BIOSAFETY MANUAL

Fitchburg State University

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LIST OF ABBREVIATIONS AND ACRONYMS

BBP	bloodborne pathogens
BL	biosafety level
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	biological safety cabinet
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
ECP	Exposure Control Plan
EH&S	Environmental Health and Safety
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GMO	Genetically Modified Organism
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
LAA	laboratory animal allergies
NIH	National Institutes of Health
NRC	Nuclear Regulatory Commission
NSF	National Sanitation Foundation
OSHA	U.S. Occupational Safety and Health Administration
PHS	U.S. Public Health Service
PI	principal investigator
PPE	personal protective equipment
rDNA	recombinant DNA
TB	tuberculosis
USDA	U.S. Department of Agriculture
WT	Wild-type
°C	degrees Celsius

CONTACT INFORMATION AND USEFUL WEBSITES

CONTACT INFORMATION		
Name/Title	Phone Number	E-mail
Environmental Health and Safety Officer	978-665-3756	Lfernan7@fitchburg.edu
Health Alliance Hospital (Burbank Campus)	978-343-5000	

WEBSITES	
Department	Web Address
Fitchburg State Exposure Control Plan	http://www.fitchburgstate.edu/uploads/files/CapitalPlanningMaintenance/FSU_Bloodborne_Pathogens_Exposure_Plan_June_30_2011.doc
The National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)	http://osp.od.nih.gov/office-biotechnology-activities/biosafety
NIH/Centers for Disease Control and Prevention (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL)	http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
OSHA Bloodborne Pathogen Standard	http://www.osha.gov/SLTC/bloodbornepathogens/index.html

1.0 INTRODUCTION

1.1 BACKGROUND

Work with tissues, cells, microorganisms, and animals comprises a wide variety of routine activities in many biological research and teaching laboratories. Exposures to potentially infectious materials during many of these activities can present underestimated health hazards to researchers and students. 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 members at increased risk for infections from 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 the 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 or Synthetic Nucleic Acid Molecules* (http://osp.od.nih.gov/office-biotechnology-activities/biosafety). The second is *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), which is published jointly by the CDC and the NIH (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm) 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. 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 suggested 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 bodily fluids. All laboratories that work with human tissues, cells, blood and other bodily fluids must adhere to the OSHA BBP Standard (http://www.osha.gov/SLTC/bloodbornepathogens/index.html).

The use of Universal Precautions is a key element of any BBP program and must be followed at all times in BL2 laboratories. All human blood, fluids, or cells and tissues that contain these fluids, are considered BL2. Universal Precautions means that all samples of blood or other human materials 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 on bloodborne pathogen safety, including Universal Precautions techniques, at orientation and on an annual basis. This training is offered through the Environmental Health and Safety (EH&S) Office. For more information, call 978-665-3756.

Safe practices for studies involving the use of rDNA are governed by the NIH Guidelines. It is Fitchburg State University policy that all laboratories comply with these Guidelines, which are law in the City of Fitchburg.

1.2.2 Commonwealth of Massachusetts

Regulations from the Commonwealth of Massachusetts (Title 105 Code of Massachusetts Regulations [CMR] Part 480.000—Storage and Disposal of Infectious or Physically Dangerous 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 Fitchburg

All rDNA work conducted in the City of Fitchburg is subject to regulations outlined in the Code of the City of Fitchburg, Chapter 68, "Biotechnology, Regulation of". At Fitchburg State, these regulations are enforced by the EH&S Office and the Institutional Biosafety Committee (IBC). In general, the requirements set forth in the city code agree with NIH and CDC guidelines. Further information can be found at http://ecode360.com/10430395.

1.3 INSTITUTIONAL BIOSAFETY COMMITTEE

The IBC is a committee made up of faculty, technical staff and community representatives, and the purpose of the IBC is to conduct specific review and oversight of biological research activities in compliance with the following guidelines and regulations:

- NIH—NIH Guideline for Research Involving Recombinant or 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
- Code of the City of Fitchburg—Biotechnology, Regulation of

1.3.1 Registering Research Projects with IBC

All biological research involving the use of recombinant DNA (rDNA), microbes, human and nonhuman primate materials, and biological toxins must be registered with the IBC. Please contact the Dean of Health and Natural Sciences for information about how to register a protocol with the IBC.

Each completed registration will be reviewed by the IBC Chair who may request additional information or clarification from the submitter or PI. Once the review is complete, the submission will be reviewed to determine if the research can be administratively approved. 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. Additionally, research with pathogens or toxins is typically reviewed at a convened meeting.

1.4 RESPONSIBILITIES

The following section outline the specific responsibilities associated with the 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 and facilities are available for laboratory members and are used properly. Each principal investigator 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 members of the laboratory and the surrounding community. They must also arrange for appropriate employee training regarding the safe use of potentially hazardous biological agents and require that all employees handling BBP receive the annual training mandated by OSHA.

In addition to the above, when research involves the use of rDNA, 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 IBC is notified prior to beginning any work with biological materials.
- Conduct an initial risk assessment of all hazards associated with the laboratory's work with biological materials.
- Report any significant problems, violations of the NIH Guidelines, or any laboratory-related accidents, illnesses, or potential exposures to the EH&S Office. The EH&S Office should be contacted immediately, as there may be additional reporting requirements to outside agencies. There is an incident report form in Appendix B.
- Instruct and train laboratory staff and students 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 and students 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 rDNA are located in the NIH Guidelines (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). Failure to comply with the NIH Guidelines by one PI could affect all NIH-funded projects at Fitchburg State University; therefore, compliance is absolutely mandatory.

1.4.2 Laboratory Staff Responsibilities

Laboratory members are responsible for following university health and safety policies and the procedures and instructions from their PIs and the EH&S Office. They need to comply with all

NIH, CDC and OSHA regulations, use safe laboratory practices, and inform the PI, laboratory supervisor, or EH&S Office regarding any potentially hazardous situations or conditions.

1.4.3 Biosafety Officer

Per IBC Policies and Procedures, the BSO is the primary intermediary between investigators and the IBC. The Chair of the IBC acts in the capacity of BSO if one is not present on campus. BSO responsibilities include:

- Managing the biosafety program and implementation of IBC policies and procedures. If acting as BSO, the IBC Chair will work with the Dean of Health and Natural Sciences, the EH&S Officer, and a credentialed consultant to implement the program.
- Assisting laboratories in conforming to pertinent regulatory guidelines and IBC policies by providing training, facility inspection, and communication of program requirements.
- Making annual inspections of laboratory containment, procedures, records, and equipment for laboratories using biological agents or toxins. In the absence of a BSO the Dean of Health and Natural Sciences in conjunction with the EH&S Officer will contract for training and facility inspections from a third party.
- Screening research protocols proposed by PIs and submitting to 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
- 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, or directing affected employees to the EH&S or a credentialed consultant for assistance.
- Summarizing the results of the biosafety inspections of laboratories in biosafety reports. The IBC Chair will be given the results of the inspections and will be responsible for communicating the results to individual laboratories.
- Distributing biosafety report results to the PI.
- Acting as the liaison between 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

PIs are responsible for conducting an initial risk assessment of their work with biological materials. They will include the results of this risk assessment in their IBC registration.

2.1 LIMITED INFORMATION

There are situations when the information is 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, consider the following:

- 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 ORGANISMS

When a PI proposes to work with genetically modified organisms (GMOs), they should consider the characteristics of donor and recipient/host organisms. In addition, s/he should consider whether or not the GMO is more hazardous than the wild-type (WT) organism. For example, if GMO is a pathogen, has the genetic modification increased the virulence or expanded the tropism of the organism? Is the GMO hardier than the WT organism? What are the potential consequences if the GMO was released into the environment outside the lab? This information should be included in the risk assessment.

2.4 HUMAN BLOOD AND BLOODBORNE PATHOGENS

Human blood, body fluids, tissues, and materials that may be contaminated with this materials, are considered potentially infectious. Any laboratory activity that includes the collection or manipulation of these materials must be conducted using Universal Precautions. Transportation of these materials through common-use or non-laboratory areas should be done in such a way as to securely contain the material and minimize or eliminate the chance of spills or accidental release.

3.0 WORKING WITH ANIMALS IN THE LABORATORY

3.1 INTRODUCTION

Working with animals in a laboratory setting can present risks from infections and injuries to all personnel. Personnel working with laboratory animals must be aware of the potential risks and implement measures to prevent injury or illnesses related to laboratory animal use. The purpose of this section is to communicate the risks involved with laboratory animal use and the protective procedures in place.

All handling and use of animals must be conducted safely, humanely, and in compliance with all institutional and federal regulations. Any hazardous or non-compliant behavior or work conditions regarding the use of animals needs to be reported to the Animal Care Facility Manager, Attending Veterinarian/Director, or the IACUC.

3.2 ALLERGIES

Allergic reactions to animals are among the most common adverse health effects associated with the care and use of animals in biomedical research.[1,2] The development of laboratory animal allergies (LAA) commonly begins with the inhalation of animal allergens, such as dander and urinary proteins. Skin and eye contact with allergens can also result in symptoms. Although most animal allergens are found in urine, dander, hair, serum, and saliva, coexisting allergies and tobacco smoking can exacerbate the development of LAA. All possible measures or controls must be implemented to decrease or eliminate the exposure of personnel to allergens when working with laboratory animals.

Symptoms of LAA can range from minor to life threatening. Rhinitis (runny noses), conjunctivitis, asthma or other breathing difficulties, fever, skin rashes or bumps (atopic dermatitis), and gastrointestinal disorders can all be the result of LAA. Be aware that symptoms can be delayed up to 12 hours after animal exposure.[3] Promptly report any suspicious clinical symptoms to your healthcare provider.

Guidelines for working with animals are summarized as follows.

- Wear required PPE at all times when working with animals.

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[1] Wolfle TL and Bush RK. 2001. The Science and Pervasiveness of Laboratory Animal Allergy. *ILAR Journal*, 42:1-3.
[2] National Research Council. 1997. *Occupational Health and Safety in the Care and Use of Research Animals*. Washington, DC: National Academy Press.
[3] Bush RK. 2001. Assessment and Treatment of Laboratory Animal Allergy. *ILAR Journal*, 42:55-64.

- Do not wear PPE outside of the animal facility.
- Wear gloves at all times when handling animals.
- Do not distribute animal bedding in your immediate work environment. All cage cleaning procedures should be performed in a manner that prevents bedding debris from entering the work environment. Change bedding in a BSC or fume hood.
- Ensure that animal cages are properly closed and that static microisolator cage lids properly fit.
- Do not overpopulate animal cages.
- Conduct work with animals in a BSC when required and whenever possible.
- When work cannot be conducted in a BSC, conduct work with animals in well ventilated areas and see facility supervisor.
- Clean and disinfect all equipment after use.
- Wash hands frequently and always after handling animals (even when wearing gloves).
- Avoid touching your face when working with animals.
- Keep work areas clean.
- Keep animal cages and transport containers properly covered at all times.
- Do not handle common items (i.e., door knobs) with gloved hands that have had animal contact.
- Do not house any animals overnight in any research laboratory.

3.3 ZOONOSES

Zoonoses are diseases that are communicable from lower animals (e.g., rats and mice) to humans under natural conditions.

3.3.1 Mice

No known risk of zoonotic diseases are known to be caused by usual animal care and handling exposures to the microbial flora of laboratory-reared mice. Two diseases of concern when working with wild mice are lymphocytic choriomeningitis virus and hantavirus. Laboratory animals at Fitchburg State are known to be free from these diseases and the Animal Health Monitoring program evaluates for these agents on a regular basis.

3.3.2 Rats

No known risk of zoonotic diseases results from typical exposure to the microbial flora of laboratory-reared rats. Two diseases of concern when working with wild rats are hantavirus and rat-bite fever. Laboratory animals at Fitchburg State are known to be free from hantavirus and the Animal Health Monitoring program evaluates for the virus on a regular basis.

Rat-bite fever is caused by two bacteria, *Streptobacillus moniliformis* and *Spirillum minor*. These bacteria are present in the upper respiratory tract and mouths of rats. Rats are asymptomatic, as the bacteria do not cause disease in them. Commercial vendors have virtually eliminated these bacteria from their animals.[4]

3.4 BITES AND SCRATCHES

Bites and scratches are hazards associated with all animals. A thorough understanding of species-specific behaviors and habits is the best preventative measure against bites and scratches. All personnel handling animals are required to go through species-specific training according to the requirements set forth by the IACUC and the Animal Facility's Policies and Procedures.

Injured and sick animals and certain strains of mice and rats may display unusually high levels of aggression towards one another and towards humans. When working with these animals, even experienced handlers must exercise caution. Diseases such as rat-bite fever are transmitted through bites and scratches. All bite wounds and scratches should receive immediate first aid; an evaluation for more extensive medical care may be needed. Please report all bites and scratches, and seek proper medical care. Also report such incidents to the EH&S Office, as in some cases reports must be filed within 30 days to the National Institutes of Health.

3.5 WORK WITH UNMONITORED ANIMAL POPULATIONS

In addition to the hazards listed above, there are specific safety and regulatory issues associated with the collection and manipulation of unmonitored animal populations such as wild fish, insects, birds, reptiles or mammals. Since the prevalence of infectious disease in these populations is not consistently recorded or monitored, field work may expose students or researchers to disease-bearing insects and animals.

Wild animals may carry a variety of diseases that can affect humans and/or other animals. The specific pathogens will depend on the type of animal and the location in which it is being collected. Prior to handling any wild animals or samples from these animals, a risk assessment must be conducted by the PI, in conjunction with the EH&S Office, to determine what hazards may be present and how these hazards can be minimized or eliminated. PIs must describe these risks and preventative measures to their students and employees prior to sending them to areas where these hazards may be present.

Permits from state or federal agencies may be required to collect wild animals or specimens from wild animals. These permits must be obtained by the PI prior to any field collection.

[4] National Research Council. 1997.

3.6 RESPONSIBILITIES

3.6.1 Institutional Animal Care and Use Committee

The IACUC will review all animal care and use protocols to ensure a safe working environment for laboratory personnel. The IACUC will work with animal facility staff to ensure that the animal care and use program complies with current regulations and standards.

3.6.2 Principal Investigator

The PI is responsible for ensuring that research is conducted in accordance with university policies and safe laboratory practices. The PI is responsible for completing all appropriate hazardous agent protocols (chemical and biological hazards) and the IACUC protocol prior to the start of the research. If field collection of animals will occur, the PI is responsible for obtaining all required permits and ensuring that they are up-to-date, and for identifying any hazards associated with these animals or the area in which they're collected and addressing these hazards with students and employees. The PI and/or a designee is responsible for obtaining necessary safety equipment and maintaining awareness of safety policies and procedures.

3.6.3 Laboratory Staff

Laboratory staff members are responsible for conducting all animal work in a safe and humane manner in accordance with university policies and safe laboratory practices. The staff member is responsible for informing the PI, animal facility management, laboratory supervisor, IACUC, or EH&S Office regarding any potentially hazardous situations or conditions. The staff member is also responsible for reporting any work-related injuries or incidents in accordance with university policies.

4.0 BIOLOGICAL RESEARCH INVOLVING PLANTS

Biosafety principles and regulations also apply to work with plants and plant pathogens. Steps must be taken to ensure that plant pathogens, exotic or invasive plants, transgenic plants, etc. are properly contained and are not released into the environment. All plant experiments must be designed to prevent the release of viable seeds, spores, microorganisms, etc. from the research area.

The creation and study of transgenic plants is subject to the NIH Guidelines, and all work with transgenic plants or plant pathogens must be registered with the IBC. In addition, other permits may be required to work with plant pathogens or exotic plants. Prior to field collection of any plants, ensure that any required permits have been obtained and are current.

5.0 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 are summarized in Table 5.1.

Table 5.1 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
BL3	Individual risk: HIGH Community risk: MODERATE	Mycobacterium tuberculosis West Nile virus
BL4	Individual risk: HIGH Community risk: HIGH	Ebola virus

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

5.1 BIOSAFETY LEVELS 1 AND 2

Laboratory work at Fitchburg State University is conducted using BL1 and BL2 containment and procedures. 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 5.2 provides a brief summary of the biosafety level criteria for BL1 and BL2.

Table 5.2 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 decontamination (i.e., chemical or autoclave) must be available.

BL biosafety level

6.0 LABORATORY PRACTICES

6.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) is an essential element in laboratory safety, and must be provided to all lab members. The selection of appropriate PPE is done through a risk assessment. PPE may include, but is not limited to:

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

At a minimum, anyone working in the lab shall wear gloves and a laboratory coat whenever handling biological agents, cells and tissues. Safety glasses with side shields, goggles, or a 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. However, it is always good practice to wear safety glasses with side shields at all times while present in the laboratory. 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 biohazard waste when discarded. If PPE is not disposable, PPE shall be cleaned and disinfected before and after use.

6.2 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 protect both laboratory personnel, the material in use, and the environment.

Class II A2 BSCs are the type most commonly found in laboratories. 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 6.1). 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

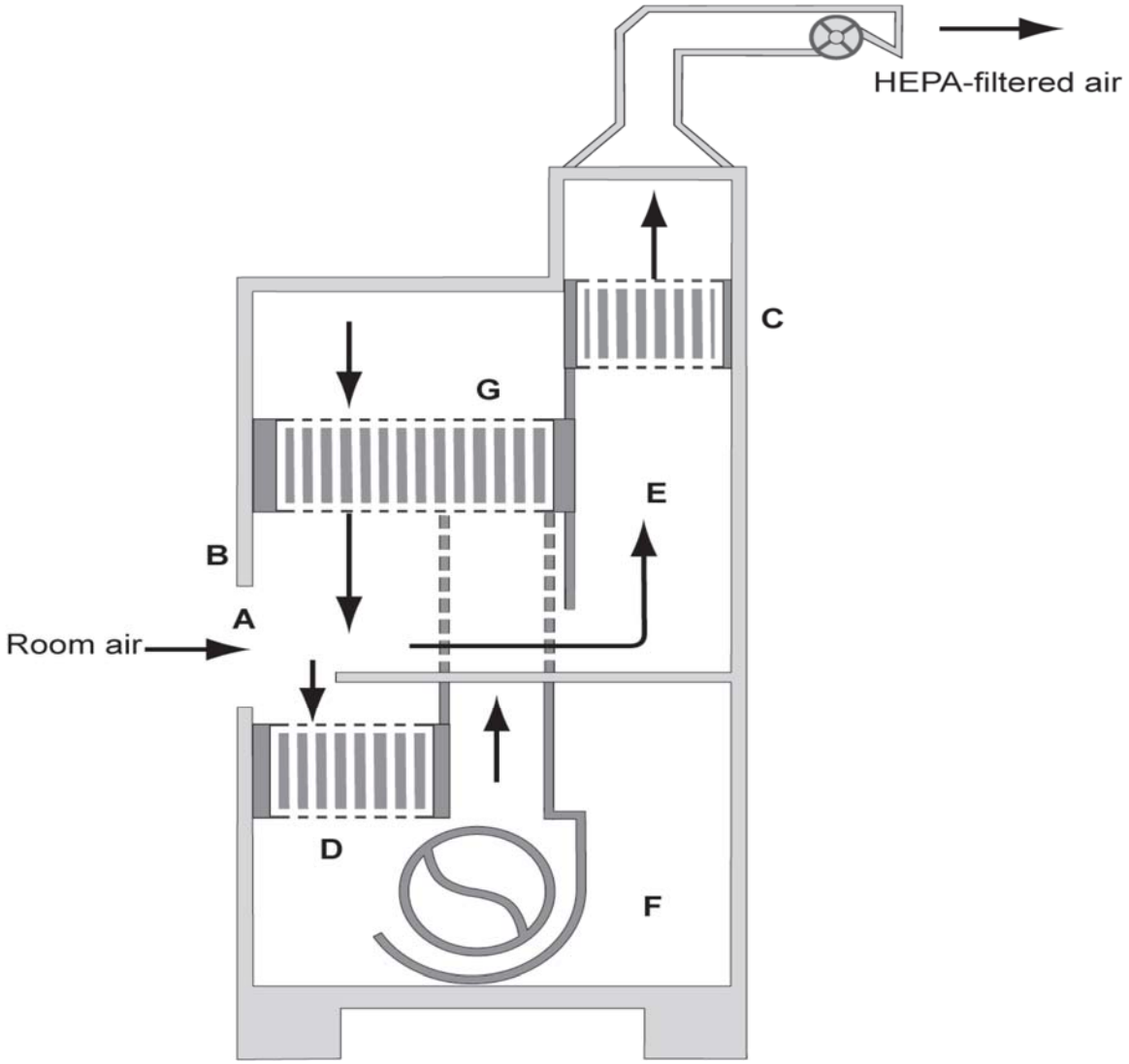
filters are 99.97% efficient which means that they remove 99.97% of the particles from the air. Because it is difficult to disperse or aerosolize single viral particles and because of the particle collection mechanisms of HEPA filters, particles larger and smaller than a filter's most penetrating size are collected with greater efficiency. This filtration level will capture a majority of bacteria, spores, and viruses from the filtered air. Figure 6.1 illustrates typical airflow patterns in a BSC.

Table 6.1 Biological Safety Cabinet Characteristics[1]

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])
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.



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

Figure 6.1 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 must be free of clutter to allow proper air intake.
- Sash should not be raised above the specified level, unless you are moving materials into or out of the cabinet prior to or after working.
- Make all attempts to avoid the use of flames such as a Bunsen burner within a BSC. Open flames disrupt airflow, leading to product and personnel contamination. The heat from open flames will damage the HEPA filter in the BSC, and can lead to uncontrolled fire within the laboratory.

- Certification must be performed annually and whenever the BSC is moved or repaired.

BSCs are required to be tested and certified annually by qualified technicians. 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 EH&S Office for additional information about BSC certifications.

6.3 DISPOSAL OF BIOLOGICAL WASTE

6.3.1 Biological Waste

Biological waste may be disposed of in one of three ways:

- Designated biological waste box
- Chemical disinfection
- Steam sterilization/autoclave

PIs will work with laboratory technicians to ensure that an appropriate disposal method is used for the biological agents in use in the laboratory.

Potentially infectious solid waste and solid waste containing rDNA must be disposed of in designated biological waste boxes. Each box is labeled with the universal biohazard symbol (Figure 6.2) and is lined with two red plastic bags to reduce the likelihood of leakage.

When a biological waste box is between two-thirds (2/3) and three-quarters (3/4) full, the two bags must be individually sealed. The box must be sealed with two-inch-wide tape. **Do not overfill the boxes**. Boxes that leak any liquid or that weigh more than 55 pounds will not be removed for disposal.

The biological waste vendor removes full, closed boxes. Contact the EH&S Office for more information about biological waste collection.

Figure 6.2 Universal Biohazard Symbol

Liquid biological and rDNA waste must be rendered non-infectious by steam sterilization or chemical disinfection prior to sink disposal. If chemical disinfection is selected, full-strength

household chlorine bleach 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.

Note: If bleach is not an adequate disinfectant for the biological agent in use, an alternative disinfectant must be selected that is effective against the agent in use. Ensure the proper contact time is met prior to disposal.

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.

6.3.2 Biological/Chemical Waste

The approach for managing biological waste containing hazardous or potentially hazardous chemicals is similar to radioactive biological 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. Contact the EH&S Office with any questions.

6.4 SHARPS MANAGEMENT

Some of the most serious accidents in biological laboratories are those caused by puncture wounds through skin (percutaneous exposures). All objects that can puncture skin are designated as sharps and require special disposal treatment. Examples of sharps include 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, even if it is not contaminated with biological materials. 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 sharps container.

Federal regulations concerning sharps primarily focus on work with human bodily fluids. Research work conducted with animals only is not required to utilize engineered sharps; however, it is recommended that engineered devices be used whenever practical. 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.
- Discard the entire syringe-needle combination in a 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. Laboratories are required to evaluate the use of safety needles whenever possible, and if feasible, select safety needles for use. Please refer to the Exposure Control Plan for details.

6.4.1 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 biohazard red bags should be disposed of in Sharps Boxes, regardless of whether they are contaminated with a biological material. **Please do not dispose of sharps that may contain mercury or other metals in sharps containers. Contact EH&S for proper disposal**. 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. All filled disposable sharps containers need to be placed into a larger reusable sharps container.

Figure 6.3 Sharps Container

6.4.2 Broken Glassware Disposal

Place clean broken glassware into the standard recycling boxes for glassware. Contaminated broken test tubes or other glass items may be placed directly into sharps containers.

6.4.3 Pasteur Pipettes Disposal

Pasteur pipettes are a special case because Massachusetts law requires that they be considered as a sharps waste no matter what their previous use. Discard glass Pasteur pipettes directly into sharps containers; **do not** use cardboard broken glassware boxes. Plastic pipettes and serological pipettes that could puncture the red waste bags should also be disposed of in sharps containers.

6.5 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 6.2.

Table 6.2 Summary of Disinfectants and Their Uses

Disinfectant	Final Concentration	Effective On	Ineffective On
Sodium Hypochlorite Bleaches: e.g., Clorox™*	1:10	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., Quatricide®*	Follow manufacturers' directions for dilutions	Bacteria, spores, viruses	

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.

6.6 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 sealed biohazard bags are placed in an autoclave. There are two simple solutions: 1) cut open the bag, or 2) place about 200 milliliters of water in the bag before sealing.

Typically, bags (24" x 36") of solid plastic waste take from 45 minutes to one hour to reach sterilizing temperatures throughout its contents.

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. Bags and other containers must have openings to allow for steam to penetrate.
- Place contaminated materials in autoclave bags. Place bags inside plastic or metal trays when autoclaving.

- Use only containers designed for sterilization. Use plastic or metal trays.
- When placing containers in the autoclave, please leave room for steam to circulate. Do not fill the autoclave too tightly as this will not allow for proper sterilization.

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.

6.6.1 Autoclave Testing and Validation

Massachusetts regulations 105 CMR 480 requires that if you use an autoclave for the treatment of infectious waste, 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 regulations 105 CMR 480 requires autoclaves used for decontaminating biological waste must be tested quarterly 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. 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.
The following paragraphs are the specific requirements as stated in 105 CMR 480.150:

(B) The methods which rely on heat shall be evaluated for each load or cycle by using a recording thermometer, thermocouple, parametric monitoring device, thermal indicator strip, or by an equivalent method approved in writing by the Department.

(C) For any wastes that are rendered noninfectious by chemical disinfection, the chemical used shall be of demonstrated efficacy, as determined by the Department, against the challenge testing target or indicator organism and registered with:

(1) The U.S. Environmental Protection Agency, Office of Pesticide Programs pursuant to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); and
(2) The Massachusetts Department of Agricultural Resources, Pesticide Bureau.

(D) All parametric monitoring equipment utilized in conjunction with any approved disinfection methods, including autoclaves, shall be calibrated at a minimum annually, by an individual who has received training from the manufacturer in the operations and maintenance of the equipment.

(E) Quarterly qualitative (growth/no growth) biological challenge testing shall be conducted during standard operations for all approved disinfection methods including autoclaves, but not incineration. Specifically:

(1) Testing shall consist of spore strips or a retrievable alternative medium approved by the Department, which contain a 1.0 x 104 minimum challenge population of a bacterial indicator organism that is most resistant to any aspect of the treatment technology as outlined in the most recent medical waste treatment technology guidelines established by The State and Territorial Association on Alternative Treatment Technologies (STAATT) or its successor The International Society of Analytical Analysis of Treatment Technologies (IStAATT).

(2) Testing methodologies including the number, type and locations shall be in accordance with manufacturer's guidelines and procedures approved by the Department;
(3) Analytical testing results (growth/no growth) should demonstrate a minimum bacterial spore reduction of 4 log 10;

(4) When a 4 log 10 bacterial spore reduction has not been demonstrated (results indicate bacterial growth), an operations and mechanical systems assessment shall be conducted by a qualified individual who has received training from the manufacturer in the operations and maintenance of the equipment. Appropriate corrective actions shall be implemented, when warranted, including but not limited to mechanical adjustments and when applicable, recalibration of all parametric monitoring devices followed by re-treatment of the waste and additional challenge testing to confirm the effectiveness of any implemented corrective action;

(5) In accordance with 105 CMR 480.500(B)(1)(f), the analytical test results shall be documented on the required record-keeping log form for medical or biological waste treated on site in conjunction with the date and all applicable corresponding process parameter results.

(6) When implemented, corrective actions pursuant to 105 CMR 480.150(E)(4) shall be documented in detail, including the date, name of the individual implementing the corrective actions and a description of the work performed, on the back of the applicable record-keeping log form for medical or biological waste treated on-site.

(7) All analytical test results shall be retained in the required record-keeping log for a period of three years.

6.7 SPILL MANAGEMENT

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 biological material is spilled, or if equipment malfunctions while processing biological materials, call the EH&S Office for immediate consultation on implementing 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 the spill contains broken glass or other sharps, these should be removed and discarded without contact with the hands. Rigid sheets of cardboard used as a "pusher" and "receiver" may be used to handle such objects and should be discarded 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., 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 hospital disinfectant, such as a dilution of household bleach (see Table 3.1). 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 disinfectant against the material, then you are required to use an 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.

6.7.1 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.

- Put on 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.
- Cover the spill with absorbent materials such as paper towels. Carefully pour or 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 paper towels.
- 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.

6.7.2 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 EH&S Office.
- Complete an incident report form (Appendix B).

7.0 IMMUNIZATIONS, MEDICAL RESTRICTIONS, AND REPORTING OF INJURIES/ILLNESSES

Work with certain biological materials may require personnel working with them to receive immunizations and/or have medical surveillance.

Personnel working with certain biological materials or the potential for exposure are recommended to receive immunizations for protection. Certain conditions may also require additional protective equipment.

7.1 HEPATITIS B VACCINE

Under the OSHA BBP Standard, hepatitis B vaccine is recommended for all employees working with human blood, body fluids, or tissues. It is provided free of charge to employees with the potential for occupational exposure to human materials. Those employees declining vaccination will be asked to sign the OSHA waiver indicating that hepatitis B vaccine has been offered and refused. Any questions about the vaccination program should be directed to the EH&S Office.

7.2 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 contaminated items.

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
- Listeria monocytogenes
- Toxoplasma gondii

This list is not all inclusive. Please contact the EH&S Office 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 EH&S Office for further information.

- Women who wish to become pregnant, or who become pregnant while working in laboratories are encouraged to consult with their personal healthcare provider regarding additional precautions recommended while working with infectious materials. The EH&S Office can be contacted for precautions when working with hazardous chemical or biological materials during pregnancy.

7.3 OTHER MEDICAL RESTRICTIONS

Examples of some conditions that might warrant special precautions when working in a laboratory setting are immunosuppressive conditions, allergies, or drug therapy that suppresses the immune system. Employees are encouraged to discuss any medical concerns or contraindications to working in a laboratory with their medical provider.

7.4 REPORTING OF INJURIES/ILLNESSES

All workplace injuries and illnesses must be reported to the EH&S Office and injured or ill employees and students should report to Healthcare Alliance Hospital immediately. This will ensure that the injured or ill person is provided with the appropriate medical care and ensure that any necessary reports are filed with the IBC, as well as the City of Fitchburg and the National Institutes of Health (NIH), in a timely fashion.

8.0 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.

Laboratory staff can receive awareness level training from the EH&S Office for the shipment of hazardous materials. Individuals packaging specimens/hazardous materials for shipment must also receive function-specific training. The training is required every two years or when there is change in the regulations. Any biohazardous materials that are being shipped must only be done so by someone who has completed the DOT/IATA training. For assistance regarding training and other requirements for the legal shipping of hazardous materials, please contact the EH&S Office.

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 principal investigator 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 refer to http://phmsa.dot.gov/hazmat; for more information about shipping packaging materials, go to the Saf-T-Pak® website http://www.saftpak.com.

9.0 GENERAL LABORATORY SAFETY AND BIOLOGICAL SAFETY INSPECTIONS

All laboratories are inspected on a routine basis, with BL2 laboratories being inspected at least annually. Laboratory inspections are typically scheduled beforehand to ensure the visit to the laboratory does not create a disruption; however, the EH&S Office 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 anytime to make certain that all conditions are corrected.

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.* Biosafety Levels 1 and 2 will be highlighted, since no work requiring Biosafety Level 3 or 4 containment will be performed at Fitchburg State.

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 1 of this section [of the BMBL] 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 [but is not required to] 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.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. When appropriate, a baseline serum sample should be stored.

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.

1. 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:

1. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

3. 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.

1. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

2. 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.

3. 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.

4. 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.

5. 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.

6. An eyewash station must be readily available.

7. 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.

8. 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.

9. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

APPENDIX B

INCIDENT REPORT FORM

INCIDENT REPORT FORM

Date: _____ **Time of Incident:** _____
Location of Incident: _____
Name: _____ **Phone#:** _____
Person Initiating Call to EH&S: _____ **Phone #:** _____
Brief Characterization of Incident:

_____

Describe the Incident:

_____
_____
_____
_____
_____
_____

Response Summary:

_____
_____
_____
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_____

Recommended Corrective/Preventive Action:

_____
_____
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_____
_____
_____

Comments:

_____
_____
_____
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_____
_____

EH&S Responder: _____

Date/Time of Resolution: _____

☐ ***Check here if this requires further action by P.I.***

Signature of Person Filling Out Report: _____