*., Category A, Category B, or exempt human or animal specimen), and the sending and receiving laboratory identification (*e.g*., PI name and room number).

- Each individual container shall have enough label information to identify its contents. In addition, a sheet containing a description of contents shall be placed inside the container between the outer and secondary packaging.

- Any dry ice or other coolant can now be added between the secondary and outer packaging layers. This coolant material shall be placed in a shipping box that contains a cooler compartment to ensure that the outer box is not damaged by moisture from cold packs or other coolants.

- All required DOT/IATA labeling and marking information shall be on the outside of the package.

- If the package contains exempt human or animal specimens, or materials that fall under the "Category B Infectious Substances" category, the package may be moved over U.S. roadways by a member of the laboratory. This exclusion, called by the U.S. Department of Transportation as "exclusive use" allows some materials that are exempt or Category B Infectious materials to be transported by a research or clinical laboratory personnel or courier service. This exclusion does not apply to Category A infectious substances or other categories of Dangerous Goods. This individual must have undergone shipping training in the last two years. This package must follow all requirements as described above. Communicate with the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 for further information and questions about this DOT exclusion.

- The container shall be shipped directly to the intended laboratory and not taken to offices, cafeterias, or other public or inappropriate locations. **Under no circumstances shall delivery of dangerous goods be left in hallways or rooms unattended.**

- Upon delivery, the receiving laboratory personnel shall be informed and the material properly stored. The package shall be carefully inspected for signs of leakage or other contamination and, if necessary, decontaminated before opening.

- **Packages of "dangerous goods" shall not be delivered after hours or on weekends and holidays.**

Packaging and Shipping Infectious Agents via Domestic Flights

Occasions do arise when a PI must either ship or receive biological materials from another institution. Such activities are governed by strict federal and international guidelines.

The International Civil Aviation Organization (ICAO) is the United Nations entity that governs all international civil aviation matters. The ICAO's *Technical Instructions for the Safe Transport of Dangerous Goods by Air* governs the shipping of dangerous goods. These technical instructions

have been incorporated into U.S. law and are an acceptable method of transport in the United States (49 CFR 175).

Packaging and shipping biological materials involves certain risks with numerous potential liabilities. The International Air Transport Association's (IATA) *Dangerous Goods Regulations* (DGR), latest edition, is the worldwide gold standard for shipping. The IATA regulations apply to *all* air transport, both domestic and international flights. Following IATA's DGR ensures that a package will also meet U.S Department of Transportation requirements for ground transport.

All responsibilities for packaging and shipment of these agents have been assigned to the shipper/the Principal Investigator or his or her designee.

Only approved and certified trained personnel shall be involved in the shipment of infectious materials for transport. The following is a summary of the requirements for packaging and shipping infectious agents and **does not** constitute proper training.

Definitions and Applicability

- *Dangerous goods:* Articles or substances capable of posing significant risk to health, safety, property, or the environment when transported by surface or air. Most infectious or biological materials are considered dangerous goods and therefore subject to shipping regulation.

- *Infectious substances*: Substances known or reasonably expected to contain pathogens. Pathogens are defined as micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents, such as prions, which can cause disease in humans or animals.

Shipping Classification

For the purposes of shipping classification, infectious substances are broken into two categories:

> **Category A:** An infectious substance transported in a form that, when exposed, is capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals.
>
> **Category B:** An infectious substance that does not meet the criteria for inclusion in Category A.

- *Biological products*: Those products derived from living organisms manufactured and distributed in accordance with the requirements of national governmental authorities (*e.g.*, the FDA). They may have special licensing requirements and are used either for

prevention, treatment, or diagnosis of disease in humans or animals, or for developmental, experimental, or investigational purposes related thereto.

Biological products manufactured and packaged in accordance with the requirements of appropriate national authorities; transported for the purposes of final packaging or distribution; and for personal healthcare use by medical professionals are NOT subject to dangerous goods regulation. However, biological products not governed by national authorities and that are known or reasonably believed to contain infectious substances MUST be classified and shipped according to dangerous goods regulations.

- *Exempt Patient Specimens:* Patient specimens for which there is a minimal likelihood that pathogens are present are exempt from most of the shipping regulations. However, such packages shall at all times must be marked with the words "exempt human specimen" or "exempt animal specimen" and must be triple-packed as described below.

- *Completely Exempt Substances:* materials that are totally exempt for consideration under the shipping regulations:

    - Substances containing micro-organisms that are non-pathogenic to humans or animals
    - Substances in a form so that any present pathogens have been neutralized or inactivated such that they no longer pose a health risk
    - Environmental samples (including food and water samples) that are not considered to pose a significant risk of infection, and
    - Dried blood spots, fecal occult blood screening tests, blood or blood products intended for transfusion, and tissues or organs intended for transplantation.

Classification and Identification – Exempt, Category A, Category B

The substance to be shipped must be classified as completely exempt from regulation, an exempt patient specimen, or a Category A or B infectious substance. Once classified, proper shipping names and identification numbers can then be assigned to the material. Exempt patient specimens do not require shipping names and identification numbers. However, Category A and B materials are assigned the following names and numbers:

- **Category A**: Assign one of two identifiers, depending on whether the material infects humans:
    - Infectious substance affecting humans: UN 2814
    - Infectious substance affecting animals: UN 2900

Note*: If a material infects both humans and animals, use the Infectious substance affecting human code, UN 2814.*

- **Category B:** Biological substance category B: UN 3373

Packaging

All regulated infectious substances, including Category A, Category B, and exempt patient specimens, must **CONTAIN PRIMARY, SECONDARY AND TERITARY CONTAINERS – namely triple packed:**

- The innermost primary receptacle(s) shall be leak-resistant.
- A leak-resistant secondary receptacle with absorbent material placed between the primary and secondary receptacles to prevent the release of liquid during transport and to shield or prevent multiple primary receptacles from coming in contact with one another.
- Rigid, tertiary outer packaging that is at least 100 mm (4 in) in its smallest external dimension.
- Additionally, shipments of Category A and Category B materials must be packaged according to IATA Packing Instructions 602 and 650, respectively. Those guidelines require the following:

    - Shipments shall be prepared in such a manner that they arrive at their destination in good condition and present no hazard to persons or animals during shipment.
    - Outer packaging shall meet structural strength requirements and carry defined specification markings.
    - Packages shall be at least 100 mm (4 in) in their smallest external dimension.
    - An itemized list of contents shall be enclosed between the secondary container(s) and the outer packaging.
    - All packages containing infectious substances shall be marked durably and legibly on the outside of the package with the name and telephone number of a person responsible for the shipment.
    - The shipper shall make advance arrangements with the recipient and the operator to ensure the shipment can be transported and delivered without unnecessary delay.
    - Substances shipped at ambient temperatures or higher must be in primary receptacles made only of glass, metal or plastic, with a positive means of ensuring a leak-proof seal. Screw caps shall be reinforced with adhesive tape.
    - Substances shipped refrigerated or frozen shall carry the refrigerant between the secondary container and outer packaging. Wet ice is not recommended for shipping as it may cause the package to leak during transport, thus delaying or causing rejection of the package by the transporter. If dry ice is used, the packaging shall permit the release of CO_2 gas.
    - Primary and secondary containers shall retain their integrity across the full range of pressures and temperatures experienced under normal and loss-of-refrigerant conditions.

Labeling

Package labeling is in the form of standardized pictures that shall be affixed to the outside of the container. The color and design of each label is prescribed in the IATA regulations. All labels shall be at least 2 inches on the smallest side.

For the purposes of infectious substances, five different labels must be considered:

Category A: https://www.federalregister.gov/articles/2002/08/14/02-20118/hazardous-materials-revision-to-standards-for-infectious-substances

Category B:
http://www.google.com/imgres?q=UN+3373&hl=en&gbv=2&biw=1006&bih=539&tbm=isch&tbnid=TAmBSH4jLPuemM:&imgrefurl=http://www.casingcorp.com/un3373_labels.htm&docid=0CnBl-WEZZOQqM&imgurl=http://www.casingcorp.com/Combined%252520UN3373%252520Biological%252520Substance%252520Cat%252520B%252520Screen.jpg&w=245&h=281&ei=WkR-T8bCJYXPiAK2tdX5Aw&zoom=1&iact=hc&vpx=115&vpy=16&dur=1328&hovh=224&hovw=196&tx=106&ty=91&sig=112198184757033146101&page=1&tbnh=161&tbnw=140&start=0&ndsp=10&ved=1t:429,r:0,s:0,i:67

Dry Ice:
http://www.google.com/imgres?q=dry+ice+label&hl=en&gbv=2&biw=1006&bih=539&tbm=isch&tbnid=B5IhbzzTUhFJPM:&imgrefurl=http://bama.ua.edu/~ehs/Shipping/NonHazIce.htm&docid=EXLcEqUf2BmxZM&imgurl=http://bama.ua.edu/~ehs/Shipping/placard9.jpg&w=400&h=400&ei=c0F-T-y_G4iqiAL7jPn5Ag&zoom=1

Cargo Aircraft Only: The attached symbol shall be affixed if shipping volumes greater than 50 ml of a Category A substances.
http://www.google.com/imgres?q=cargo+aircraft+only+label&hl=en&gbv=2&biw=1006&bih=539&tbm=isch&tbnid=HmMkMxsV6enJrM:&imgrefurl=http://www.diamondlabels.com/servlet/-strse-1659/4.25x4.25-Cargo-Aircraft-Only/Detail&docid=cyM2RzRIxBK0PM&imgurl=http://www.diamondlabels.com/catalog/D401.jpg&w=300&h=300&ei=NUJ-T_3MK4_8iQKGrqjtAg&zoom=1&iact=hc&vpx=514&vpy=180&dur=19470&hovh=225&hovw=225&tx=140&ty=163&sig=112198184757033146101&page=1&tbnh=114&tbnw=114&start=0&ndsp=12&ved=1t:429,r:9,s:0,i:92

Orientation Arrows: If shipping liquids, two such labels shall be affixed to the package, on opposing sides.
http://www.google.com/imgres?q=Orientation+arrows+for+shipping&hl=en&gbv=2&biw=1006&bih=539&tbm=isch&tbnid=PYomicoYkB1ABM:&imgrefurl=http://www.jjkeller.com/webapp/wcs/stores/servlet/categorySearch_10151_-1_10551_Hazardous%2520Materials%257C16532_Shipping%2520Labels_153_18505&docid=olW1OBNsgIGFgM&imgurl=http://www.jjkeller.com/wcsstore/CVCatalogAssetStore/images/catalog/small/00085_sm.jpg&w=180&h=180&ei=S0N-T4W9JsaaiAK5rIihAw&zoom=1&iact=hc&vpx=341&vpy=2&dur=1250&hovh=144&hovw=144&tx=60&ty=97&sig=112198184757033146101&page=2&tbnh=144&tbnw=142&start=9&ndsp=15&ved=1t:429,r:1,s:9,i:94

Marking

Markings are the words and numbers required to be on the outside of a package. The following markings shall be present on any package containing a Category A or Category B material:

- *UN Number and Proper Shipping Name*:
    - UN 2814 Infectious substance affecting humans
    - UN 2900 Infectious substance affecting animals
    - Biological substance category B

Note*: The UN number is part of the label for Category B substances.*

- *Contact Information*
    - Name and telephone number of the responsible person
    - 24-hour emergency telephone number in case of transportation emergency
    - "To" and "from" information (Origin and Destination Information)

If shipping a material under dry ice, the following additional marking is required:

- UN 1845 Dry Ice (the weight in kilograms of the dry ice present shall also be noted)

If shipping an exempt patient specimen, the only marking required is:

- Exempt Human Specimen
  or
- Exempt Animal Specimen

Training Requirements

Laboratory Research Staff involved in the packaging and shipping of infectious substances shall undergo training every two to three years or when activities change. It is the investigator's responsibility to ensure training is completed. The Environmental Health and Safety (EHS) Specialist at (323) 357-3659 can provide additional training. Additional resources are required in order to accomplish appropriate and effective training. The shipper is obligated to receive further proficiency and qualification when shipping hazardous materials of a class or division where current training is insufficient.

Shipping Documents

Shipping papers describing the material in transit shall accompany all shipments of dangerous goods. For ground transport, a Bill of Lading is required. For air transport, an Airway Bill takes the place of a Bill of Lading. However, air transport of a Category A material also requires that a *Shipper's Declaration of Dangerous Goods* be completed. The full and accurate completion of the Shipper's Declaration is essential, as these are legal documents signed by the shipper, which creates a contract between the shipper and the carrier. The document shall be accurate, legible, and neat and without any spelling errors.

- The declaration form must be completed in English.

- Three copies of the declaration shall be completed. One copy will remain with the shipper (PI). Two copies will be sent with the shipment. If the declaration is not a three-part NCR form, photocopies must be made.

	INSTRUCTIONS
1	**Shipper's:** • Name • Address • Phone number
2	**Receiver's:** • Name • Address • Phone number
3	**Line out the item that does not apply.** Passenger aircraft can only be used to ship quantities less than 50 ml. Cargo aircraft shall be used to ship quantities between 50 ml and 4 L.
4	**Line out the item that does not apply.**
5	• Proper Shipping Name (infectious substance, affecting humans or infectious substance, affecting animals) • Identify the specimen by name in parenthesis *e.g.*, Infectious substance, affecting humans (rabies virus)
6	**Class or Division *** Always 6.2
7	**UN Code *** UN 2814 or UN 2900 (UN 3373 does not require shippers dec.)
8	**Packaging Group *** There is no packaging group for biological agents.
9	• Identify by stating the number of containers by the quantity in each container. (*e.g.*, 5 X 10ml) • Identify type of outer container for the shipment
10	**Packaging Instructions *** 602 or 650 (also 904 if dry ice included)
11	• 24-hour emergency contact number for the shipper (PI, Lab Supervisor), • The statements, "Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made." And "Prepared according to

	ICAO/IATA."
12	**Name and Signature of the shipper (PI or PI designee).**

*As described in the latest edition of the IATA Dangerous Goods Regulation

Note:

- When shipping biomaterials on dry ice, remember that dry ice is itself considered a dangerous good and shall also be listed on the shipping documents as UN1845, Packing Group III, Packing Instruction 904.

- CDU have adopted additional shipping requirements for "high hazard materials" with strict requirements for approved carriers, including a dedicated vehicle, point-to-point delivery, and specified shipping routes. For more information, contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659.

- *The transportation must also meet the NIH guidelines.*

CDU Materials Transportation Mitigation Management Policy

1. Purpose and Applicability

- The purpose of this policy is to define the procedures used to manage the shipping, receiving, and transportation of items determined to be high-risk by the Environmental Health and Safety (EHS) Specialist and governance committees in accordance with CDU policies and procedures and all applicable laws and regulations.

- This policy applies to all items determined to be high-risk and to all employees and staff, including those who are visiting users of CDU facilities and those who are contracted services involved in the shipping, receiving, handling or other use of subject materials as described below.

- This policy defines the protocols for the selection of contracted services to be used in the shipping, receiving, and transport of subject materials. It also includes standards for packaging, transporting, delivery routes and the quality controls to be utilized to ensure that all those involved in the management of subject materials transport adhere to these standards.

2. Definitions

Subject Materials: A substance or material in a quantity and form that may pose a high level of risk to health, safety or property when received, transported and/or stored. These materials include, but are not limited to, toxic/infectious substances (including select agents), radioactive

materials, chemicals, compressed gases, and any other materials that governance committees in collaboration with the Environmental Health and Safety (EHS) Specialist deems a material that shall be managed throughout its transport.

Select Agents: Biological agents and toxins that have the potential to pose a threat to public health and safety if used for bioterrorism purposes. The list includes over 80 bacteria, viruses, toxins, rickettsia, and fungi. The program is regulated by the Department of Health and Human Services (DHHS) and Department of Agriculture (USDA) under the Federal Regulation for Select Agents [42 CFR 73.0; 7 CFR 331; 9 CFR 121] and the Department of Homeland Security.

Shipper: The shipper is the person who packages the subject material and signs the shipper's declaration form. This person is responsible for the material to be classified, identified, packaged, marked and labeled, with all appropriate documentation included with the package. This individual is required to have shipping training, and notify the receiver regarding the planned shipment of high-risk material.

Transporter: The transporter is the individual, operator or contracted service that obtains the package from the shipper, verifies that it has been packaged correctly, and carries the package to the receiver.

Receiver: The receiver, for the purposes of this policy, is the individual who receives the package. This individual is required to have shipping training. The receiver notifies the shipper upon receipt of the planned delivery of high-risk material.

Shippers Declaration Form: The documentation that a high-risk material will be shipped. These documents shall be maintained in accordance with all laws, regulations and CDU policies including standards for the maintenance of original forms to be maintained by the shipper, the transporter and the receiver.

Qualified Vendor: A vendor who meets or exceeds the criteria in Chapter 6.

3. Roles and Responsibilities

3.1 Environmental Health and Safety (EHS) Specialist

The Environmental Health and Safety (EHS) Specialist, in collaboration with oversight committees, is responsible for the management and oversight of the Materials Transportation Management Policy and for ensuring compliance with the procedures outlined within this policy by all employees and staff, visiting users of CDU facilities and contracted services including associated transporters.

3.2 CDU Mail Services

The CDU Mail Services will provide support to the Environmental Health and Safety (EHS) Specialist with the screening/examination of delivered packages, with the staffing of designated locations, and with the management of contracted services.

3.3 Office of Finance - Procurement

Procurement will be responsible for facilitating the selection of contracted service providers who are capable of providing services in accordance with this policy and in compliance with all applicable laws and regulations. Procurement will select, monitor, manage and discharge all contracted services that are involved in the management and transport of subject materials.

3.4 Campus Security and Facilities

Environmental Health and Safety (EHS) Specialist shall deploy, direct, manage, and/or monitor compliance evaluation and quality assurance associated with this program. CDU Campus Safety and Facilities will be responsible for maintaining the security of locations determined to be appropriate for the receiving, shipping and storage of designated materials as well as the screening and examination of vehicles, packages and personnel. CDU Campus Safety will provide security at the point of receipt of the high hazard material and escort the package from the point of entry to the final destination in CDU.

3.5 Emergency Planning and Response

The Hazardous Materials Specialist and the Environmental Health and Safety (EHS) Specialist shall provide to Facilities Manager, and members of the Emergency Response Team recommendations related to emergency management planning, training, and response coordination. In addition, the Hazardous Materials Specialist in collaboration with the Environmental Health and Safety (EHS) Specialist shall participate in the development and implementation of emergency response plans, exercises, risk reduction initiatives and risk prevention measures. This Emergency Planning program shall be overseen by the charged Oversight Governance Committee.

3.6 The Shipper/PI or designee

The Shipper shall be responsible for ensuring that the material being shipped is appropriately packaged including classifying, identifying, marking, labeling and providing appropriate documentation with the package. The shipper shall be trained in accordance with all applicable laws, regulations and CDU policies including those that address the type and frequency of training and necessity of additional training should laws, regulations or CDU policies change at any time.

3.7 The Transporter

The Transporter shall be required to do the following: accept, store, load, inspect and deliver packages to an approved location using approved access routes; report any and all violations of law, regulation or policy; retain all records; and have proper shipping training. The inspection of packages includes requirements involving damage to packages, reporting guidelines and immediate communication to the shipper and receiver, public health and regulatory authorities. In addition to these requirements, transport companies may have their own specific safety requirements for subject material transport.

4. Procedures

Environmental Health and Safety (EHS) Specialist will determine the best location for the receipt, control, audit, transport, and shipping of all items under this policy. Such location(s) will be operated or provided with oversight by the Environmental Health and Safety (EHS) Specialist and other related user departments. These areas will be routinely audited. Transport to and from this location will be by major routes of travel that immediately border CDU.

Environmental Health and Safety (EHS) Specialist with appropriate staff shall train all users of the laws, regulations, polices and requirements involved in the shipping and receiving of subject materials and shall manage the tightly controlled, pre-approved, scheduling of shipment and delivery times. Environmental Health and Safety (EHS) Specialist and qualified staff shall train all CDU users in the approved procedures for the packaging of materials, the approved contracted services to be used in the transport of such materials and the penalties of failing to follow all aspects of this policy. Appropriate qualified staff shall be actively involved in the development of curriculum courses taught in accordance with federal, state and local guidelines.

Environmental Health and Safety (EHS) Specialist and technical resource staff shall determine the packaging requirements to be used in the shipping and receiving of subject materials. These requirements will comply with all applicable regulatory standards. These mandated packaging requirements shall only be altered after obtaining any required approval from all relevant regulatory authorities.

Transport of select agents shall not be allowed from within or outside of CDU.

Environmental Health and Safety (EHS) Specialist and technical resource staff/qualified administrative staff, and Procurement will select contractors for the transportation of subject materials based on criteria including, but not limited to, the following:

- Past performance on similar contracts.
- Appropriate references based on past work performance.

- Ability to provide services as a qualified vendor for transport of all subject materials.
- The managerial and technical ability to provide transport services in accordance with all applicable regulatory standards.
- Ability to provide transport services in accordance with all applicable CDU governance standards.
- CDU requires that the DOT-compliant triple packaging be placed in a non-crushable liquid tight solid container for an added layer of safety.
- CDU requires that packages be secured in the vehicle away from potential impact on outer walls.
- Ability to provide competent staffing that has undergone, and continues to undergo on an annual basis, appropriate background checks.
- Ability to provide courier services that may require that a single individual pick up and deliver packages.
- Ability to provide GPS tracking of packages or vehicles as determined appropriate and approved by CDU.
- Ability to provide vehicles that are inspected in accordance with all applicable inspection and regulatory standards at least every six months.
- Ability to provide customized services that require adherence to CDU determined routes of travel, audit procedures and strictly defined schedules for both pick-ups and deliveries.
- Ability to maintain and to provide an all-inclusive chain of custody document upon delivery of each package.
- Ability to provide resources to participate in CDU audits of services.
- Any transportation vendor personnel having relative proximity to the package must report all occurrences of illness to the County of Los Angeles, Department of Public Health, Center for Disease Control and Prevention (CDC), Homeland Security and in some cases NIH for a period of three weeks from the delivery departure date.

Tracking Shipments: Environmental Health and Safety (EHS) Specialist in collaboration with the Shipping and Receiving Office shall schedule all deliveries and will track the delivery with the

contracted service performing the transportation by means of contractor-provided tracking methods. CDU department offices, as cited above shall initiate its own tracking methods at its discretion and shall determine the type of packaging that the shipper, receiver and transportation company uses, and that it is in compliance with all laws and regulations.

Off Peak Delivery: Environmental Health and Safety (EHS) Specialist shall schedule all deliveries to arrive at off peak traffic hours through the City of Los Angeles to ensure transport and reduce the possibility of accident or delay due to traffic congestion.

Delivery shall be during normal working hours and never on weekends and holidays.

Clear Loading Dock: Facilities Manager will ensure that the loading dock or other facility where the transporter is delivering the high hazard material is free and clear of all parked vehicles to enable safe, secure transfer and receipt. Areas used for deliveries will include secure loading or vehicle inspection areas in which the delivery vehicle can be isolated from movement.

All deliveries of dangerous and hazardous materials shall be delivered directly to the laboratories with prior notification given to the DRO's office by the shipper.

Delayed Receipt: Failure to receive package within the specified time range of delivery shall result in an immediate investigation involving the transport contractor, the shipper, CDU and all applicable regulatory personnel.

Receipt of Packages: Packages shipped to CDU laboratories shall be inspected, verified, documented by qualified resource personnel and transported to the appropriate laboratory location. The PI and laboratory staff shall conduct a survey of the package to verify that no contamination or spill has occurred. The PI shall complete a log verifying no contamination has occurred. The log report shall be forwarded to the attention of the Environmental Health and Safety (EHS) Specialist and retained for records management purposes. Should contamination or spill of the shipped material occur, a report shall be prepared and submitted to the Environmental Health and Safety (EHS) Specialist for records retention. Decontamination shall also occur immediately with notification given to all involve parties (shipper, PI, receiver and all response agencies).

Prior to receipt of the package, shipping and receiving shall verify with the driver that the package's integrity is intact. In the event that the package's integrity is compromised, the transport compartment will be sealed and the transporter's emergency protocols will be followed. Environmental Health and Safety (EHS) Specialist shall notify all appropriate local response agencies and initiate the CDU Emergency Response Plan and Incident Command System.

Problems or Incidents En Route: The transporter shall contact the relevant law enforcement agency having jurisdiction for any problem or incident that may occur during transit or

transport of the subject material. The transporter shall also notify CDU immediately of any such event.

- The transporter shall ask that the public safety agency having jurisdiction notify the local emergency responders having jurisdiction where any incident occur.

- Upon notification of an incident en route to CDU, Environmental Health and Safety (EHS) Specialist shall ensure that the local emergency response departments having jurisdiction over this matter are notified.

- The transporter shall employ reputable hazardous materials cleanup contractor available on a 24-hour by seven days a week basis for response for a biological incident mitigation. The contractor shall coordinate those mitigation efforts with the local emergency responder incident commander.

Notice of Successful Transport: Upon the successful receipt of a shipment under this policy, The Environmental Health and Safety (EHS) Specialist shall notify all the appropriate public safety agencies of the conclusion of the transport.

5. Key References and Resources

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition, December 2009
- U.S. Department of Transportation, 49 CFR Part 171 Final Rule, 03/18/05
- Current Revised International Air Transport Authority, Dangerous Goods Regulations U.S. Public Health Service (HHS)/ CDC 42 CFR Part 73.0, "Possession, Use & Transfer of Select Agents and Toxins," 03/18/05
- Morbidity and Mortality Weekly Report Vol. 1 No. RR-19, "Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents" 12/06/02 National Institutes of Health *Guidelines for Research Involving Recombinant DNA Molecules*

6. Web Sites

- Centers for Disease Control and Prevention: www.cdc.gov/
- Federal Express, Dangerous Goods Program: www.fedex.com/us/
- International Air Transport Authority: www.iata.org/Pages/default.aspx
- United Parcel Service, Hazardous Materials Support Center: www.ups.com/
- United States Postal Service: www.usps.gov/
- United States Public Health Service: www.usphs.gov/
- U.S. Department of Transportation: www.dot.gov/
- U.S. Department of Homeland Security: www.dhs.gov/

Appendix A

IBC Forms

IBC Forms are located at http://www.cdrewu.edu/IBC/Forms.

1. Project and Material Registration for Research Involving the Use of Recombinant or Synthetic Nucleic Acid Molecules and/or Biological Materials (Form 2, Version 5/2013) (IBC Registration Form)

2. Inventory of Microorganisms, Cell Lines, Primary Cell Culture, Human/Animal Materials, Recombinant or Synthetic Nucleic Acid Molecules (Inventory)

3. Continuing Review Form

4. Incident Report Form

5. Appendix A and B – Risk Group Classification (Reference)

Policies, Guidance, and Information are located at http://www.cdrewu.edu/IBC/Guidances.

1. Emergency Numbers for Biological Safety

2. Biosafety Manual

3. IBC Standard Operating Procedures

4. Needlestick Policy

5. Medical History Questionnaire

6. Respiratory Medical Questionnaire

7. Laboratory Ergonomics Questionnaire

8. List of Decontamination Solutions

9. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Appendix B

Laboratory Ventilation and Containment for Biosafety

Contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 for maintenance and servicing and certification of biosafety cabinets.

Please see the Chemical Hygiene Program Manual and the Global Harmonization Hazardous Communication Program.

Appendix C

Autoclave Quality Assurance Program

The sterilization monitoring program managed by the University is a certified and permissible program accredited by the state of California. Sterilization is a microbiological procedure acceptable and approved for the decontamination of bio-hazardous waste. Potentially infectious materials, biological cultures and stocks, contaminated solid and liquid waste can be sterilized by an approved sterilization monitoring process.

After biohazardous materials undergo the sterilization process in a steam autoclave by applying the sterilization monitoring parameters, potentially infectious materials are rendered non-infectious.

At CDU, all autoclaved waste is placed into the solid biohazard waste stream, which does not permit the mixing of hazardous chemicals and radioisotopes waste.

To ensure that biohazardous waste is properly decontaminated during autoclaving, the following procedures are employed by the Occupational Hygiene Technologist.

1. Infectious waste shall be treated in an autoclave for a **minimum of 30 minutes at 125°C (250°F)**; however, the total processing time required to decontaminate infectious waste depends on the specific loading factors (type of biohazard, container type, water content, quantity, physical form, etc.). A total processing time of 60 minutes is recommended for gravity displacement autoclaves and 10 minutes for vacuum-type autoclaves (132°C).

   Sterilization Monitoring:

   Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Because steam will not penetrate a sealed plastic autoclave bag, bags containing dry loads shall not be tightly sealed (rubber band closures will allow bags to “breathe”) or adequate amounts of water must be added to the load. Consult the manufacturer’s instructions for sterilizing materials inside plastic autoclave bags. Liquid waste may also be autoclaved in lieu of adding an appropriate chemicals disinfectant. The parameters for sterilizing liquid waste is different from solid waste. Consult with the Occupational Hygiene Technologist prior to sterilizing liquid waste.

   Liquid waste shall not be thrown down the sink.

   No animal carcasses are autoclaved at CDU. They are collected and stored in a freezer and disposed by a contracted vendor.

2. All autoclaved waste shall include a steam sterilization indicator (the use of biohazard bags with a "built-in" indicator is recommended).

3. Steam autoclaves used to treat infectious waste shall operate at a minimum temperature of 125°C. The operating temperature of the autoclave shall be verified for each cycle by maintaining a record of the temperature as a chart or paper tape recording and a manual recording in a logbook. CDU uses the "ATTEST SYSTEM" for sterilization monitoring system.

4. On a monthly basis, quality assurance test are performed to confirm that adequate compliance sterilization conditions are implemented through the use of ampoules containing heat-resistant spores (*Geobacillus stearothermophilus*) placed in the center of an autoclave load. In conjunction with the *G. stearothermophilus* testing, the maximum temperature achieved during the autoclave cycle are measured and recorded through the use of a maximum registering (or "holding") thermometer or calibrated data logger for full cycle.

5. Maintain records of *G. stearothermophilus* testing and maximum autoclave temperature recordings for a minimum of one year (see Autoclave QC Log at end of appendix).

Monthly Spore Testing Procedure

1. Place ampoule of *G. stearothermophilus* spores and holding thermometer or data logger in the center of an autoclave load.

2. Process the load under normal operating procedures.

3. The highest temperature indicated on the holding thermometer is entered on the Autoclave QC Log. If this temperature is less than 125° C, the autoclave is not to be used to treat infectious waste until it has been repaired and passes service and maintenance "retesting" with appropriate calibration and certified report. In the interim, tag the autoclave as "Not Approved for Infectious Waste."

4. Incubate the autoclaved ampoule and a non-autoclaved, control ampoule according to the manufacturer's instructions (normally 55° - 60°C for 24 to 48 hours).

5. If a color change occurs, the sterilization process was unsuccessful. Discontinue use of the autoclave until it is repaired and passes maintenance retesting. Label the autoclave as "Not Approved for Infectious Waste" until the autoclave passes service and maintenance and retesting.

6. Indicate test results on Autoclave QC Log (see end of appendix) and retain for at least one year for state inspection or site visit.

AUTOCLAVE QC LOG (STERILIZATION MONITORING LOG)

Year: _____; Autoclave Location: _____

Manufacturer: _____; Model: _____Serial Number: _____

Autoclave Testing Instructions:

1. Perform autoclave QC tests monthly.
2. Place *G. stearothermophilus* spore ampoule and holding thermometer in the center of an autoclave load.
3. Process the load under normal operating procedures.
4. Record the highest temperature indicated on the holding thermometer. If this temperature is less than 125° C, the autoclave is not to be used to treat infectious waste until it has been repaired and passes retesting.
5. Incubate the autoclaved ampoule and a non-autoclaved control ampoule according to the manufacturer's instructions (normally 55°- 60° C for 24 to 48 hours).
6. If a color change occurs, the sterilization process was unsuccessful. Discontinue use of the autoclave (for infectious waste) until it is repaired and passes retesting.
7. Record testing results on Autoclave QC Log and retain for at least one year.

Date	Operator	Cycle Time	Cycle Temp	Results	Comments
Jan.					
Feb.					
Mar.					
Apr.					
May					
June					
July					
Aug.					
Sept.					
Oct.					
Nov.					
Dec.					

Appendix D

Biosafety Level 2 (BSL-2) Requirements

Biosafety Level 2 (BSL-2) is suitable for experiments involving agents of moderate potential hazard to personnel and the environment.

For example:

- Microorganisms of low biohazard potential, such as those in Risk Group 2 or BSL-2.
- Recombinant DNA activity requiring BSL-2 physical containment including animal studies that involve the construction of transgenic animals.
- Non-recombinant cell and/or tissue culture systems that require this level of containment.
- Oncogenic viral systems classified as low risk.
- Production activities with Risk Group 1 organisms.

The control of potential biohazards at the BSL-2 level is provided by use of standard microbiological practices with the addition of personnel protective equipment (lab coat and gloves).

The following are procedures used with BSL-2 containment requirements. They are based on the recommendation of the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition, December 2009.

Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director or PI when experiments are in progress.
- All lab staff shall wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- Personnel who use contact lenses shall consult with Occupational Health and Safety if required to use additional eye protection in the lab or whether face shield shall be used.

Additional eye protection shall depend on the lab procedures/biotechnique utilized or planned to be performed.

- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated upon completion of work, or at the end of the day, and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
- A pest control program such as insect and rodent control program shall be implemented and managed effectively.

Special Practices

- Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, lab staff who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, lab staff who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director/PI, in consultation with the Environmental Health and Safety (EHS) Specialist and Research Occupational Health Program/Veterinarian has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
- The Principal Investigator or Laboratory Director implements the policies and procedures whereby only lab staff who are enrolled in the Occupational Health Program and have been advised of the potential hazards and meet specific entry requirements (*e.g.*, immunization) shall enter the laboratory.
- A biohazard sign shall be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use; the biosafety level; the required immunizations; the investigator's name and telephone

number; any personal protective equipment that must be worn in the laboratory; and any procedures required for exiting the laboratory.

- Laboratory staff shall receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (*e.g.*, hepatitis B vaccine or TB skin testing).

- Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director/PI. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

- The Principal Investigator or Laboratory Director ensures that laboratory and support personnel receive appropriate training about the potential hazards associated with the work involved; the necessary precautions to prevent exposures; and the exposure evaluation procedures. Lab staff shall receive annual "updates" or additional training as necessary for procedural, or policy and compliance changes in order to meet the level of competency and proficiency required.

- A high degree of precaution shall always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

- Needles and syringes or other sharp instruments shall be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware shall be substituted for glassware whenever possible.

- Only needle-locking syringes or disposable syringe-needle units (*i.e.*, needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they shall be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps shall be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

- Syringes that re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

- Broken glassware shall not be handled directly by hand, but shall be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal according to any local, state, or federal regulations.

- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

- Laboratory equipment and work surfaces shall be decontaminated with an effective approved disinfectant on a routine basis; after work with infectious materials is finished; and especially after overt spills, splashes, or other contamination by infectious materials. Prior to its removal from the facility, contaminated equipment shall be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations.

- Spills and accidents that result in overt exposures to infectious materials are immediately reported to the Principal Investigator/Laboratory Director and the Environmental Health and Safety (EHS) Specialist. Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.

- Sinks in the BSL-2 area shall be cleared routinely using appropriate disinfectant such as a chlorine-containing abrasive and flushed with a suitable chemical decontaminant.

- Water baths and all water reservoirs shall be washed periodically with a suitable chemical decontaminant.

- Once a month, work spaces that do not get daily decontamination with germicide shall be cleaned, as well as other lab areas where clutter accumulates (*e.g*., storage areas).

- The laboratory shall set up a routine schedule to perform surface cleaning with appropriate chemical disinfectant of large equipment (such as incubators) as part of laboratory good practices. A cleaning log shall be maintained by the PI of the laboratory.

- Supplies shall be rotated and outdated material discarded. Unlabeled material shall be eliminated.

- Good housekeeping practices shall be followed.

- Custodial services: Only personnel with appropriate authorization may enter a BSL-2 facility while BSL-2 research activity is in progress.

- Animals not involved in the work being performed are not permitted in the lab.

Safety Equipment (Primary Barriers)

- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are to be used when:

    - Procedures that have the potential to create infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intravenously, and harvesting infected tissues from animals or embryonate eggs.
    - High concentrations or large volumes of infectious agents are used. Such materials shall be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

- Face protection (goggles, mask, face shield, or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the biological safety cabinet.

- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (*e.g.*, cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it shall never be taken home by personnel.

- Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces, or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.). They shall not be worn outside the lab. Alternatives to powdered latex gloves shall be available. Hands are washed following removal of gloves.

Procedures for Receiving and Inspecting Samples

- The PI shall designate a responsible lab staff for the purchase of all infectious materials to be used in the BSL-2 lab.

- Infectious materials shall be shipped to the laboratory in accordance with the appropriate Department of Transportation (DOT) and the International Air Transportation Association (IATA) standards for shipping of infectious biological materials.

- Upon receipt of the package, it shall be placed on a tray covered with absorbent material and opened in the biological safety cabinet to prevent any potential exposure to personnel in case the container leaked during transport.

- Personnel assigned to open packages shall wear lab smock, gloves, and eye protection.

- If any containers are found to be damaged, leaking or otherwise contaminated, they shall be immediately isolated into a plastic bag along with all packaging materials. The spill shall be disinfected and decontaminated. The Principal Investigator, lab director or designee will be notified immediately. The incident shall be reported to Occupational Health and Safety, the Environmental Health and Safety (EHS) Specialist at (323) 357-3659, and, as necessary, to all appropriate agencies. An incident log report shall be prepared and submitted to the Environmental Health and Safety (EHS) Specialist for review by the appropriate governance committee such as the IBC.

- If, after inspection, the samples are intact, they can be placed into labeled secondary containers (unbreakable plastic containers or metal tubes) and then transferred to a storage area.

- Only authorized staff can remove samples from storage. Removal and use of all such materials shall be entered into the logbook.

- Unused cultures can be returned to storage after the outer container has been properly disinfected.

Laboratory Facilities (Secondary Barriers)

In a BSL-2 lab, the following conditions are to exist:

- Lockable doors shall be provided for facilities that house restricted areas.

- Consideration shall be given to locating new laboratories away from public areas.

- Each laboratory contains a sink for hand washing.

- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

- Bench tops are impervious to water and resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work shall be covered with a non-fabric material that can be easily decontaminated.

- Biological safety cabinets shall be installed in such a manner that fluctuations of the room's air supply and exhaust air do not cause them to operate outside their parameters for containment. Locate biological safety cabinets away from doors, windows that can be opened, heavily traveled laboratory areas, and other potentially disruptive equipment so as to maintain the biological safety cabinet's air flow parameters for containment.

- An eyewash station shall be readily available.

- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

- There are no specific ventilation requirements. However, planning of new facilities shall consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they shall be fitted with fly screens.

Appendix E

List of Biological Agents with the Potential to Cause Laboratory Acquired Infection (LAI)

The List of Biological Agents with the Potential to Cause Laboratory Acquired Infection (LAI) contains many BSL-2 agents in use within the CDU research community.

Principal Investigators and research staff listed on approved IBC protocols involving these biological agents with the potential to cause LAI shall receive agent specific training; agent specific identification cards to be carried by those personnel; and Agent Information Sheets (AIS) providing safety and handling instructions.

- The list of biological agents with the potential to cause LAI is dynamic and shall be routinely reviewed for pathogens that researchers are proposing to use. As new agents approved by the IBC are introduced into the laboratory environment, they shall be added to the list or as agents are no longer being used they shall be removed from the list. Contact the IBC Office at biosafety-ibc@cdrewu.edu for the current list.

Appendix F

Summary of Requirements for Biosafety Levels

Safety Guideline	BSL1	BSL2
Laboratory personnel must wash their hands after handling cultures, removing gloves, and before leaving the laboratory.	Y	Y
Eating, drinking, smoking, and application of cosmetics are prohibited.	Y	Y
Personnel must be familiar with basic biosafety procedures, including this manual.	Y	Y
Personnel should wear goggles or face shields if the possibility of splashes and aerosols exists.	Y	Y
Pipetting by mouth is prohibited.	Y	Y
All laboratory procedures should be performed to minimize aerosol generation.	Y	Y
Work surfaces must be decontaminated at least daily, after each use for infrequent users, and after any spill of viable materials.	Y	Y
Sharps must be placed in specially designed puncture- and leak-proof sharps containers and disposed of appropriately as medical waste.	Y	Y
Laboratories must be kept neat; good housekeeping procedures must be in place and in regular use.	Y	Y
All medical waste is decontaminated before disposal by an approved decontamination method or disposed of as medical waste.	Y	Y
Insect and rodent control programs are instituted.	Y	Y
Laboratory contains a sink for handwashing.	Y	Y
Laboratories are designed for ease of decontamination (*e.g.*, no carpets, sealed surfaces, no unreachable areas, etc.).	Y	Y
Bench tops are impervious to water, moderate heat, and chemicals.	Y	Y
Laboratory furniture must be secured, and spaces between benches, cabinets, and equipment must be accessible for decontamination.	Y	Y
All laboratory windows must be fitted with fly screens.	Y	Y
Laboratory coats or gowns and gloves must be worn.	Y	Y
Autoclaves are required for waste treatment prior to disposal as non-biohazardous waste.	N	N
Autoclave quality control program is required for use specified above.	Y	Y
Instructions for safety precautions are posted by the Principal Investigator.	Y	Y
Animals not involved in the experiment are not permitted in the laboratory.	N	Y
Biological safety cabinets are required and must be certified annually.	N	Y
Laboratory personnel require specific training in the handling of pathogenic materials.	N	Y
Safety centrifuge cups are required.	N	Y
Access to facility is limited or restricted during experiments.	N	Y
The universal biohazard symbol must be posted on the access door to the laboratory.	N	Y

Immunization and/or serological testing for agents to be handled may be required.	N	Y
All laboratory procedures must be performed in a properly certified biological safety cabinet.	N	N
Laboratory requires controlled entry, unidirectional air flow, and other special design features.	N	N
Windows must be closed and sealed.	N	N
No material or equipment can leave the laboratory unless it is autoclaved or decontaminated.	N	N
Autoclaves must be located inside the laboratory.	N	N
Access is through an airlock system.	N	N

Appendix G

Summary of Requirements for Animal Biosafety Levels

Safety Guideline	BSL1	BSL2
Access is limited or restricted at the discretion of the laboratory or Animal Care Facility Director.	Y	Y
Personnel must wash their hands after handling cultures and animals, removing gloves, and before leaving the facility.	Y	Y
Eating, drinking, and application of cosmetics are prohibited.	Y	Y
Personnel must be familiar with basic biosafety procedures, including this manual.	Y	Y
Personnel should wear goggles or face shields if the possibility of splashes and aerosols exists.	Y	Y
Pipetting by mouth is prohibited.	Y	Y
All procedures should be performed to minimize aerosol generation.	Y	Y
Work surfaces must be decontaminated at least daily, after each use for infrequent users, and after any spill of viable materials.	Y	Y
Sharps must be placed in specially designed puncture- and leak-proof sharps containers and disposed of appropriately as medical waste.	Y	Y
Facilities must be kept neat; good housekeeping procedures must be in place and in regular use.	Y	Y
All medical waste is decontaminated before disposal by an approved decontamination method or disposed of as medical waste.	Y	Y
Insect and rodent control programs are instituted.	Y	Y
Doors to animal rooms are kept closed when experimental animals are present.	Y	Y
Facilities are designed for ease of decontamination (*e.g.*, no carpets, sealed surfaces, no unreachable areas, etc.).	Y	Y
Bedding materials from animal cages are removed in a manner that minimizes aerosol production and are disposed of as medical waste.	Y	Y
Instructions for safety precautions are posted by the Principal Investigator.	Y	Y
Facility windows that open must be fitted with fly screens.	Y	Y
Laboratory coats or gowns and gloves must be worn.	Y	Y
Autoclaves are required for waste treatment prior to disposal as non-biohazardous waste.	Y	Y
Autoclave quality control program is required for use specified above.	Y	Y
Cages are washed prior to release or reuse.	Y	Y
Air is exhausted to the outside without recirculation.	N	Y
Personnel baseline serum samples may be required.	N	Y
Facility personnel require specific training in the handling of pathogenic materials.	N	Y
Safety centrifuge cups are required.	N	Y
Access to facility is limited or restricted during experiments.	N	Y

The universal biohazard symbol must be posted on the access door to the facility.	N	Y
Immunization and/or serological testing for agents to be handled may be required.	N	Y
All procedures must be performed in a properly certified biological safety cabinet.	N	N
Facility requires controlled entry, unidirectional air flow, and other special design features.	N	N
Windows must be closed and sealed.	N	N
No material or equipment can leave the facility unless it is autoclaved or decontaminated.	N	N
Autoclaves must be located inside the facility.	N	N
Access is through an airlock system.	N	N

Appendix H

Bloodborne Pathogen Standard

Hepatitis B viral infection is one of the most frequent laboratory-associated infections, and laboratory personnel are recognized as a high-risk group for acquiring this infection (Centers for Disease Control and Prevention). Avoiding occupational exposure to human blood, body fluids, and tissues is the primary way to prevent transmission of bloodborne pathogens. The goal of the initial and annual standard precautions training is to present information on how to prevent such exposures by administrative controls, workplace engineering controls, proper work practices, personal protective equipment, and a hepatitis B vaccine immunization program.

Personnel can be exposed to blood borne pathogens by being stuck with contaminated needles, lacerations from contaminated sharp instruments, or being splashed with blood or body fluids on the mucous membrane of the eye, nose or mouth, or on abraded, non-intact skin (i.e., chapped skin or skin affected by dermatitis). Any direct contact (i.e., contact without barrier protection) to concentrated hepatitis B, hepatitis C, HIV, or any other infectious virus in a research laboratory or production facility is considered an exposure that requires clinical evaluation. All employees working with human cell cultures from human tissue samples should be offered hepatitis B vaccination and be evaluated if an exposure occurs.

The Cal/OSHA Bloodborne Pathogens Standard applies to all employees who might come into contact with blood or other bodily fluids, including:

- Human blood
- Human blood components
- Products made from human blood, or other potentially infectious materials (OPIM) such as the following human body fluids:
    - Semen
    - Vaginal secretions
    - Cerebrospinal fluid
    - Synovial fluid
    - Peritoneal fluid
    - Amniotic fluid
    - Saliva
    - 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.
- Any unfixed tissue or organ (other than intact skin) from human (living or dead).

- HIV-containing cell or tissue cultures, organ cultures, and HIV-, HBV-, or HCV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals with HIV, HBV, or HCV.

Program Elements

The Bloodborne Pathogens Standard requires that an Exposure Control Plan be written and implemented. The Exposure Control Plan must have the following elements:

- Policies and procedures for elimination, or minimization, of exposure
- Evaluation of employee exposure potentials
- Medical surveillance program
- Routine training

The following is a general outline of the CDU Exposure Control Plan.

Roles and Responsibilities

The PI/laboratory director/employee supervisor must identify employees under his or her supervision who may be at risk.

Upon identifying these employees, the supervisor must

- Reduce potential risks by providing personal protective clothing and equipment.
- Provide HBV vaccinations at no cost to the employee.
- Train the employees.
- Develop an effective hazard communication program.
- Ensure engineering controls, such as a biological safety cabinet or sharps container.
- Develop safe work practices and procedures, as well as internal notification procedures to report accidents.
- In conjunction with the academic department, review the list of at-risk employees on an annual basis to ensure that the list is current.

Key Definitions

- Other potentially infectious materials (OPIM) are those listed in Appendix L.

- Regulated waste means liquid or semi-liquid blood or other potentially infectious materials and contaminated items that would release blood or other potentially infectious material in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

- Sharps waste means any device having acute rigid corners, edges, or protuberances capable of cutting or piercing, including, but not limited to, all of the following:

    - Hypodermic needles, syringes, blades, and needles with attached tubing
    - Broken glass items, such as Pasteur pipettes and blood vials contaminated with other medical waste

At-risk Employee Identification

The Exposure Control Plan requires each employer to identify, in writing, all tasks, procedures, and job classifications where occupational exposure to blood may occur and to document the methods of compliance that will minimize the potential of occupational exposure.

Incident Reporting

All incidents must be documented and a copy be kept in the laboratory and a copy forwarded to the Environmental Health and Safety (EHS) Specialist at (323) 357-3659. Accident response procedures are described in Chapter 8.

Written Policies and Procedures

This Manual is intended to act as a primary source of policies and procedures designed to eliminate, or minimize, potential employee exposure to all biological materials, regardless of their hazard level. Employees are required to read and implement all sections of this Manual that are relevant to their work environment, and be fully familiar with universal precautions. Each PI must further develop site-specific SOPs to address local programmatic needs.

Medical Surveillance

The Cal/OSHA Bloodborne Pathogens Standard requires that all personnel with potential exposure to bloodborne pathogens be offered immunization against the hepatitis B virus.

- HBV vaccinations must be offered to an employee within 10 days of assignment.

- Personnel must indicate their acceptance or declination on the Hepatitis B Vaccine Compliance Form; the PI must retain this form on file for the duration of the employee's employment plus thirty (30) years.

- An employee who declines hepatitis B vaccination may, at any time thereafter, change his or her mind and receive the vaccine. The acceptance statement must be signed at that time.

- The PI/laboratory supervisor must not make participation in a prescreening program a prerequisite for receiving the vaccination.

- The HBV vaccination is available at no cost to the employee.

CDU Environmental Health and Safety (EHS) Specialist at (323) 357-3659 and Occupational Health Committee and IBC shall be notified of all presumptive exposures.

Training Requirements

The PI must ensure that all employees with the potential for occupational exposure participate in a training program provided by the Environmental Health and Safety (EHS) Specialist at no cost to the employee during working hours.

Training must be given in accordance with the Bloodborne Pathogens Standard upon initial assignment, on an annual basis thereafter, and whenever modification of an existing job description may affect the employee's potential for occupational exposure.

HIV/HBV research laboratories must ensure that their employees demonstrate proficiency in standard microbiological procedures prior to being allowed to work in the laboratory.

Training must include a comprehensive discussion of this standard, including epidemiology, symptoms and transmission of bloodborne diseases; the Exposure Control Plan; the uses, limitations of, and procedures for using personal protective equipment; a discussion of the HBV vaccination (including the benefits of vaccination and efficiency of the vaccine to prevent disease); emergency procedures involving blood exposure or contamination and post-exposure follow-up procedures; hazard communication; and a question-and-answer discussion opportunity.

Environmental Health and Safety (EHS) Specialist provides Bloodborne Pathogens training on a regularly scheduled basis. For more information and scheduling, please call 323-357-3659.

PI Responsibilities for Occupational Health Issues

In keeping with the Cal/OSHA Bloodborne Pathogen Standard, this policy requires annual standard precautions training, a hepatitis B immunization program, and a post-exposure medical management program.

It is the PI's responsibility to ensure that researchers, technicians, students, or volunteers who work in the laboratory and who have contact with animals, infectious agents, or bloodborne pathogens are medically evaluated prior to starting work and that anyone working with bloodborne pathogens is offered the hepatitis B vaccination series administered by Occupational Health Services in compliance with the Bloodborne Pathogen Exposure Policy for CDU.

An appointment can be made with a medical provider by calling Bayside Medical Center, 2301 W. El Segundo Blvd., Hawthorne, CA 90250, (323) 757-2118.

If the research project is located at a facility at another institution, occupational health services should be available on-site at that institution. If these services are not available on-site, please contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 for assistance in arranging access to appropriate services.

It is the PI's responsibility to ensure that:

- Any person present in a CDU laboratory who has an incident involving potential exposure to an infectious agent is offered ***immediate*** access to a medical evaluation at Bayside Medical Center, Industrial Medicine or Hospital Emergency (after hours, holidays, and weekends- call (310) 900-4525 for Emergency). An immediate evaluation is important, as efficacy of post-exposure medication for HIV and other infectious agents may be less effective if the initiation of treatment is delayed.

- Personnel working with non-human primates or their tissues have a physical examination, an evaluation for tuberculosis, and an immunization screen. Additionally, such individuals must provide serum for banking prior to commencing work with these animals, be informed about the risks and preventive services available, and wear appropriate personal protective equipment.

- When required, each individual completes a medical clearance for respirator use prior to fit-testing for a respirator.

- Personnel who develop symptoms of allergy or asthma that occur upon exposure to experimental animals are referred to Bayside Medical Center for evaluation.

Under the Cal/OSHA Bloodborne Pathogen Standard, CDU is required to offer the hepatitis B vaccine to all employees at risk within 10 days of starting their work assignment. Employees must be informed of the vaccine's benefits and risks, and if they choose not to receive it at the initial evaluation time, they must sign a declination form. If the employee has had the vaccine

previously, but has not had a blood antibody titer to confirm his or her immunity in the past, the employee will be offered the opportunity to have a titer drawn. An employee who declines the vaccine may at any time elect to have the vaccine if his or her job tasks or work setting continue to have the risk of potential exposure to bloodborne pathogens.

If during research, any employee has an exposure to bloodborne pathogens, he or she MUST report immediately to the Environmental Health and Safety (EHS) Specialist at (323) 357-3659, Human Resources, Risk Management, IBC Chair, PI, and Bayside Medical Center during regular business hours, after hours, on holidays, or weekends. An immediate evaluation is important, as efficacy of post-exposure medication for HIV may be less effective if the initiation of treatment is delayed. For more information, call the Environmental Health and Safety (EHS) Specialist at (323) 357-3659.

Contacting Occupational Health Services

Bayside Medical Center at (323) 757-2118
2301 West El Segundo Blvd., Hawthorne, CA 90250

Questions may be directed to:

Environmental Health and Safety (EHS) Specialist at (323) 357-3659
Risk Manager at (323) 326-4988

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OSHA Bloodborne Pathogen Program
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Appendix I

Working Safely with Animals

Working with animals poses potential additional health and safety hazards that require extra precautions. The specific requirements will depend on the types of activities (*e.g.*, surgery, feeding animals, use of anesthetic agents, etc.) and the specific species used. Personnel should follow the following guidelines:

- Follow the specific requirements established in the IACUC-approved protocol and the facility requirements.

- Wash hands after handling an animal or anything that an animal has touched. The most common way of contracting an animal-transmitted infection is placing the infectious material directly into the mouth.

- Never smoke, drink, or eat in an animal area or before washing hands.

- Wear protective clothing as recommended by the facility for the species and operations.

    - Do not take protective clothing home.
    - Protective clothing helps prevent potentially contaminated material from leaving an animal area.

- Use the personal protective equipment (PPE) recommended for the species and operations.

    - Workers shall wear the appropriate PPE (*e.g.*, gloves, face shields, masks, and respirators) when required and follow their supervisor's instructions scrupulously.

- Participate in the Research Occupational Health Medical Surveillance Program (see Appendix K – Medical Surveillance Program) that provides medical evaluations, testing, immunizations, and periodic screenings for allergies, infections, and other medical problems related to animal exposure.

    - Seek medical attention promptly when injured. Follow the specific recommendations for the facility.
    - Workers engaged in work involving vertebrate animals should inform their physician of their work when seeking treatment for illness, even if uncertain whether the illness is work related.

- If there is any possibility of work-related illness or disease, the Environmental Health and Safety (EHS) Specialist must be notified immediately at (323) 357-3659.

- Get the appropriate training and contact a supervisor with any questions.

Risks in Working with Animals

Most experimental animals are housed for a long period of time in the animal care facility, but there are still potential risks in working with animals, such as bites, scratches, or infection by zoonotic diseases. Before using various animals in experiments, researchers must understand and be familiar with the species' characteristics. Staff in animal care facility needs to closely observe each animal on a daily basis. Staff must notify the veterinarian if the animal exhibits any abnormal conditions. In addition, researchers must pay attention to personal hygiene and protection, which is the most important prevention step from risks of getting infected by an animal.

Most rodents used in the animal care facility are mice. The most common injury caused by working with rodents is scratches and bites. Rodents will often bite the handlers if they experience discomfort, pain or anxiety due to incorrect techniques. Allergy to the rodents is another risk faced by researchers. The main zoonotic diseases infection from rodents are lymphocyte choroidal meningoencephalitis (LCM), Hantaan virus infection, Leptospirosis and Rat bite fever.

1) Lymphocytic Choriomeningitis (LCM)

Pathogenic Lymphocytic choriomeningitis virus can infect rats, mice, hamsters and guinea pigs. Experimental rodents can be infected through contact with field rodents with pathogen. The contaminated cell stains are the potential source of infection. The virus exists in the dejecta, urine, body fluids of infected rodents. Humans can be infected through the contact of mucous membrane with pathogen or inhalation of virus particles.

The incubation period after infection on human is about one to three weeks. The early onset of symptoms is like a flu, which is then followed with skin papules, lymphatic node disease and meningitis. Severe central nervous system symptoms can cause death.

2) Hantaan Virus Infection

Pathogen of Hantaan virus infection includes Hantaan virus, Seoul virus, Sin Nombre virus and others. The Hantaan virus is widely distributed in the world. Rodents infected with Hantaan virus are asymptomatic, but exudes large quantities of virus from the dejecta, urine, saliva and respiratory secretions. Humans can be infected through the inhalation of virus particles, such as dry saliva or urine, or dust particles contaminated by the urine of infected rodents. Human infection of Hantaan virus can be fatal with symptoms of hemorrhagic fever with renal

syndrome (HFRS). The main symptoms include fever, back pain, mucous membrane bleeding, and nephritis. The Sin Nombre virus in North America affects the respiratory system.

3) Leptospirosis

Rodents can be infected by some strains of pathogenic Leptospira bacteria. Rodent infection does not cause symptoms, but the bacteria is secreted via the urine. Contact with these infected urine through wounds or mucous membrane can cause the infection in humans. The symptoms of human infection include fever, headache, and myalgia. Severe infection can cause sepsis and affect the kidney, liver, and even cause jaundice.

4) Rat bite fever

The main hosts of rat bite fever are rat and mice infected with *Streptobacillus moniliformis* or *Spirillum minus*. These two kinds of bacteria exist in the rodent upper respiratory tract and mouth. Rodents infected with *Streptobacillus moniliformis* or *Spirillum minus* remain asymptomatic. Humans acquire infection through the bite of the infected rodents. The main symptoms include headache, fever, myalgia, regional lymph node disease and wound swelling.

Protection from Infection

Prevention method from infection of zoonotic disease is personal protection; sustained animal health monitoring; prevention of field rodents entering into animal rooms; testing cell lines before injecting into rodents, and avoiding the use of contaminated cell lines.

Basic Safety for the Necropsy of Infected Animals

- Ensure that the necropsy of infected animals is carried out in biological safety cabinets by trained personnel.
- Wear a surgeon’s wrap-around gowns over laboratory clothing.
- Use a surgeon’s mask and eye protection.
- Use other PPE recommended by the facility for the infectious agents present.
- Wear gloves.
- Wet the fur of the animal with a suitable disinfectant.
- Pin down or otherwise fasten small animals to metal in a tray.

- Before and after necropsy, disinfect the necropsy table, inside the biosafety cabinet (BSC), and other potentially contaminated surfaces with a suitable germicide.

- Upon completion of necropsy, place all potential biohazardous materials in suitable containers and then sterilize the materials.

- Segregate contaminated mixed waste and store for appropriate disposal.

- Place contaminated instruments in a bath that contains a suitable disinfectant.

- Follow the facility requirements for sterilization.

- Clean contaminated rubber gloves in disinfectant before removal from the hands.

- Wearing gloves is not a substitute for hand washing; wash hands after necropsy and carcass disposal.

- Follow the facility's guidelines for the disposal of dead animals.

Appendix J

Procedures for Working in an Animal Biosafety Level 2 (ABSL-2)

Before starting any Animal Biosafety Level 2 (ABSL-2) work at CDU, a PI must:

- Obtain IBC and IACUC approval
- Make appropriate housing arrangements with the DCM director

The following Standard Operating Procedures (SOP) have been developed to provide guidance to those individuals working in rooms in which animals involved in chemical and biological hazards determined to be ABSL-2 are housed.

Definitions

ABSL-2: Animal Biosafety Level 2 includes pathogenic agents of moderate hazard potential (CDC Biohazard Class 2) and chemical hazard agents of moderate hazard potential.

BSC: Biosafety Cabinet

PPE: Personal protective equipment

Parenteral: Taken into the body or administered in a manner other than through the digestive tract, as by intravenous or intramuscular injection.

Overview

Access to the room where the work with animals is to be conducted is restricted. Laboratory personnel must have training in aseptic micro-isolator techniques, when applicable, and use of biological safety cabinets, in addition to specific safety training in handling the pathogenic and/or chemical agent(s) with which they are working.

Research and DCM personnel should receive appropriate immunizations or tests for any agents handled or potentially present in the room prior to initiating the ABSL-2 portion of their project.

Procedures must be conducted in a Class II BSC.

Equipment and Supplies

Personal Protective Equipment (PPE)

- Solid front gown
- Hair cover

- Shoe covers
- Mask
- Double gloves

In addition:

- N95 respirators may be required
- Face shield or other specific eye or face protection may be required

Biohazard stickers for cage cards

- MB-10 (chlorine dioxide) disinfectant or Virkon-S
- Biological safety cabinet: Class II

Responsibilities

It is Environmental Health and Safety (EHS) Specialist's responsibility to ensure that all necessary project-specific safety training is provided to research and DCM staff prior to any project being initiated. It is also Environmental Health and Safety (EHS) Specialist's responsibility to provide documentation to the DCM of such training.

The PI and individuals working in the facility are responsible for ensuring they have received proper training and that they are adhering to this SOP, as well as to posted precautions and guidelines in the facilities.

Procedure

Entry

1. Remove the lab coat worn in the DCM facility and hang it on the garment rack provided outside of designated ABSL-2 animal space.

2. PPE *must* be worn while working in ABSL-2 animal housing and procedure space. PPE is provided just outside specific ABSL-2 rooms. Don designated PPE prior to entry into the ABSL-2 areas.

3. Proceed into the designated ABSL-2 room using an access card or key.

Conducting a Procedure

1. General Information
    a. Investigators using the room will be assigned a cubicle and/or rack and shelf/shelves where their animals will be housed.

i. A cubicle or rack may hold cages belonging to more than one investigator.
ii. Biohazard projects are not housed in cubicles that house ongoing chemical hazard projects.

b. DCM personnel will perform daily health checks of animals on studies involving infectious agents if the animals are housed on racks not held in cubicles.
    i. DCM personnel will perform daily health checks of animals on studies involving chemical agents by viewing the animals through the window of the isolation cubicle.
c. DCM will notify PIs of any animal health issues.
d. All animal work will be conducted within the confines of the Class II BSC.
    i. Always open animal cages in the Class II BSC using aseptic micro-isolator technique.
e. Hypodermic needles and syringes are used only for parenteral injection or aspiration of fluids from laboratory animals and bottles with plastic/rubber diaphragms.
    i. Only needle-locking syringes or disposable needle syringe units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids.
    ii. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use.
    iii. The needle and syringe should be promptly placed in a puncture-resistant sharps container.

2. Always spray or wipe down all interior surfaces of the Class II BSC with MB-10 or Virkon-S **before** and **after** working in the hood. Allow 10 minutes of contact time prior to wiping the surfaces with disposable towels. Do not spray the top grille of the Class II BSC (this is where the filter is located). Discard used towels in the waste container.

3. Immediately following infection of animals with pathogenic agents, place a biohazard sticker on their cage card(s) (DCM provides these specific stickers). Fill out the following information on the biohazard sticker for cage cards:

    - PI name
    - 24-hour contact phone number
    - Protocol number
    - Pathogenic agent
    - Dose per animal
    - Date(s) infected
    - Husbandry by PI or DCM (circle one)

4. Report all spills and accidents that result in overt exposure to infectious materials to the DCM Director and the DCM office.

Removal of Dirty Cages and Bottles from Room

1. Biological agents
    a. Place a cage inside the BSC, remove the water bottle from the cage, and replace the lid.
        i. Put soiled cage, wire lid, and bedding in a semi-clear biohazard autoclave bag and place on a cart.
        ii. Put water bottles in a separate, semi-clear biohazard autoclave bag on a cart.
    b. Closure ties are stored adjacent to the biohazard autoclave bags on the supply rack in the corridor outside rooms and in rooms.
    c. **Do not use** autoclave tape to close the bag.
    d. Thoroughly spray the exterior of all bags with MB-10 or Virkon-S after closing the bag with a nylon tie, beaded tie, or twist tie. Spray all surfaces and wheels of the cart(s).
    e. Cart(s) should be moved to the soiled side of the cage wash room.

2. Chemical agents
    a. Place a cage inside the BSC, remove the water bottle from the cage, and replace the lid.
        i. Put soiled cage, wire lid, and bedding in a red biohazard bag and place on a cart.
        ii. Put water bottles in a separate red biohazard bag on a cart.
    b. Closure ties are stored adjacent to the biohazard autoclave bags on the supply rack in the corridor outside W-838/839 and in both rooms.
    c. **Do not use** autoclave tape to close the bag.
    d. Thoroughly spray the exterior of all bags with MB-10 after closing the bag with a nylon tie, beaded tie, or twist tie. Spray all surfaces and wheels of the cart(s).
    e. Cart(s) should be moved to the soiled side of the cage wash room on the 8th floor of W Building (W-8).

3. Disposal of carcasses
    a. Any dead animals must be removed from their cage (while in the BSC) and placed in a small, leak-proof red biohazard bag.
    b. Place a sticker with animal identification information on the outer bag, then thoroughly spray the bag with MB-10 or Virkon-S and place in the refrigerator. A cage card with a sticker showing the same animal identification information will be placed on the cage.
    c. DCM will remove carcasses for incineration three days after they are found in the refrigerator.

Exiting Procedures

1. When work is complete and the BSC and all other work spaces have been decontaminated with MB-10 or Virkon-S, outer gloves should be removed and placed in the biohazard waste container.

2. To exit the room, open the door and remove one shoe cover, stepping over the room threshold into the hallway with that foot.

3. Remove the shoe cover from the other foot as it is brought into the hallway but before stepping into the hallway with the second foot.

4. Remove gown from the shoulders, turning it inside out.

5. Discard disposable face protection, hair cover, mask, gown and gloves in the red biohazard trash receptacle in the hallway outside ABSL-2 room.

6. After leaving ABSL-2 room and discarding PPE, hands should be washed using the alcohol hand sprayer located on the wall immediately to the left of the doors to each ABSL-2 room

Reference

Biosafety in Microbiological and Biomedical Laboratories (5th edition, 2009).

Appendix K

Medical Surveillance Program

Please see Medical Surveillance Manual.

Appendix L

Laboratory and Equipment Decontamination Procedures

Decontamination of Lab Space and Equipment List

1. Designate a Move Coordinator.

2. Contact an outside vendor for the decontamination of biological safety cabinets (tissue culture hoods).

3. Have appropriate personal protective equipment available (lab coat, gloves, eye protection).

4. Dispose of old chemicals and all other chemical waste as hazardous waste. Notify the Occupational Health Office upon termination of a hazardous waste accumulation area or with any questions on this issue.

5. Decontaminate all equipment that is either to be moved or left behind.

6. Contact Occupational Health regarding the discarding of equipment.

7. On the decontamination certificate, list all decontaminated equipment by room (one sheet per room).

8. Small pieces of equipment can be decontaminated and boxed by lab personnel.

9. Any working equipment to be left behind without a new owner shall be reported to Facilities Management and Occupational Health.

10. Contact the Radiation Safety/Protection Office (RPO) for the decontamination and moving of radiological materials and work spaces.

11. Decontaminate all labs, including fume hoods, the outside of tissue culture hoods, cold/warm rooms, darkrooms, etc.

12. If perchloric acid was used in a fume hood, contact Occupational Health at (323) 563-5990 or 9631.

13. Fill out one decontamination log or sheet for each room, tape one copy to the outside of the lab door (if it is a section of a lab, tape to bench), fax one copy to the Environmental Health and Safety (EHS) Specialist at (323) 357-3659, and keep one copy for the records.

14. Disinfectants: the most common are 10% freshly diluted bleach (leave on for 20-30 minutes, then wash off), 70% ethanol, or isopropanol. Phenolic agents are not recommended.

15. If refrigerators, freezers, incubators, etc., are to be moved with content inside, make sure the content is well protected from sliding, breaking, etc.

16. The Move Coordinator must ensure the following emergency procedures are covered:
    - Chemical spills
    - Biological spills
    - Fire
    - Personal injuries, such as slips, falls, cuts, etc.

17. Protective clothing and spill absorbent materials must be on hand.

18. Follow and complete the Laboratory or Equipment Decontamination certification form.

19. Occupational Health shall inspect the laboratory space or equipment to ensure they have been appropriate cleaned and decontaminated.

20. Occupational Health shall affix a decontamination sticker on equipment that had been properly cleaned and disinfected. The equipment shall be moved out within 15 days that the sticker is issued. The equipment will not be moved if the 15 days issuance lapses. Occupational Health Office shall inspect the equipment again and issue a new sticker.

21. Biohazard sticker will be removed once the equipment has been properly decontaminated.

LABORATORY OR EQUIPMENT DECONTAMINATION CERTIFICATION

Location and Department:

Date:

1. I certify that the rooms or equipment listed below, previously used by my laboratory, have been emptied of biological and chemical materials:

   EQUIPMENT:

   _____
   _____

1. The surfaces of these rooms/equipment have been decontaminated (if equipment: inside and outside) with: (specify decontaminants and percentages, (*i.e.*, 70% Ethanol, if 10% bleach is used, it must be freshly made up).

   _____
   _____

2. All chemicals contained within the rooms or equipment have been removed or drained and collected for proper disposal (including but not limited to):

   - *Oil* – if the equipment contains a pump or other oil reservoir, oil must be drained and collected as Hazardous Waste. Contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659.

   - *Mercury* – If there is a thermometer or other device inside or associated with the equipment or space the device must be removed and collected as Hazardous Waste. Contact DRO for assistance.

   - *Refrigerant Gas* – If the equipment involved cooling and relied on refrigerant gas, this gas must be removed prior to disposal. Facilities Management must be contacted as only licensed mechanics can perform this service.

   - *Lead Shielding* – If the equipment used lead as a shielding agent, this material must be removed prior to disposal. Contact DRO to assist in lead removal.

     _____ Yes _____ No _____ N/A

3. If the space or equipment contained or was used with any radioactive materials (isotopes, sealed sources, etc.), the laboratory personnel have decontaminated the area and equipment. Radiation Safety has been contacted, has surveyed the equipment, and has certified it free of detectable Radioactive contamination and arranged for the removal of any shielding:

4. All sink traps (including those in fume hoods) have been bleached and flushed with water (use 1 cup of concentrated bleach, wait 20 minutes, then flush thoroughly with water):

   _____ Yes _____ No _____ N/A

5. Does the equipment contain fluid (*e.g.*, water bath, antifreeze, etc.)?

   _____ Yes _____ No

Name (print):		**Phone Ext:**	
Signature:		**Date:**	
Dept., Bldg., Room #:			
Decontaminated by:		**Principal Investigator:**	

Please return/fax completed form to the Environmental Health and Safety (EHS) Specialist williamwong@cdrewu.edu or call (323) 357-3659

Laboratory Decommissioning and Relocation Procedures

Purpose and Applicability

- It is the policy of CDU that laboratory decommissioning take place prior to the relocation of any laboratory space or upon vacating laboratory space or leaving either institution. In addition, safe-moving practices shall be adhered to at all times.

- This policy is intended to minimize research and clinical lab downtime due to moving of a laboratory, and to protect contractors, laboratory personnel, and any other personnel involved in the process from laboratory hazards.

- This policy applies to all CDU employees and tenants occupying laboratory space within CDU buildings.

Definitions

- *Abandoned Laboratory*: A clinical or research laboratory that is left vacant by a Principal Investigator or Laboratory Director and his or her laboratory staff, and has laboratory materials (biological, surplus chemical, radioactive), equipment or waste that has not been disposed.

- *Biological Materials*: All human, plant, and animal pathogens; all human blood, blood components and products, tissues and body fluids; all human and animal cultured cells; all infected animals and animal tissues; all cultures/stocks of biological agents, including recombinant DNA materials; and all biological toxins. Also includes biomedical waste and physically dangerous (sharp) waste.

- *Decommissioning*: The process whereby a Principal Investigator or Laboratory Director and his or her laboratory staff decontaminate existing laboratory space and make a clinical or research laboratory safe prior to vacating the space.

- *Decontamination*: The process whereby the Principal Investigator or Laboratory Director and his or her laboratory staff clean and disinfect laboratory surfaces and equipment so they are safe to handle.

Roles and Responsibilities

- The Principal Investigator or Laboratory Director is responsible for the complete decommissioning of the laboratory space prior to vacating the laboratory. In cases where an abandoned lab is identified, the department that the PI or Laboratory Supervisor reported to shall be responsible for the decommissioning and all costs associated with the process.

- The Environmental Health and Safety (EHS) Specialist and appropriate assigned and appointed staff resources shall distribute this policy and attachments and advise Principal Investigators, Laboratory Directors, and laboratory personnel on how to implement the various aspects of the policy. They shall also verify that a lab has been appropriately decommissioned before a Principal Investigator or Laboratory Director may leave or move his or her laboratory.

- The Move Coordinator for the laboratory is appointed by the Principal Investigator or Laboratory Director and is responsible for coordinating the laboratory decommissioning and move. The Move Coordinator is the primary contact with DRO.

- Other personnel (facilities, moving personnel, and contractors) shall be aware of this policy and shall not handle laboratory materials, equipment, or waste unless instructed to do so by their supervisor and/or DRO.

Procedures: Preparation

- Prior planning is key to a successful laboratory decommissioning and move. Preparation and communication with DRO shall be a major factor in minimizing delays, protecting property against damage and loss, and most importantly, reducing the potential for personal injury. Contact DRO at (323) 563-5990 with any questions or for assistance.

Procedures: Waste Disposal

- All biological waste and hazardous waste must be disposed of according to current DRO policies and procedures. All radioactive waste must be disposed of according to Radioaction Safety Committee policies and procedures. Boxes and trash must not be left in corridors. Prior arrangements for regular trash must be made with Custodial Services.

- Chemical waste must be labeled with hazardous waste stickers regardless of whether or not they are labeled from the manufacturer.

- Unwanted, unopened, or uncontaminated chemicals shall be offered to other labs that may be able to use them before the chemicals are considered for disposal.

- Any unknown chemical must be identified and labeled as hazardous waste. For chemical unknowns that cannot be identified by the Principal Investigator, Laboratory Director or laboratory personnel, the laboratory may be assessed a service fee for hazardous waste analysis prior to disposal.

- Darkroom tanks shall be drained and the contents disposed of as hazardous waste. Empty compressed gas tanks must be returned to the distributor prior to the move.

Mercury thermometers shall be disposed of as hazardous waste, and vacuum pumps shall be drained of oil and the oil disposed of as hazardous waste.

Procedures: Decontamination

- All laboratory bench-top surfaces must be decontaminated prior to vacating the laboratory, and all laboratory equipment that is either remaining in the laboratory or being moved to a new laboratory shall be decontaminated if potentially contaminated with biological, chemical, or radioactive materials.

- Lab equipment requiring decontamination includes, but is not limited to, animal cages, centrifuges, fermenters, fish tanks, incubators, water baths, refrigerators, and freezers (if not moving intact).

- Fume hoods must be decontaminated. Contact the Occupational Hygiene Technologist at (323) 563-4817 for decontamination and certification advice. Notify the Occupational Hygiene Technologist if there is any current or past practices that may reveal potential problems. Certain chemicals such as perchloric acid and mercury may remain on surfaces or equipment or in building systems.

- Biological safety cabinets and glove boxes that have been used with potentially infectious materials shall be decontaminated using paraformaldehyde gas before moving. This shall be done by a qualified outside contractor. If BSCs are either being moved to new laboratory areas or being left behind, contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 to discuss decontamination well in advance of the move. BSCs that are moved must be re-certified after installation.

- An appropriate disinfectant must be utilized in cases where biological materials were in use. A disinfectant is deemed appropriate if it targets the biological materials that were in use in the laboratory. In most cases, 70% alcohol, bleach solution (1:10 made fresh), or a phenolic disinfectant should be adequate for disinfection of lab furniture and equipment potentially contaminated with biological materials. Call the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 with questions or concerns.

- A *CDU Equipment Decontamination Record* sticker must be affixed to all equipment that has been decontaminated. This will allow moving personnel to safely move the equipment to the new laboratory space. Only equipment with this sticker shall be moved. Stickers may be obtained from DRO at (323) 563-5990.

- The Principal Investigator or Laboratory Director shall complete the “Laboratory Decontamination Certification Form” and submit the form to DRO when decontamination and decommissioning activities are completed. This will allow DRO personnel to review the decommissioning activities, visit the decommissioned

laboratory, and alert the appropriate administrative personnel that the decommissioning has been performed. Upon receipt of the completed form, DRO will contact the Principal Investigator or Laboratory Director to schedule a tour of the laboratory to confirm the decommissioning activities.

- For more information regarding proper disinfection or decontamination procedures, contact DRO at (323) 563-5990.

Procedures: Designation of New Laboratory Space

- The Principal Investigator or Laboratory Director shall inform DRO of any new laboratory space, so that the appropriate safety signage may be provided.

- The Principal Investigator is responsible for notifying all applicable CDU research committees and outside agencies, as necessary, of the move to new laboratory space. Research projects approved by the IBC must have updated laboratory location information. USDA Veterinary Service are laboratory site specific. Contact the Environmental Health and Safety (EHS) Specialist at (323) 357-3659 for assistance.

Procedures: Packing and Moving Laboratory Materials

- Laboratory personnel are responsible for collecting all packaging items needed before the move date. Carts, plastic bags, toweling, or other cushioning, absorbent materials, sealable plastic or plastic-lined boxes, labels (*e.g*., Fragile, Universal Biohazard, ID, Location, Caution, Radioactive Material), sturdy tape, and spill kits shall be readily accessible. Each container or piece of equipment must be labeled. Labels shall identify the agent, hazard, and necessary precautions.

- The Principal Investigator or Laboratory Director is responsible for establishing safety and emergency procedures for all phases of the move. Potential emergencies include material spills, fires, slips and falls, and cuts. Protective clothing and spill absorbent materials shall be available during packing, moving, and unpacking.

Procedures: Packing and Moving Laboratory Chemicals

- In order to minimize the amount of chemicals that need to be packed and moved, new chemicals shall be ordered only as necessary and in small quantities. Laboratory personnel should plan in advance to minimize the inventory of liquid volume and weight of materials being moved. In addition, reduction of active materials shall be planned the week prior to the move. Laboratory chemicals shall be packed and moved by an outside contractor approved by DRO. Prior to the packing and moving, laboratory personnel are responsible for labeling each chemical container with the chemical identity.

- **Compressed gas** tanks that are to be moved shall have regulators removed and caps secured prior to moving. If possible, have old tanks collected prior to a move and arrange for future tanks to be delivered to the new location.

- **Thermometers** shall be removed from refrigerators, water baths, and incubators prior to equipment moving.

- **Vacuum pump oil** shall be drained from pumps prior to equipment moving.

Procedures: Packing and Moving Biological Materials

- **Biological materials** shall be appropriately packed and moved by the laboratory personnel. Regulated materials and biological materials include all human, plant, and animal pathogens; all human blood, blood components and products, tissues and body fluids; all human and animal cultured cells; all animal carcasses and unfixed animal tissues; all cultures/stocks of biological agents including recombinant DNA materials; and all biological toxins.

- **Proper packaging** consists of a primary sealed container placed within a secondary sealed, unbreakable container, with enough absorbent material in between to contain and absorb any spill. ***Some examples of proper packaging include*** petri dishes in a plastic sleeve within a plastic-lined box using paper towel spacers; stabs in a sealed Tupperware container with paper towels to cushion vials; sealed tubes in a rack placed into plastic sealable container with enough paper towels to absorb any spilled contents; tissue culture dishes placed into a plastic-lined dishpan or a sealable cardboard box with an absorbent. Freezers can be moved intact, provided all contents are in sealed, unbreakable containers and the freezer remains closed. Because shifting of contents may occur, enclose loose items in boxes, or fix in some other way to avoid breakage and spills when the freezer is reopened. Other equipment, such as fermenters, refrigerators, incubators, and biological safety cabinets must be empty and decontaminated prior to the move.

- **Labeling:** Once packaged, all biological materials must be properly labeled. ***Labels shall include*** the name, Principal Investigator (PI), new location, ID of agent, biosafety level, telephone number for assistance in the event of any breakage, and a FRAGILE notice if applicable. Also the **universal biohazard label** shall be used whenever packaging a BSL-2 or higher agent. Questions concerning the biosafety level of biological materials or requests for biohazard labels shall be directed to the Environmental Health and Safety (EHS) Specialist at (323) 357-3659.

Procedures: Laboratory Furniture and Equipment

- **Furniture:** The Move Coordinator shall be informed if there is any furniture of particular concern (fragile, valuable, requires dismantling) not already mentioned. Different moving companies may have different requirements that shall be ascertained in advance of the move.

- **Special Requirements:** The Move Coordinator shall be informed in advance of any equipment under service contract, as well as equipment not under contract but requiring servicing and/or special handling.

- **Alarms:** Laboratory personnel shall disconnect alarms on freezers (if moving intact) and any other sensor alarms on or before the day of the move.

- **Keys and Combinations:** Laboratory personnel shall keep keys and combinations to locks readily accessible.

Appendix M

Laboratory Door Signage

Please see Global Harmonization Manual and Radiation Safety Manual.

Appendix N

Institutional Biosafety Committee Standard Operating Procedures

Various regulatory agencies with oversight of research activities involving the use of etiologic agents or recombinant DNA, funding agencies, and CDU policies require that a comprehensive, ongoing inspection and audit program be in place to review the compliance record of the users and the facility.

This includes:

- The review of procedures to ensure compliance with the terms of approved protocols (*e.g.*, biological materials, animals)
- Inventory controls
- General facility conditions
- Training of individuals engaged in research
- Other specific mandates required by the particular agency or IBC

Oversight

The IBC has responsibility for the oversight program that will fall under one of the three broad categories defined below:

Inspections

Inspections are conducted as a result of a specific issue or concern and could be prompted by receipt of a complaint, request from a regulatory agency, or the Institutional Biosafety Committee. All these instances will be investigated in accordance with the protocols established by IBC and the results will be reported to the IBC.

The IBC's chair or the vice chair and Director of Research Integrity and Compliance will be notified immediately at the initiation of any inspection. Upon notification, the IBC chair or vice chair will review the nature of the event leading to the investigation and determine if any immediate action is required. Such actions might include, but are not limited to:

- Establishment of a sub-committee to participate in the inspection or to discuss the violation before the next convened full committee meeting.
- Temporary suspension of activities or closure of the facility.
- Other actions as necessary.

Audits

Audits are part of the routine quality control program during which staff conduct ongoing audits of approved protocols.

The frequency, extent, and content of the audits will vary depending on the specific protocol being audited and will be developed by the IBC and the Environmental Health and Safety (EHS) Specialist.

At the end of each audit, the staff will:

- Discuss their findings with the PI or the alternate responsible person named in the protocol when appropriate. The discussion will include any corrective actions needed.
- Send, within five working days, the PI or the alternate responsible person a written report describing any findings, corrective actions required, and the deadline for a written response.
- Determine, upon receipt of the responses from the PI, if a follow-up visit is necessary to conclude the audit. In the event of failure by the PI to respond to the report in a timely manner, staff will contact the PI by phone or in person.
- Determine the type and severity of the findings and corrective actions taken.
- Report the findings to the committee.

Lab Review

Reviews are site visits conducted to observe certain procedures or activities and may be requested by the IBC or the PI. In general, the purpose of these reviews is to observe an activity (*e.g.*, a PI is starting a procedure that he or she has not conducted before) and provide feedback to the IBC or a PI. Depending on the nature of the request, these reviews are often excellent forums for training and may or may not require a formal report to the IBC.

IBC Review and Enforcement

At the conclusion of an inspection or audit, the Environmental Health and Safety (EHS) Specialist, or designee, will report the findings to the IBC, or the sub-committee if one was appointed, for review and action. The report will include any corrective actions taken or in progress.

The IBC will review the findings and determine the appropriate corrective actions depending on a number of factors, such as the severity of the infractions, nature of the violation, or the history of PI and/or laboratory compliance.

In general, the IBC views the violations as:

- *Major deviations:* These are the type that have the potential for causing health or safety problems and may include deviations such as failure of monitoring; departure

from approved protocol; use of unapproved biological agents; unauthorized removal of agents; repeat history of violations within the laboratory, etc.

- *Moderate Deviations*: These are the type that are typically first time deviation that are either major administrative or have a likelihood of causing minor health and safety problems and may include personnel qualified but not added to protocol, missing inventory records, QC not performed in a timely manner, etc.
- *Minor Deviations*: These are generally of the type that are administrative in nature and have insignificant potential for causing health or safety problems and may include incomplete records (*e.g.*, missing dates or initials), posting infractions, etc.

Enforcement

In any category, the PI will be given a deadline to respond to the IBC report with an explanation of the reason for failure and plans for correction and/or protocol modification as necessary.

Note*: The PI has an opportunity to present his or her case to the full IBC should he or she so desire.*

After review of the inspection report and the PI's response, and after reviewing the facts surrounding the violation, the IBC will take appropriate corrective action and may impose sanctions.

This action may range from, but is not limited to:

- Requiring more frequent laboratory inspections and/or monitoring.
- Mandated additional training.
- Requiring the PI and/or authorized users to re-take the user certification test.
- Permanent termination of the protocol.
- Placing the PI on probation for a period of time.
- Removing certification of certain individuals who were responsible for a major violation including repeat offenders
- Suspension of the approval of the protocol.*

* Only the IBC can authorize reinstatement. In making a finding regarding reinstatement, the IBC will consider the PI's corrective actions (taken or planned) and the results of an additional inspection.

Note*: **The IBC has been given full authority to suspend any activity that is judged to be:***

- Unapproved
- A clear violation of the approved protocol or regulatory requirements
- Have adverse health or environmental impacts

Notification

At the initiation of any inspections, the following notifications must be done immediately:

- Director of Research Integrity and Compliance, who will initiate any agency notification necessary.
- IBC chair or vice chair, who will determine immediate actions required.
- Occupational Health Officer, if there is a potential for employee exposure. The Occupational Health Officer will initiate relevant health agency notifications.

Appendix O

Criteria for Development of Laboratory Standard Operating Procedures (SOP)

In this Manual, there are a number of sections where the laboratory is required to prepare standard operating procedures (SOP). This appendix is intended to provide guidelines on the development of such documents. It is not mandatory to follow these procedures.

Introduction

What is a Standard Operating Procedure (SOP)?

A standard operating procedure (SOP) document is a comprehensive set of instructions written to provide employees with guidelines to follow to complete a job safely. SOP should be written in a manner that provides the user with a clear set of guidelines that ensure the task is performed as desired by the institution and that meet regulatory compliance standards. Institutions write SOPs for the following reasons:

- to provide individuals who perform operations with all the safety, health, environmental, and operational information required to perform a job properly;
- to ensure that operations are done consistently to maintain quality control of processes and products;
- to ensure that processes continue and are completed on a prescribed schedule;
- to ensure that no failures occur in manufacturing and other processes that would harm employees or anyone in the surrounding community;
- to ensure that approved procedures are followed in compliance with company and government regulations;
- to serve as a training document for teaching users about a process;
- to serve as a historical record of the how, why, and when of steps in a process for use when modifications are made to that process and when a SOP must be revised;
- to serve as an explanation of steps in a process that can be reviewed in incident investigations that seek to improve safety practices and operating conditions.

Purpose and Scope of this Document

The purpose of this document is to provide guidance and a template for drafting SOPs.

Developing a SOP

Except for the simplest operations, a SOP must be developed for each of the operations for reasons described above. A SOP is best developed by a team that includes the worker, the job supervisor, a safety and health professional, etc. When an SOP has been properly written, the result is satisfactory completion of the work with regard to efficiency, risk, and safety.

The first step in preparing to write a SOP should have the worker demonstrate how he or she will accomplish a particular procedure. The worker must be someone who is already doing that job or who has done similar work. The supervisor acts as an advisor to monitor the required efficiency and contributes necessary information about the correct use of the equipment involved. The safety person notes the hazards of the job and lists the protective equipment that should be required.

The SOP should include identifying information (*e.g.*, title and/or number) and all the procedure's steps, including associated hazards and precautions. Precautions for the employee's overall health and safety must be addressed, especially in terms of training and personal protective equipment and what to do in emergencies. The SOP also must address the precautions needed to prevent any impacts to the environment, whether it is the immediate workplace environment, the waste disposal system, or the surrounding community.

Note*: Detailed information does not need to be provided on some of the areas where there is already another document describing the procedure. For example, when describing the operation of a particular piece of equipment, a notation could be included that refers to the operating manual for that equipment or another SOP describing the operation. In these instances, it is important to ensure that these referenced documents are readily available*.

SOP Template

No standard SOP templates exist nationally, so each institution develops its own. The template presented at the end of this SOP is compliant with the requirements of Good Laboratory Practices (GLP) and is strongly recommended for use by others.

General Information

This is the top section of the SOP template and includes information about:

- *Unit*: The unit that develops and owns the SOP.
- *SOP Title*: The full title of the SOP.
- *SOP number*: Based on a standard numbering system.
- *Version*: The version of the SOP with V1.0 as the initial number and each subsequent revision having a new number such as V1.1.

- *Implementation Date*: The date the current version of the SOP went, or will go, into effect.
- *Approval*: The name and title of the individual who is responsible for approving the SOP. The responsible individual must sign or initial this section to indicate approval.
- *Page Number*: Indicates the page number using the notation "Page 1 of X."
- *Expiration date*: If the SOP is for a given operation that is for a specific duration, it should indicate that date; otherwise it should note "*until revoked.*"

Purpose and Scope

There should be brief statement on the purpose and scope of the SOP.

References

This section should list any additional resources that may be useful in performing the procedures. These may include:

- **Regulations:** Regulatory references should be listed here.
- **Policies:** All relevant CDU policies should be listed here.
- **Other SOP:** SOP referred to in any other section of this SOP should be listed here.
- **Supplementary Documents**

Definitions

Definitions for the major terms used in the SOP should be included to provide the reader a clear understanding. Spell out the acronyms fully and show the abbreviations in parentheses; this format should also be followed each time a new term is introduced in any part of the SOP.

Roles and Responsibilities

If the particular procedures require that individuals from various sections participate, the roles and responsibilities for each should be clearly defined. For example, if a procedure requires a cage washer to deliver clean cages to the rooms before the technicians responsible for changing the cages can do so, define the roles. If Environmental Health and Safety (EHS) Specialist must perform hazard evaluation as part of the SOP, the Environmental Health and Safety (EHS) Specialist's role and responsibility should be defined.

Special Requirements

- **Equipment and Supplies Required**

This section should list all equipment and supplies needed for performing the task or procedures. In a SOP with extensive supply list, it might be more appropriate to include the supplies in the description of each procedure.

- **Safety Requirements**

  This section should define all health, safety, and environmental protection measures that must be followed while performing the procedures, including spill and accident response procedures relevant to the particular operation defined in the SOP.

- **Training**

  Clearly define all the training requirements (i.e., courses), including the schedule for training (*e.g.*, prior to the start of performing the procedures), re-training frequency, and how to obtain the courses.

- **Monitoring Requirements**

  This section should define the need, frequency, and methods of conducting personnel or environmental monitoring.

- **Personnel Protective Equipment (PPE)**

  List all the PPE required for performing this task, identifying which are mandatory and which are recommendations for further enhancing employees' health and safety.

- **Medical Surveillance**

  Clearly define the medical surveillance requirements for the procedures, if any.

- **Other Prerequisites**

  List any other prerequisites that exist for performing the procedures. These could include requirements for being familiar with companion polices, professional or special operating permits, etc.

Applicable Locations

List all locations where this SOP is applicable to (*e.g.*, all barrier facilities, all research laboratories, areas where hazardous chemicals are used, Rooms 111, 222 and 333 only, etc.)

Procedures or Instructions

This is the most important component of the SOP and requires a complete and step-by-step description of how the function should be performed.

When developing this section, consider the possibility of using the document as a training tool for new employees. Therefore, the details included should be such that after reading the document, a new employee could obtain a high level of understanding of how the function is performed.

Include the equipment used as part of this section and reference any SOP or operating manuals required.

Note*: Some SOP might include a listing of all equipment used at the start of this section.*
The title of any manufacturer's manuals, good practices, and professional organization guides, available or used in this procedure, should be listed here. The location of these documents should also be noted.

Note*: To avoid the need for frequent updating of the procedures, each program should designate a permanent location that acts as a reference library*.

Forms

The SOP should include all the forms required by the SOP. It is recommended that:

- All forms are included as attachments to the main SOP with a clear reference in the "Procedures and Instruction" or "Record Management" sections. This will make revisions of the SOP simpler if forms are changed.

- A form numbering system is established that correlates to the SOP numbers.

Record Management

This section incorporates record management practices, including location of active records, archived records, and record retention times.

SOP Revision History

It is extremely important to track the history of the SOP and document all its revisions. This expectation should be integral part of all SOP development and maintenance processes.
The SOP should be reviewed by the team that created it:

- When there is a change in regulatory requirements
- Operating procedures have changed significantly
- Forms used or the record management system has changed Introduction of new facilities, equipment, risks, hazards, or processes

- At least annually

The following pages provide a template for use.

Unit:	**SOP #:** **Revision #:** **Current Version:** **Implementation Date:**
Page #:	**Last Reviewed/Update Date:**
Expiration Date:	**Approval Authority:**
SOP Titles:	

Version	Section/Paragraph Changed	Changes Made	Effective Date
V.1	N/A	None, Original Version	