Biosafety Manual

Prepared for:

Office of Environmental Health and Safety
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LIST OF ABBREVIATIONS AND ACRONYMS

BBP	bloodborne pathogens
BL	biosafety level
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BPHC	Boston Public Health Commission
BSC	biological safety cabinet
BSO	biosafety officer
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CMR	Code of Massachusetts Regulations
DNA	deoxyribonucleic acid
DOT	U.S. Department of Transportation
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GMMO	Genetically Modified Microorganism
HBV	hepatitis B virus
HCV	hepatitis C virus
HEPA	high efficiency particulate air
HIV	human immunodeficiency virus
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
NIH	National Institutes of Health
NSF	National Sanitation Foundation
OEHS	Office of Environmental Health and Safety
OPIM	Other Potentially Infectious Material
ORSP	Office of Research and Sponsored Programs
OSHA	U.S. Occupational Safety and Health Administration
PHS	U.S. Public Health Service
PI	principal investigator
PPE	personal protective equipment
rDNA	recombinant DNA
RSO	Radiation Safety Officer
USDA	U.S. Department of Agriculture
UMB	University of Massachusetts Boston
°C	degrees Celsius

CONTACT INFORMATION AND USEFUL WEBSITES

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Facilities Department	617-287-5450	https://www.umb.edu/facilities	24 hours
Department of Public Safety	911 (campus phone) 617-287-1212 (cell)	public.safety@umb.edu https://www.umb.edu/public_safety	24 hours
University Health Services Quinn Administration Building, second floor	617-287-5660	https://www.umb.edu/healthservices	8:30 am to 5:00 pm
Boston Medical Center, Emergency Department	617-414-4075	http://www.bmc.org/	24 hours

WEBSITES

Department or Organization	Contact For
University of Massachusetts Boston Institutional Biosafety Committee (UMB IBC)	https://www.umb.edu/orsp/research_committees/ibc
University of Massachusetts Boston Institutional Animal Use and Care Committee (IACUC)	https://www.umb.edu/orsp/research_committees/iacuc
University of Massachusetts Boston, Office of Research and Sponsored Programs (ORSP)	https://www.umb.edu/orsp
National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA and Synthetic Nucleic Acid Molecules (NIH Guidelines)	https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf
NIH/Centers for Disease Control and Prevention (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL)	http://www.cdc.gov/biosafety/publications/index.htm
OSHA Bloodborne Pathogen Standard	http://www.osha.gov/SLTC/bloodbornepathogens/index.html
Public Health Agency of Canada – Pathogen Safety Data Sheets	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php
American Biological Safety Association	www.absa.org

1.0 INTRODUCTION TO BIOSAFETY

1.1 BACKGROUND

Work with biological materials comprises a wide variety of routine activities in many biomedical research and biotechnology laboratories. Exposures to potentially infectious materials during many of these activities can present potential health hazards to laboratory staff. Development of new products from cells and tissues for therapeutic use, isolation and identification of genes, and introduction of genes into cells, tissues, microorganisms, plants, and animals are all current and expanding biotechnologies. However, these routine activities may place laboratory staff at increased risk for exposure to bacteria, fungi, viruses, viral vectors, recombinant deoxyribonucleic acid (rDNA), and biological organisms containing rDNA.

Biosafety is defined as a group of practices and procedures designed to provide a safe environment for individuals who work with potentially hazardous biological materials. The primary goal of biosafety is to eliminate exposures to these materials through the use of containment. The term containment refers to safe methods for managing potentially infectious materials in laboratory environments. Containment includes both primary containment (e.g., good microbiological techniques and safety equipment) and secondary containment (e.g., the design and operation of the laboratory facility).

Two government agencies, the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC), have developed biosafety guidelines that provide the foundation for this manual. They are designed to protect laboratory personnel and individuals in the surrounding community and are described in two publications.

The first is the *NIH Guidelines for Research Involving Recombinant DNA and Synthetic Nucleic Acid Molecules* (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf). The second is the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), which is published jointly by the CDC and the NIH (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf); the most recent edition was published in 2009. These two publications classify work with biological agents into four distinct biosafety levels (BLs). Each of these levels is matched with progressively restrictive practices and laboratory design features that reduce health risks from exposures to potentially hazardous biological agents. These levels are further discussed in Section 3.

1.2 REGULATIONS

Federal, state, and local agencies have developed regulations for protecting laboratory workers and the general public from the potential health hazards associated with the use of biological agents in laboratories. Some of these regulations, such as those from the U.S. Occupational

Safety and Health Administration (OSHA), have the force of law, while those from NIH and CDC are recommended guidelines but may be mandatory if the institution receives federal funding and/or is located in a city where there is a requirement for compliance. As part of the grant application process, many federal and private granting agencies require applicants to certify that they adhere to all federally mandated requirements and guidelines.

1.2.1 Federal

Laboratory workers who come in contact with human blood or other human bodily fluids are at increased risk for exposures to and infections from bloodborne pathogens (BBP), such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). The OSHA Bloodborne Pathogens Standard (Title 29 Code of Federal Regulations [CFR] Section 1910.1030) was designed to eliminate or minimize occupational exposure and the risk of developing infectious diseases associated with blood and other potentially infectious material (OPIM). All laboratories that work with human blood, human tissues, human cells, certain specific human bodily fluids, and non-human primate derived material must adhere to the OSHA BBP Standard (http://www.osha.gov/SLTC/bloodbornepathogens/index.html) and the University of Massachusetts Boston (UMB) Exposure Control Plan.

The use of Universal Precautions is a key element of any BBP program and must be followed at all times when working with human and non-human primate derived materials. Universal Precautions means that all human samples are treated as potentially infectious. For example, blood from any source, including HIV-seronegative control donors, must be handled as potentially infectious. Employees are trained in Universal Precautions techniques during their environmental health and safety training. All laboratory personnel working with OPIM are required to take online BBP training module.

Safe practices for studies involving the use of rDNA and synthetic nucleic acid molecules are governed by the NIH Guidelines (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf). It is UMB policy that all laboratories comply with these Guidelines, which is also a Boston Public Health Commission requirement.

1.2.2 Commonwealth of Massachusetts

Regulations from the Commonwealth of Massachusetts (Title 105 Code of Massachusetts Regulations [CMR] Part 480.000—Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code Chapter VIII) (http://www.mass.gov/eohhs/docs/dph/regs/105cmr480.pdf) primarily focus on the management of biological waste. The principal issues deal with what constitutes biological waste and how to dispose of it properly. Overall, the state statutes agree with the NIH and CDC definitions of biological waste.

1.2.3 City of Boston

All rDNA work conducted in the city of Boston is subject to the NIH rDNA Guidelines through the Boston Public Health Commission (BPHC). This Ordinance is enforced by the UMB Institutional Biosafety Committee (IBC). In general, the requirements set forth in the city ordinance agree with NIH and CDC guidelines. Further information can be found at the URL for BPHC http://www.bphc.org/whatwedo/healthy-homes-environment/biological-safety/Pages/Biological-Safety.aspx

1.3 UNIVERSITY OF MASSACHUSETTS INSTITUTIONAL BIOSAFETY COMMITTEE

The UMB has an IBC for its researchers engaged in biological research. The purpose of the IBC is to protect the health of researchers and the community by assuring that biological research is conducted in compliance with federal, state and local laws. The IBC conducts specific review and oversight of biological research activities in compliance with the following guidelines and regulations:

- NIH—*NIH Guideline for Research Involving Recombinant and Synthetic Nucleic Acid Molecules (NIH Guidelines)*
- U.S. Occupational Safety and Health Administration—OSHA Bloodborne Pathogen Standard 1910.1030
- Massachusetts Department of Public Health—Medical Waste Regulation
- Boston Public Health Commission—Biological Safety

Additional information on the responsibilities of the IBC can be found on their website https://www.umb.edu/orsp/research_committees/ibc

1.3.1 Registering Research Projects with the IBC

All biological research involving the use of recombinant and synthetic nucleic acid molecules, biological agents, human and nonhuman primate materials, and biological toxins must be registered with the IBC. Projects are registered by completing an IBC Research Registration Form, which can be accessed on the Office of Environmental Health and Safety (OEHS) and Office of Research and Sponsored Programs (ORSP) websites. The completed form must then be sent via email to OEHS at UMBEHS@UMB.edu.

Each completed registration will be reviewed by the Biosafety Officer (BSO) and/or the IBC Chair, who may request additional information or clarification from the submitter or principal investigator (PI). The review will include a determination regarding which section of the NIH rDNA guidelines apply to the research. If the research falls under Sections III-A through III-D of the NIH Guidelines, it must be reviewed at a convened meeting of the IBC. For additional details on the review process, please contact the BSO.

1.4 RESPONSIBILITIES

The following section outlines the specific responsibilities associated with the UMB biosafety program.

1.4.1 Principal Investigator

PIs are responsible for implementation of all applicable biosafety procedures and practices in their laboratories. They must ensure that appropriate equipment, including personal protective equipment, and facilities are available for laboratory staff members and are used properly. They must also arrange for appropriate employee training regarding the safe use of potentially hazardous biological agents and require that all individuals handling BBP receive the annual training mandated by OSHA. Each PI must be aware of the potential adverse health effects of the biological materials used in his or her laboratory, the appropriate biosafety level, and any other pertinent factors that will ensure the safety of laboratory staff members and the surrounding community.

In addition to the above, when research involves the use of recombinant or synthetic nucleic acid molecules, the PI agrees to abide by the NIH Guidelines. Under the NIH Guidelines, the PI has a number of specific responsibilities, including the following:

- Ensure that the IBC is notified prior to beginning any work with biological materials.
- Report any significant problems, violations of the NIH Guidelines, or any research-related accidents, illnesses, or potential exposures to the UMB BSO or the IBC.
- Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents. Instruction may be required when procedures are changed, new procedures are implemented, or when accidents occur. This instruction should be specific to the agents and materials used in the research project.
- Make available to all laboratory staff protocols that describe the potential biohazards and the precautions to be taken with the agents to be used.

Additional responsibilities of the PI when working with recombinant or synthetic nucleic acid molecules are located in the NIH Guidelines (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf). failure to comply with the NIH Guidelines by one PI could affect all NIH-funded projects at UMB; therefore, compliance is absolutely mandatory.

1.4.2 Laboratory Staff Responsibilities

Laboratory staff members are responsible for following the UMB health and safety policies and the procedures and instructions from their PIs and BSO. They need to comply with all NIH, CDC

and OSHA regulations, use safe laboratory practices, and inform the PI, laboratory supervisor, BSO, or other UMB OEHS team member regarding any potentially hazardous situations or conditions.

1.4.3 Biosafety Officer

The BSO is the primary intermediary between investigators and the IBC. BSO responsibilities include:

- Managing the biosafety program and implementation of applicable policies and procedures at UMB.
- Assisting laboratories in conforming to pertinent regulatory guidelines and applicable UMB policies by providing training, facility inspection, and communication of program requirements.
- Performing annual inspections of laboratory containment, procedures, records, and equipment for laboratories using BL1, BL1-N, BL2, BL2-N, BL2 with stipulations (a.k.a. BL2+) practices and procedures.
- Screening research protocols proposed by PIs and submitting to the IBC for approval. The BSO will determine whether more information is necessary and, if so, will communicate this need to the PI. Once the revised application is complete, the BSO completes a risk assessment for the IBC summarizing the salient characteristics of the study and recommending an appropriate biosafety level to reviewers and/or the full committee.
- Reporting to the IBC on the program status.

In addition, the BSO is responsible for:

- Preparing a Biosafety Officer Memorandum to the PI explaining the requirements associated with their IBC recommendations.
- Providing advice on safe methods for new procedures.
- Recommending emergency response procedures in the event of an infectious spill or an exposure to a biological material.
- Summarizing the results of the biosafety inspections of laboratories in biosafety reports.
- Distributing biosafety report results to the laboratory biosafety contact and PI.
- Acting as the liaison between the IBC, IACUC, and researchers.

2.0 HAZARD ANALYSIS/RISK ASSESSMENT

In order to determine which practices and procedures are required when working with biological materials, a risk assessment should be conducted. At a minimum, the risk assessment should include the following:

- Pathogenicity and infectious dose of the biological material
- Consideration of the outcome of an exposure
- Natural route of exposure
- Other routes of exposure (parenteral, airborne, ingestion, etc.)
- Stability of biological material in the environment
- Concentration of biological material and amount to be manipulated
- Presence of a suitable host
- Information available from animal studies and reports of laboratory-acquired infections or clinical reports
- How the biological material will be used (concentration, sonication, aerosolization, centrifugation, etc.)
- Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens
- Local availability of effective prophylaxis or therapeutic interventions

2.1 LIMITED INFORMATION

There are situations when the information may be insufficient to perform a risk assessment. For these situations, the following conservative approach must be used:

- Universal precautions must be followed, and barrier protections applied (gloves, gowns, eye protection), regardless of the origin of the samples.
- Biosafety level 2 will be the minimum requirement for the handling of specimens.

2.2 BIOLOGICAL EXPRESSION SYSTEMS

Since biological expression systems consist of vectors and host cells, the following should be considered.

- The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the organism.
- Inserted DNA sequences are not well characterized, e.g., during the preparation of genomic DNA libraries from pathogenic microorganisms
- Gene products may have potential pharmacological activity
- Gene products may code for toxins

2.3 GENETICALLY MODIFIED MICROORGANISMS

When a PI proposes to work with genetically modified microorganisms (GMMO), the characteristics of donor and recipient/host organisms should be considered. In addition, consider the hazards:

- Arising directly from the inserted gene (donor organism):
  - Toxins
  - Cytokines
  - Hormones
  - Gene expression regulators
  - Virulence factors or enhancers
  - Oncogenic gene sequences
  - Antibiotic resistance
  - Allergens

- Associated with the recipient/host
  - Susceptibility of the host
  - Pathogenicity of the host strain, including virulence, infectivity, and toxin production
  - Modification of the host range (i.e., tropism)
  - Recipient immune status
  - Consequences of exposure

- Arising from the alteration of existing pathogenic trails
  - Is there an increase in infectivity or pathogenicity?
  - Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?
  - Does the foreign gene encode a pathogenicity determinant from another organism?
  - If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMMO?
  - Is treatment available?
  - Will the susceptibility of the GMMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?
  - Is eradication of the GMMO achievable?

3.0 PRINCIPLES OF BIOSAFETY

The BMBL classifies work with biological agents into four distinct BLs that have increasingly restrictive practices and facilities. Each BL designation is based on the potential health risks for individuals handling the biological materials. The four BLs and the associated risks for individuals and community members including BL2 with stipulations (a.k.a. BL2+) are summarized in Table 3.1.

Table 3.1 Biosafety Level Classifications for Biological Agents

Biosafety Level	Risk Group	Examples
BL1	Individual risk: LOW Community risk: LOW	*Escherichia coli* K12 (lab strain) Adeno-associated viruses
BL2	Individual risk: MODERATE Community risk: LOW	*Streptococcus* *Staphylococcus* Hepatitis B and C viruses Adenoviruses Most retroviral and lentiviral vectors
BL2 with stipulations (a.k.a. BL2+)	Individual risk: MODERATE Community risk: LOW	Human immunodeficiency virus Oncogenic inserts Prions
BL3	Individual risk: HIGH Community risk: MODERATE	Mycobacterium tuberculosis West Nile virus
BL4	Individual risk: HIGH Community risk: HIGH	Ebola virus

Note: Biosafety Level 3 and 4 work is currently not permitted at UMB.

Appendix A contains specific information drawn from the BMBL concerning BL1 and BL2.

Laboratory work at UMB is conducted using BL1, BL2, and BL2+ containment and procedures. There are no BL3 and BL4 laboratory facilities on the UMB campus.

3.1 BIOSAFETY LEVELS 1 AND 2

BL1 is applicable to work involving well-characterized agents not known to consistently cause disease in healthy adult humans; these agents present minimal potential health hazards to laboratory personnel and the surrounding community. BL2 is recommended for work involving agents that present moderate potential health hazards to laboratory personnel and the surrounding community. BL2 includes all of the practices and procedures of BL1 and then builds upon these guidelines. Table 3.2 provides a brief summary of the biosafety level criteria for BL1 and BL2.

Table 3.2 Summary of Biosafety Level Criteria for BL1 and BL2

Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
BL1	Not known to consistently cause disease in healthy adults.	Standard Microbiological Practices.	Personal protective equipment (PPE) includes laboratory coats; gloves; eye protection as needed.	Open bench top. Sink required.
BL2	Associated with human disease. Potential hazards from percutaneous injury, ingestion, and mucous membrane exposure.	BL1 practices plus: • Limited access • Biohazard signs • PPE • Sharps precautions • Biosafety manual that defines any biological waste decontamination policies.	• Primary barriers include Class I or II biosafety cabinets or other physical containment devices for all manipulations of agents that cause splashes or aerosols of infectious materials. • PPE includes laboratory coats; gloves; face protection as needed.	BL1 plus: • Method of disinfection (i.e., chemical or autoclave) must be available.

BL biosafety level

3.2 BIOSAFETY LEVEL 2 WITH STIPULATIONS

BL2 with stipulations (BL2+) includes work that is performed in a BL2 facility using BL3 procedures and work practices, including the appropriate safety equipment (safety centrifuge cups, biosafety cabinets, disposable labware, etc.). BL2+ affords a greater margin of safety for personnel in instances when BL3 containment is not required.

BL2+ is used when working with infectious agents that may cause serious illness, but that do not have a documented aerosol route of exposure. This containment level may also be suitable for activity with agents where there is insufficient information available about the agents in question and/or about worker safety when using these agents.

Biological agents that may require BL2+ conditions may include HIV and viral vectors expressing oncogenes and toxins. BL2+ is defined as the use of BL2 practices plus selected BL3 practices such as but not limited to:

- The BL2+ laboratory must be self-contained. If BL2+ materials must be transported outside of the BL2+ laboratory, they must be kept in sealed secondary containment.
- Strict needle and sharps precautions must be observed. Plastic is substituted for glass whenever possible.
- All work must be done in a biosafety cabinet.

- Sealed rotors and centrifuge safety cups must be used when centrifuging BL2+ materials and the rotors/cups must be opened inside a biosafety cabinet within the BL2+ laboratory.
- Vacuum lines must be protected with high efficiency particulate air (HEPA) filters.
- Gloves (2 pair), closed-front gowns, and safety glasses must be worn.
- Solid and liquid waste materials must be autoclaved prior to disposal, unless they contain material(s) that may cause a hazardous situation when autoclaved (e.g., bleach, phenolics, radioactive isotopes or materials that release formaldehyde gas from formaldehyde containing waste streams). The initial risk assessment conducted by the BSO will include recommendations for managing laboratory waste streams. Any changes to laboratory protocols that could change the hazards associated with this waste must be reported to the BSO, and an additional risk assessment will be conducted.
- Before beginning *in vitro* work with replication incompetent vectors, the PI must provide the IBC with a protocol for testing the viral vector preparations for replication competence. The sensitivity of the assay must be indicated.

4.0 LABORATORY PRACTICES

4.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) is an essential element in biosafety and must be provided to all employees free of charge. PPE provided includes, but is not limited to:

- Gloves
- Laboratory coats (impervious)
- Face shields/masks
- Safety glasses with side shields
- Prescription safety glasses with side shields
- Goggles
- Hoods
- Sleeve covers
- Shoe covers
- Respiratory protection (through the UMB Respiratory Protection Program)
- Other site-specific personal protective equipment

At a minimum, laboratory, personnel shall wear gloves and a laboratory coat whenever handling biological agents, cells and tissues. Safety glasses with side shields, goggles, or face shield shall be worn when manipulating these materials in such a manner that droplets could form and/or materials splashes could occur, or if the agent in use can be easily transmitted through ocular exposure. Laboratory personnel should wear other PPE (apron, face shield, mask, etc.) as needed or required to prevent potentially infectious materials from reaching their clothes, skin, eyes, mouth, or other mucous membranes. PPE must be removed prior to leaving the work area and placed in designated areas. PPE must be treated as medical waste when discarded. If PPE is not disposable, PPE shall be cleaned with disinfectant before and after use.

For the UMB BL1and BL2 laboratories, a laboratory coat, gloves and safety glasses must be worn when handling biological materials. For the BL2+ laboratories, a disposable solid front gown, gloves and eye protection must be worn in working in the laboratory and by all persons who enter the laboratory when work is in progress. Contact the BSO if you have any questions on selection, use and disposal of PPE.

4.2 BIOLOGICAL SAFETY CABINETS

Biological safety cabinets (BSCs) provide a primary level of containment for working safely with potentially hazardous biological materials. When combined with good microbiological practices, BSCs can provide protection to laboratory personnel, the environment, and products being manipulated.

BSCs are designated as Class I, II, or III based on specific airflow patterns within the BSC and on the locations of HEPA filters within the unit (Table 4.1). HEPA filters are usually composed of a pleated sheet of borosilicate fiber material that has been treated with a wet-strength water-repellant binder. These filters are specifically designed to remove particles equal and greater than 0.3 microns with an efficiency of 99.97%. This filtration level will capture a majority of bacteria, spores, and viruses from the filtered air. Figure 4.1 illustrates typical airflow patterns in a BSC.

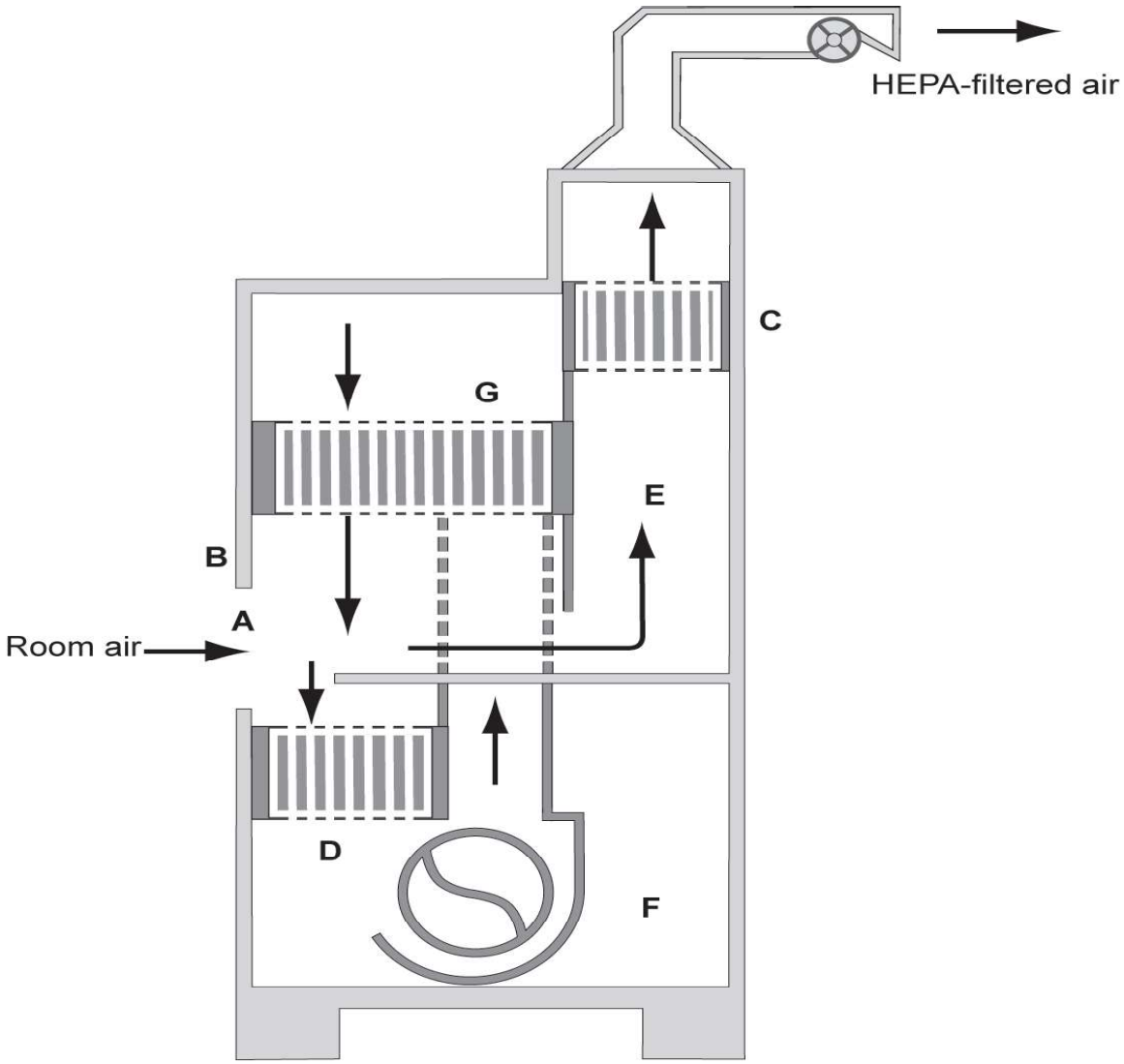
Table 4.1 Biological Safety Cabinet Characteristics[1]

New NSF Class and Type	Previous NSF Class and Type	Face Velocity (linear ft/min)	Airflow Pattern	Use of Volatile Toxic Chemicals and Radionuclides
A1	II, A	75	70% of intake air recirculated; 30% exhausted from a common plenum to the room. Plenum contaminated with biological materials under positive pressure.	No
A2	II, A/B3	100	70% of intake air recirculated; 30% exhausted from a common plenum to the room. Plenum contaminated with biological materials under negative pressure or surrounded by negative pressure.	Yes (small amounts[2])
A2	II, B3	100	70% of intake air recirculated; 30% exhausted from a common plenum to the room. Plenum contaminated with biological materials under negative pressure or surrounded by negative pressure.	Yes (small amounts)
B1	II, B1	100	40% of intake air recirculated; 60% exhausted from cabinet; exhaust air pulled through dedicated exhaust duct into facility exhaust system. All plenums contaminated with biological materials are negative to the room or surrounded by negative pressure plenums.	Yes (small amounts[2])
B2	II, B2	100	No intake air recirculated; 100% exhausted from cabinet. Exhaust air pulled through dedicated exhaust duct into facility exhaust system. All ducts and plenums are under negative pressure; all ducts contaminated with biological materials are under negative pressure or surrounded by directly exhausted negative pressure ducts or plenums.	Yes (small amounts[2])

NSF National Sanitation Foundation
ft/min feet per minute

1 Information from The Baker Company
2 Under no circumstances should the chemical concentration approach the lower explosion limits of the compound.

The Baker Company https://www.bakerco.com/introduction-biological-safety-cabinets



(Figure taken from *Biosafety in Microbiological and Biomedical Laboratories*, Fifth Edition, 2009)

Figure 4.1 The Class II, Type B1 Biological Safety Cabinet (classic design)
(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air.
Note: The cabinet exhaust needs to be hard connected to the building exhaust system.

Implementation of the following procedures will ensure optimal operation of a BSC:

- Front and rear grills should be free of clutter to allow proper air intake.
- Sash should not be raised above the specified level.
- Disinfect work surfaces before and after working in the BSC.
- Use slow and deliberate arm movements when moving hands/arms in and out of the BSC.
- Avoid Bunsen burner use to prevent airflow disruptions and damage to the HEPA filter.
- Wear PPE at all times for personal and product protection.
- Certification must be performed annually and whenever the BSC is moved or repaired.

BSCs are required to be tested and certified annually by technicians accredited by the National Sanitation Foundation (NSF). Additionally, BSCs will be certified when they are installed and whenever they are moved, even to a nearby laboratory, because the HEPA filters may be dislodged from their proper fitting during these moves. Please contact the UMB OEHS for additional assistance with BSC certifications.

4.3 DISPOSAL OF BIOLOGICAL WASTE

4.3.1 Biological Waste

Non-sharp biological waste may be treated and disposed of in a variety of ways:

- Designated biological waste box
- Chemical disinfection
- Steam sterilization/autoclave followed by depositing in the regular trash

The PI is responsible for selecting and using an appropriate disposal method for the biological agents in use in his or her laboratory. Follow the biological waste disposal guidelines posted in the laboratory. The BSO is available to provide technical advice. In addition, the IBC may require a certain method of decontamination for biological waste.

Non-sharp potentially infectious solid waste and solid waste containing recombinant or synthetic nucleic acid molecules that is not autoclaved must be disposed of in designated biological waste boxes. Each box is labeled with the universal biohazard symbol (Figure 4.2). Cardboard boxes must be lined with two red plastic bags to reduce the likelihood of leakage.

When a biological waste box is three-quarters (3/4) full, the bags must be individually sealed. Cardboard boxes must be sealed with two-inch-wide tape. Hard plastic boxes must have the lid flaps closed. **Do not overfill the boxes**. Boxes that leak any liquid or that weigh more than maximum allowable weight listed on the box will not be removed for disposal by the vendor.

Once the box is taped or closed, contact OEHS for pickup. The vendor picks up the closed boxes and removes them for treatment and disposal off campus.

Figure 4.2 Universal Biohazard Symbol

Biological waste that has been autoclaved in a **validated** UMB autoclave may be placed in a regular trash bag for disposal. Validation records must be maintained as well as a log of the waste that has been autoclaved. Contact the OEHS for advice.

Liquid biological and recombinant or synthetic nucleic acid molecule waste must be rendered non-infectious by steam sterilization or chemical disinfection prior to sink disposal. If chemical disinfection is selected, full-strength chlorine bleach recommended by OEHS may be added to the waste container, such as an aspiration flask, so that the **final** solution concentration of bleach will be 10%. Contact time should be at least 30 minutes prior to sink disposal. Best practice entails adding bleach **before** infectious waste is added to aspiration flasks so that waste is actively being rendered non-infectious.

Note: If bleach is not an adequate disinfectant for the biological agent in use, a U.S. Environmental Protection Agency (EPA)-approved disinfectant must be used. Ensure the proper contact time is met prior to disposal. Contact the OEHS for advice.

Prior to sink disposal, the pH of the disinfected solution must be checked to ensure that it is within the permissible pH range under the Massachusetts Water Resources Authority (MWRA) discharge permit (5.5 – 12.0 standard units). If it is within this range, then sink disposal should be done while the water is running in order to minimize possible plumbing damage due to the corrosive effects of the disinfectants. Autoclaving solutions containing bleach is **forbidden** due to the potential for production of toxic chlorine gas and damage to the autoclave.

4.3.2 Biological/Radionuclide Waste

Solid waste should be rinsed (glass or plastic) or sprayed (paper) with a suitable disinfectant. Follow the manufacturer's or regulatory recommendations regarding appropriate contact time for the disinfectant being used. After chemical disinfection, contact the Radiation Safety Officer (RSO) for additional guidance.

Liquid waste should be treated with a 1:10 dilution of household bleach for at least 30 minutes. Add concentrated bleach to the waste liquid until a final dilution of 1:10 is achieved. Evaluate the liquid waste for presence of radioactivity. Contact the RSO if help is needed in determining the best method for measuring radioactivity in liquids. If the levels are below Massachusetts Radiation Control Program sink disposal limits, it may be possible to dispose of the waste in a designated sink. Record the disposal. If the radioactivity exceeds the permissible sink disposal limits, contact RSO for further guidance.

4.3.3 Biological/Chemical Waste

Disinfect the infectious material with chemical disinfectant and dispose of as chemical waste. Select chemical disinfectants carefully because some disinfectants can react with chemicals. Consult the UMB OEHS if you have any questions.

4.4 SHARPS MANAGEMENT

Some of the most serious accidents in laboratories are those caused by puncture wounds through skin (percutaneous exposures). It is recommended to replace glassware with plastic whenever possible and select and use "safety sharps" such as retractable needles/syringes or self-sheathing scalpel blades. All objects that can puncture skin are designated as sharps and require special disposal treatment. Examples of sharps include but are not limited to hypodermic needles, glass Pasteur pipettes, razor blades, broken glass, and suture needles. **Massachusetts regulations classify any item that may cause punctures or cuts as a sharp, regardless of whether it is contaminated with a biological material.** Sharps must be disposed of separately from all other waste streams, and sharps containers cannot be mixed with other biological waste. All filled disposable sharps containers must be placed into a larger reusable secondary containment.

Federal regulations concerning sharps primarily focus on work with human bodily fluids. Because the majority of laboratory biohazard injuries are due to hypodermic needles, special attention has focused on their use and disposal. Some guidelines include:

- Minimize use of needles and syringes.
- Do not bend, shear, or break needles.
- Do not recap needles.
- Do not remove needles from syringes.
- Throw away the entire syringe-needle combination in the sharps container.
- Be careful during cleanup; some sharp items may be hidden in the waste materials.
- If you do stick yourself, wash the area, and then get medical attention immediately.

In 2001, in response to the *Needlestick Safety and Prevention Act*, OSHA revised the BBP Standard 29 CFR 1910.1030. The revised standard clarifies the need for employers to select safer needle devices and to involve employees in identifying and choosing these devices. The updated standard also requires employers to maintain a log of injuries from contaminated sharps. Further information can be found at http://www.osha.gov/SLTC/bloodbornepathogens/evaluation.html. The UMB laboratories are required to evaluate the use of safety sharps whenever possible, and if feasible, select safety sharps for use. Please refer to the UMB Exposure Control Plan for details and contact the OEHS.

4.4.1 Sharps Disposal

To protect yourself and others from injury from sharps, place all needles, Pasteur pipettes, syringes, suture needles, scalpels, and razor blades into standard sharps containers. Large volumetric/serological pipettes, or other items that can puncture biohazardous red bags should be disposed of in Sharps Boxes. **Please do not dispose of sharps that may contain mercury or other metals in sharps containers. Contact OEHS for proper disposal instructions**. Sharps containers must be red, fluorescent orange or orange-red leak proof, rigid, puncture-resistant, shatterproof containers that that are marked prominently with the universal biohazard warning symbol and the word "Biohazard" in a contrasting color. Place sharps containers in convenient locations near work areas so they will be used. **Do not overfill the sharps containers.** Containers should be sealed when they are three-quarters (3/4) full and should not contain any non-sharps waste. Contact OEHS for full sharps container pick up.

Figure 4.3 Sharps Container

4.4.2 Broken Glassware Disposal

Place clean broken glassware in broken glass box. All biological contaminated broken glassware should be placed into the sharps containers.

4.4.3 Pasteur Pipettes Disposal

Massachusetts law requires that Pasteur pipettes be considered as a sharps waste. Discard glass Pasteur pipettes directly into sharps containers; **do not** use cardboard broken glassware boxes or regular trash. Plastic pipettes and serological pipettes that could puncture the red waste bags should also be disposed of in sharps containers.

4.5 DISINFECTION AND DECONTAMINATION

Disinfection and decontamination are terms that are often used interchangeably, but they each have specific definitions. Disinfection is a chemical or physical treatment that destroys most biological agents, except spores. Decontamination refers to a chemical or physical treatment that destroys most biological agents to a low level, but not necessarily zero. A number of disinfectants are commonly used in laboratory settings, particularly to wipe down surfaces to remove infectious agents. Types of disinfectants and their uses are summarized in Table 4.2.

Table 4.2 Summary of Disinfectants and Their Uses

Disinfectant	Final Concentration	Effective On	Ineffective On
Sodium Hypochlorite Bleach: e.g., Clorox™*	1:10 freshly prepared	Bacteria, some spores, viruses, TB†, HIV	Some spores
Chlorine Dioxide: e.g., Clidox®-S*	*1:18:1~ (disinfection) or *1:3:1~ (sterilizing solution)	Bacteria, spores, viruses, TB	
Alcohols (ethanol, isopropanol)	70%	Bacteria, most viruses	Spores, TB
Quaternary Ammonium Compounds: e.g., D-125®*	Follow manufacturers' directions for dilutions	Bacteria, spores, viruses, HIV	
Phenolic Compounds; e.g., Vesphene®*	Follow manufacturers' directions for dilutions	Bacteria, viruses, TB, HIV	Spores

TB tuberculosis
HIV human immunodeficiency virus

* The use of brand names does not imply a recommendation.
† Use 1/5 dilution.
~ Please check the manufacturers' directions for specific dilutions.

4.6 AUTOCLAVING PROCEDURES

Autoclaves work by denaturing biological molecules with superheated steam; dry heat is not nearly as effective. For example, it takes 12 minutes to kill most spores with steam at 121 degrees Celsius (°C), while 6 hours are required with dry heat at the same temperature.

As a result, anything that does not come in contact with steam inside the autoclave may not be adequately decontaminated. The potential for inadequate decontamination becomes a greater concern when completely sealed biohazard bags are placed in an autoclave. Therefore, do not completely seal the autoclavable biohazard bag. Tie or secure the top of the bag loosely to allow steam to penetrate into the bag.

Typically, bags (24" x 36") of solid plastic waste take from 45 minutes to one hour to reach sterilizing temperatures throughout its contents. Each autoclave load should consist of a standard number of bags and this load should be validated with biological indicators.

In the research laboratory setting, the target organisms to be killed are usually known and they are usually heat sensitive. In practice, the same autoclave is used for sterilizing laboratory materials and waste. If sterilized materials are subsequently determined to be contaminated, it is an indication that the autoclave is not working properly.

The following tips will help prevent injury and property damage when using the autoclave.

- Do not overfill containers. Leave the top third as empty expansion space.
- Use only vented closures.
- Place contaminated materials in autoclave bags. Place bags inside shallow plastic or metal trays when autoclaving.
- Use only containers designed for sterilization. Use plastic or metal trays.

Bottles should be cool to the touch before attempting to remove them. Do not place hot bottles directly on a room temperature or cool surface. Tighten screw caps when the liquid is completely cooled.

Proper autoclave use training must be completed before operating autoclave on campus. Contact OEHS for guidance.

4.6.1 Autoclave Testing and Validation

Massachusetts regulation 105 CMR 480 requires that if you use an autoclave for the treatment of infectious waste and dispose of it as regular trash upon completion of treatment, each load must be logged with the date of the treatment, the quantity of the waste treated, the type of waste, process parameters (e.g., pressure temperature) and the signature of the operator. Examples of log-sheets are located at the Massachusetts Department of Public Health website: http://www.mass.gov/eohhs/docs/dph/environmental/sanitation/105cmr480-medical-waste-on-site-log.pdf

Massachusetts regulation 105 CMR 480 requires autoclaves used for decontaminating biological waste must be validated periodically to ensure that they are operating properly and killing the biological organisms in each autoclave load. The preferred method to check your autoclave is to test it with a commercial spore test system, also known as biological indicators. This system uses ampoules containing a bacterial species called *Bacillus stearothermophilus* that is tolerant to high temperatures and a color indicator solution. The ampoules are autoclaved under realistic conditions, such as in the middle of a bag of waste, and then incubated for two days at 56 °C. If

the spores grow, a color change will occur indicating inadequate sterilization in the autoclave. If there is no growth, no color change occurs and the autoclaving procedure is adequate. It is important to note that autoclave tape indicates only that a critical temperature was reached; it **does not** indicate the length of time at the desired temperature or whether steam was present.

OEHS will advise laboratory personnel on the periodic autoclave validation procedures and is a resource for questions on proper autoclave procedures.

4.7 SPILL MANAGEMENT

OEHS has provided labs with biological material kits to clean up small spills. The following procedures are recommended for the management of small spills of blood, bodily fluids, or other potentially infectious materials. If a large volume of a biological material is spilled, or if equipment (centrifuge/homogenizer/biosafety cabinet) malfunctions while processing biological materials, contact UMB OEHS for immediate consultation to implement appropriate measures to contain the spill.

- **Wear gloves and proper protective clothing.** Heavyweight, puncture-resistant, utility gloves are recommended to be worn over disposable latex or nitrile gloves if they do not reduce dexterity to a degree that increases risk. If the spill contains broken glass or other objects, these should be removed and discarded without contact with the hands; the use of dustpan and broom or forceps to collect the sharps is recommended. Additionally, rigid sheets of cardboard used as a "pusher" and "receiver" may be used to handle such objects and should be discarded with the objects into an appropriate biohazard container. If the spill is large and/or there is a potential of contaminating the worker’s shoes, water-impermeable shoe covers should be worn.

- **Absorb the spill.** Because most disinfectants are less active, or even ineffective in the presence of high concentrations of protein that are found in blood and serum, the bulk of the spilled liquid should be absorbed prior to disinfection. Absorb the spilled material with disposable absorbent material (e.g., spill pads, paper towels, gauze pads, or tissue paper wipes). If the spill is large, granular absorbent material may be used to absorb the liquid. Absorbent granular material, such as an Isolyzer, containing a chemical that releases chlorine upon wetting is commercially available. The efficacy of such material for disinfection is not known and, therefore, should not be relied upon to disinfect a spill. After absorption of the liquid, all contaminated materials should be discarded as biological waste.

- **Clean the spill site** of all visible spilled material using an aqueous detergent solution. Any household detergent may be used. The intent is to dilute the spilled material, lyse red blood cells, and further remove proteins from the contaminated area. Absorb the bulk of liquid prior

to disinfection to prevent dilution of the disinfectant. The use of a disinfectant detergent is not necessary.

- **Disinfect the spill site** using an appropriate intermediate to high-level disinfectant, such as a freshly prepared dilution of household bleach (see Table 3.1). Carefully flood the spill site or wipe down the spill site with disposable towels soaked in disinfectant to make the site "glistening wet."

  Note: If bleach is not an effective disinfect for the material you are working with, then you are required to use another EPA-approved disinfectant. Ensure the proper contact time prior to disposal.

- **Rinse the spill site** with water to remove any noxious chemicals or odors. Dry the spill site to prevent slipping.

- **Dispose** all disposable materials used to decontaminate the spill into a biological waste container. Handle the material in the same manner as other infectious waste.

- **Maintain a biological spill kit** in every laboratory comprised of disinfectant (e.g., bleach, prepare a 1:10 dilution when required), absorbent material (e.g., paper towels, spill pillows), a waste container (e.g., biohazard bags, sharps containers), PPE (e.g., laboratory coat, gloves, eye and face protection, booties) and mechanical tools (e.g., forceps, dustpan and broom).

4.7.1 Management of Small Spills

The following procedures are recommended for the management of small spills of blood, body fluids, or other potentially infectious materials in the laboratory or in a biosafety cabinet.

- Remove contaminated clothing/PPE and place them in a red bag.
- Put on new protective clothing (laboratory coat, gloves, face and eye protection, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, and paper towels).
- If the spill has occurred in a biosafety cabinet, keep the cabinet turned on and close the sash.
- Carefully spray the affected area with a disinfectant, such as a fresh 10% bleach solution.
- Pick up any broken glass with forceps and dispose it in a sharps container.
- Let disinfectant sit for 30 minutes.
- Soak up the disinfectant and spill with absorbent pads.
- Discard all clean-up materials in a biological waste box. Autoclave any reusable items, such as laboratory coats.
- Wash hands and exposed skin areas thoroughly with soap and water.

4.7.2 Management of Large Spills

The following procedures are recommended for a large volume biological spill in the laboratory area, in a BSC, or if equipment malfunctions while processing biological materials:

- If the spill occurs in a BSC, close the sash and leave the BSC running.
- Keep people out of the area to prevent spread of the contamination. Put up a warning sign.
- Remove any contaminated clothing and put it into a biohazard bag for decontamination later.
- Wash hands and exposed skin thoroughly.
- Contact the UMB OEHS at 617-287-5445 or email umbehs@umb.edu.
- Complete an incident report form.

5.0 IMMUNIZATIONS AND MEDICAL RESTRICTIONS

Certain biological materials may require personnel working with them to receive immunizations or participate in medical surveillance programs. Each project that is registered with the IBC will be reviewed to determine if any medical surveillance, immunizations or restrictions are required.

5.1 HEPATITIS B VACCINE

Under the OSHA BBP Standard, hepatitis B vaccine is recommended for all employees working with human blood, body fluids, or tissues. Information on how to obtain the vaccine and questions should be directed to the OEHS Office at 617-287-5445.

5.2 PREGNANCY

Several infectious agents are known to affect embryonic development. Women of childbearing age should be aware of the risks associated with studies using these agents. Men or women living with women of childbearing age should also know of the risks and should be especially careful not to bring infectious agents home on clothing or other laboratory materials.

For an infectious agent to affect embryonic development, the infectious agent must be transmitted to the child. In some cases, transmission is via the blood through the placenta. The following is a partial list of infectious organisms thought to have some adverse effects on human embryo and fetal development:

- Rubella virus
- Herpes simplex virus
- Varicella virus
- HIV

This list is not all inclusive. Please contact the UMB BSO for further information.

Infections caused by the following biological agents can cause birth defects in animals, but have not yet been shown to be teratogenic in humans:

- Influenza virus
- Mumps virus
- Parainfluenza type 2

This list is not all-inclusive. Prior to pregnancy, it would be best to discuss with your medical provider any infectious agents or chemicals you may have contact with in your work area. You may also contact the UMB OEHS for further information.

- Radiation exposure can also cause fetal damage.

6.0 LABORATORY SAFETY TRAINING INFORMATION

General laboratory safety information, including biological safety training is provided for all UMB laboratory staff by the UMB OEHS. Employees and students must be adequately trained prior to beginning any work with microbes, human source materials and other potentially infectious materials (OPIM), non-human primate materials, biological toxins and recombinant or synthetic nucleic acid molecules. Refresher training is provided as an on-line course through OEHS.

Project-specific training should include discussions about signs and symptoms of illness following an exposure to biological materials, potential hazards from exposure, and methods available to employees to prevent exposure.

PIs are encouraged to review this Biosafety Manual with their employees and students and address the following topics:

- The biology of the microbes used in experiments or that may be in the materials used, with emphasis on potential biohazards;
- Good aseptic technique;
- Proper techniques for decontamination and disinfection;
- Emergency procedures;
- A review of all relevant safety practices, the potential hazards of the work, and what to do if there is a suspected or confirmed exposure to biohazardous materials.

All non-technical staff members such as, but not limited to, maintenance personnel are trained as necessary by the OEHS about workplace hazards based on their job function. This training, in most cases, familiarizes them with the potential hazards associated with biological materials in general. The responsibility for training contract employees belongs to the contractor, but the OEHS is available to advise them. In cases where there is doubt or uncertainty about their personal safety, non-technical staff members should:

- Look for warning statements on doors, refrigerators, or other signage, such as the universal biohazard signs and avoid if at all possible areas posted with such signs;
- Be escorted into the area by a technical staff member.

Contact the UMB OEHS at 617-287-5445 for further information.

7.0 SHIPPING AND RECEIVING PROCEDURES FOR BIOLOGICAL SPECIMENS

Import, export, and interstate transport of biological materials are subject to requirements and laws from several federal agencies. The U.S. Public Health Service (PHS), U.S. Department of Transportation (DOT), U.S. Department of Agriculture (USDA), and U.S. Postal Service, regulate transport of hazardous materials by rail, air, vessel, and public highway. The guidelines and regulations of the International Air Transport Association (IATA) and International Civil Aviation Organization also apply when shipping substances by air. Import/Export Permit requirements are regulated by the Bureau of Customs; the Department of Commerce, CDC, and USDA require permits for certain agents.

The PHS defines etiological agents as viable microorganisms that cause disease in humans; infectious substances are those substances that contain etiologic agents. This terminology is used by the DOT and IATA. Diagnostic specimens are anything that the shipper reasonably believes to contain an infectious substance. Diagnostic and infectious specimens are regulated by the USDA, U.S. Food and Drug Administration (FDA), PHS, and IATA. Biological product means a product prepared in accordance with regulations that govern the manufacture of vaccines, reagents, or all viruses, serums, toxins, etc. intended for use in the diagnosis, treatment, or prevention of diseases in humans or animals. Biological products are regulated by the USDA, FDA, PHS, DOT, and IATA.

The required type of packaging, labeling, and documentation depend on the biohazardous material being shipped, how it is being shipped, and where it is being shipped. Specific packaging requirements for various biological agents should be reviewed by the PI to ensure compliance with all regulatory requirements. **Please be aware that anyone who ships restricted items improperly and without authorization may be subjected to fines and/or incarceration.** For more information of DOT Research and Special Programs Administration Office of Hazardous Materials Safety regulations (49 CFR 100-185) please see http://phmsa.dot.gov/hazmat; for more information about shipping packaging materials, go to the Saf-T-Pak® website http://www.saftpak.com.

Before shipping or receiving biological material, contact the OEHS to determine if any permits are required and the appropriate classification of the material for shipping purposes.

8.0 GENERAL LABORATORY SAFETY AND BIOLOGICAL SAFETY INSPECTIONS

Laboratory and biosafety inspections are typically scheduled beforehand to ensure the visit to the laboratory does not create a disruption; however, the UMB OEHS reserves the right to perform unannounced inspections. The surveyor will review any non-compliant conditions observed, and make recommendations for improvement. An unannounced site visit may occur at any time to make certain that all conditions are corrected. Results are documented and communicated to the appropriate personnel for follow-up and closure.

APPENDIX A

LABORATORY BIOSAFETY LEVEL CRITERIA

LABORATORY BIOSAFETY LEVEL CRITERIA

The following is excerpted from the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) Fifth Edition.

Section IV—Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 2 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. The following standard practices, safety equipment, and facility requirements apply to BSL-1:

A. Standard Microbiological Practices

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

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

None required.

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

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
    a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. **Do not wash or reuse disposable gloves.** Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

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

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment. The

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

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

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

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

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

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available.
9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).