

BIOSAFETY MANUAL

**THE WYSS INSTITUTE
FOR
BIOLOGICALLY INSPIRED ENGINEERING**



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LIST OF ABBREVIATIONS AND ACRONYMS

BBP	bloodborne pathogens
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	biological safety cabinet
BL	biosafety level
BSO	biosafety officer
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CMR	Code of Massachusetts Regulations
COMS	Harvard Committee on Microbiological Safety
DNA	deoxyribonucleic acid
DOT	U.S. Department of Transportation
ECP	Exposure Control Plan
EH&E	Environmental Health & Engineering, Inc.
EH&S	Environmental Health and Safety
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GMMO	Genetically Modified Microorganism
HBV	hepatitis B virus
HCCM	Harvard Center for Comparative Medicine
HCV	hepatitis C virus
HEPA	high efficiency particle air
HIV	human immunodeficiency virus
HUPD	Harvard University Police Department
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
LAA	laboratory animal allergies
MADPH	Massachusetts Department of Public Health
NIH	National Institutes of Health
NIH Guidelines	NIH Guidelines for Research Involving Recombinant DNA Molecules
NSF	National Sanitation Foundation (NSF International)
OBA	Office of Biotechnology Activities
OPIM	other potentially infectious material
OSHA	U.S. Occupational Safety and Health Administration
PHS	U.S. Public Health Service
PI	principal investigator
PIM	potentially infectious material
PPE	personal protective equipment
rDNA	recombinant DNA
TB	Tuberculosis
USDA	U.S. Department of Agriculture
Wyss Institute	Wyss Institute for Biologically Inspired Engineering at Harvard University
°C	degrees Celsius

CONTACT INFORMATION AND USEFUL WEBSITES

CONTACT INFORMATION

Name/Title	Phone Number	E-mail
Jessica Healey, M.S., Biosafety Officer	774-244-7018 (cell)	jhealey@eheinc.com
Christopher Neal, Manager of Environmental Health and Safety	617-432-7222 (office) or 617-293-0333 (cell)	cneal@eheinc.com
Jennifer Bosselman, M.S., Project Manager	800-825-5343* or 617-293-7640 (cell)	jbosselman@eheinc.com
Biosafety Emergency Response Pager	781-597-9786	
Mary Tolikas, Wyss Operations Director	978-457-5191	mary.tolikas@wyss.harvard.edu
Harvard Radiation Safety Emergency after hours number	617-496-3797 617-495-5560	radiation_protection@harvard.edu
Martín Montoya-Zavala, Wyss Laboratory Manager	617-233-9281	martin.montoya@wyss.harvard.edu
Occupational Health Departments: Harvard Medical School (HMS) Children's Hospital Boston (CHB) Beth Israel Deaconess Medical Center (BIDMC) Dana-Farber Cancer Institute (DFCI) Boston University (BU) Massachusetts Institute of Technology (MIT) University of Massachusetts (UMass) Medical School: Employee Health University Campus Employee Health Memorial Campus Employee Health 210 Lincoln Street	617-432-1370 617-355-7580 617-632-0710 617-632-3016 617-353-6630 617-253-8552 774-441-6263 508-334-6238 508-793-6400	
Harvard University Police Department (HUPD)	617-432-1212	
BioMed Realty Trust, Inc. (BMR) Security	617-232-0102 or 617-202-8957	
Janitronics	617-632-7243	Pager #90598
Chemical Waste pickup: Triumvirate	617-667-5143	clschemicals@bidmc.harvard.edu
Biological Waste/Sharps pickup	617-632-7243	Pager #90598
Able Engineering (Building Maintenance)	617-735-4399	
Triumvirate contact: Dennis Colarusso	617-715-8920	

NOTE: 800-825-5343 is the phone number for the main switchboard for Environmental Health & Engineering, Inc. (EH&E), which is the company supplying biosafety support. Please specify the employee when the person answers the phone. For all emergencies, please call HUPD.

EMERGENCY REPORTING

For any emergency, contact the Harvard University Police Department (HUPD) at 617-432-1212.

For medical emergencies call 911 then HUPD with the exact location of the emergency.

For lock-outs or walking escorts anywhere **on campus**, call New Research Building Security at 617-432-6119.

EMERGENCY HOSPITALS

In a life threatening emergency, personnel should go to the nearest emergency room.

For non-life threatening injuries requiring a hospital visit personnel should report to the emergency room associated with their respective institution. Please refer to the list below:

For Harvard University, Harvard Medical School, Children's Hospital Boston, Brigham and Women's Hospital (BWH), Dana-Farber Cancer Institute affiliated personnel: **BWH**

For Beth Israel Deaconess Medical Center (BIDMC) affiliated personnel: **BIDMC**

For Massachusetts Institute of Technology (MIT) affiliated personnel: **MIT Medical**

For Boston University (BU) affiliated personnel: **Boston Medical Center**

For UMass Medical School personnel: **no preferred hospital; whichever is most convenient**

Note: For MIT, University of Massachusetts Medical School, and BU affiliated personnel, patients should be taken to the nearest available hospital, if necessary.

WEBSITES

Department/Resource	Webpage
Harvard Center for Comparative Medicine(HCCM)	https://arcm.med.harvard.edu/
Harvard Committee on Microbiological Safety (COMS)	http://www.hms.harvard.edu/orsp/coms
<i>National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules</i> (NIH Guidelines)	http://oba.od.nih.gov/rdna/nih_guidelines_oba.html
NIH/Centers for Disease Control and Prevention (CDC) <i>Biosafety in Microbiological and Biomedical Laboratories</i> (BMBL)	http://www.cdc.gov/biosafety/publications/bmb15/
U.S. Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens (BBP) Standard	http://www.osha.gov/SLTC/bloodbornepathogens/index.html

1.0 INTRODUCTION TO BIOSAFETY

1.1 BACKGROUND

Work with tissues, cells, microorganisms, and animals, comprises a wide variety of routine activities in many biomedical research and biotechnology laboratories. Yet exposures to potentially infectious materials during many of these activities can present underestimated health hazards for 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 infections from bacteria, fungi, viruses, viral vectors, recombinant deoxyribonucleic acid (rDNA), and biological organisms containing rDNA.

Biosafety can be simply defined as a group of practices and procedures designed to provide safe environments for individuals who work with potentially hazardous biological materials in laboratory environments. The primary goal of biosafety is to reduce or eliminate exposures to these agents through the use of containment. The term containment refers to safe methods for managing potentially infectious materials in laboratory environments. Containment includes not only good microbiological techniques and safety equipment (primary containment), but also the design and operation of the laboratory facility (secondary containment).

Biosafety guidelines have been developed by two government agencies, the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC). These guidelines 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 *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html), which was last published in January 2011. 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/>); the most recent edition (Fifth Edition) was published in December 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 have been developed to 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 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 the suggested federal guidelines and the federal, state, and/or local mandated requirements.

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 certain 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 Section 1910.1030 [29 CFR 1910.1030]) was designed to eliminate or minimize occupational exposures to blood and other bodily fluids and the risks for developing the infectious diseases associated with them. All laboratories that work with human blood, human tissues, and certain, specific human body 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 a BBP program and must be followed at all times in the BL2 laboratories. Universal Precautions involves treating all samples as potentially infectious. For example, blood from any source, (even HIV-seronegative control donors), should be handled as potentially infectious. Training in Universal Precautions techniques is given at the time of orientation and on an annual

basis. This training is offered through the Environmental Health and Safety (EH&S) Office for the Wyss Institute for Biologically Inspired Engineering at Harvard University (Wyss Institute).

Safe practices for studies involving the use of rDNA are governed by the *NIH Guidelines* (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). It is the policy of the Wyss Institute that their staff complies with these Guidelines, which are law in the City of Boston.

1.2.2 Commonwealth of Massachusetts

Regulations from the Commonwealth of Massachusetts, Title 105 Code of Massachusetts Regulations Section 480.000 (105 CMR 480.000), Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code Chapter VIII) (<http://www.mass.gov/Eeohhs2/docs/dph/regs/105cmr480.pdf>), primarily focus on the management of biological waste. This regulation is mandated by the Massachusetts Department of Public Health (MADPH). The principal issues deal with what constitutes biological waste and how to dispose of it properly.

1.2.3 City of Boston

All non-exempt rDNA work conducted in the City of Boston must be approved by the Harvard Committee on Microbiological Safety (COMS) and be registered with the City of Boston (<http://www.hms.harvard.edu/orsp/coms/Government/BostonRegulations.htm>). The City of Boston rules conform to NIH/Office of Biotechnology Activities (OBA) and CDC guidelines.

1.2.4 Wyss Institute Regulations

The Wyss Institute has adopted the regulations from NIH/OBA, CDC, OSHA, MADPH, and the City of Boston as institutional policy. The NIH/OBA places the responsibility for implementing its guidelines in the hands of an Institutional Biosafety Committee (IBC). The Harvard COMS serves as the Wyss Institute IBC. All research involving the use of rDNA and infectious microorganisms, including BBP, must be registered with the COMS.

1.3 HARVARD COMMITTEE ON MICROBIOLOGICAL SAFETY

Harvard University has adopted all applicable regulations from NIH/OBA, CDC, OSHA, MADPH, and the City of Boston as institutional policy. The NIH/OBA places the responsibility for implementing its requirements in the hands of an IBC; the IBC for the Harvard Medical School affiliates in the Longwood Medical Area is COMS. In compliance with the *NIH Guidelines*, the COMS includes representatives from the general public as part of its Committee membership. All research involving the use of rDNA and infectious microorganisms, including BBPs, must be registered with the COMS. Additional information about COMS can be found on the website (<http://www.hms.harvard.edu/orsp/coms>).

The primary purpose of COMS is to ensure the safe handling and management of potentially hazardous biological materials. The primary responsibilities of COMS are to:

- Promote the best practices for the safe handling and disposal of potentially hazardous and infectious biological materials.
- Ensure compliance with all relevant federal, state, and local regulations for work with biohazardous materials.

The functions of COMS are as follows:

- Recommend appropriate biosafety-related policies and procedures for management of potentially hazardous biological materials.
- Serve as a resource for technical information for biological risk assessment and reduction of exposures to biohazards.
- Keep current on regulations pertaining to the use of potentially biohazardous materials.
- Assist investigators in identifying technical resources and relevant information related to biosafety.

1.3.1 Registering Research Projects with COMS

Each project, study, or experiment using potentially hazardous biological materials must be registered with the COMS. Examples of such materials include rDNA, organisms containing rDNA, unfixed human tissues, immortalized human cell lines, pathogens, and

toxins. Clinical trials involving human gene therapy, vaccine development, and xenotransplantation must also be registered with COMS. The principal investigator (PI) is responsible for completing the appropriate project registration forms. This may be delegated to another person working in the research laboratory. The PI must review all COMS applications and sign the Memorandum of Understanding prior to submittal to ensure (s)he is aware of specific responsibilities as a result of the research being performed in his/her laboratory.

COMS registration forms can be downloaded from the COMS website at <http://www.hms.harvard.edu/orsp/coms/forms.htm>. If there are questions about completion of the forms, please contact the Wyss Institute Biosafety Officer (BSO), Jessica Healey, by e-mail at jhealey@eheinc.com or by telephone: 800-825-5343 (office) or 774-244-7018 (cell).

Each registration form will be reviewed by the BSO to verify its completeness and is then submitted to COMS for final approval. The PI will receive an approval letter signed by the COMS Chair that contains specific information about biosafety procedures and containment for the project. Copies of the registrations are also maintained by the Wyss Institute EH&S Office.

1.3.2 Helpful Hints for COMS Registrations

In order to expedite the review of COMS applications, please review the following "Helpful Hints." By providing appropriate information in the application, the review process by the BSO and COMS will be completed in as timely a manner as possible.

- On the front page, be sure to give an accurate mailing address. Once the application has been approved, COMS will use this mailing address for annual renewals and other written correspondence.
- **Read the *Memorandum of Understanding and Agreement* section carefully.** The signatory agrees that (s)he will provide biosafety training, maintain a safe work environment, and notify COMS immediately of accidental exposures or if a staff member develops symptoms related to the agents used in the laboratory.

- In the “Describe the Experiment in Detail” section, provide a detailed, but brief description of the experiment. Include information on how all biological agents will be used. This should include each agent’s purpose in the experiment, route of administration (if applicable), and how the agent will be maintained and manipulated throughout the experiment. **Do not cut and paste information from other documents** such as animal protocols or grants into this section, as these documents may have information that is not relevant to the biological safety of the project.
- If the application includes citations or references scientific papers, please provide a copy of the full publication, if possible.
- List the specific strain and source (i.e., the name of the commercial company or research laboratory) of all biological agents and transgenic animals.
- If the project involves the use of animals, provide a copy of the animal protocol (Institutional Animal Care and Use Committee [IACUC] form) even if the animal application is pending the biosafety approval.
- In the “Describe the Biohazard Potential of These Experiments” section, list procedures where exposures might occur and the biosafety levels of agents in use, if known. **Leaving this section blank or stating “none” is not acceptable.** If viral vectors will be used to insert or knockout genes address the following issues in the Biosafety Section:
 - Identify any oncogenes, tumor suppressor genes, toxin producing genes, or other genes with the potential to cause harm if expressed or knocked out.
 - Indicate if the inserted gene has the potential for altering the cell cycle.
 - Indicate if the viral DNA can integrate into the host genome.
 - Discuss the probability of generating replication-competent viruses including methods to reduce this probability such as multiple plasmid systems and self-inactivation.
 - Discuss the expected consequences of a human exposure to the vector.

1.4 RESPONSIBILITIES

The following section outline the specific responsibilities associated with the Wyss Institute biosafety program.

1.4.1 Principal Investigator

PIs are responsible for implementation of the applicable biosafety procedures and practices in their laboratories. They must ensure that the appropriate equipment and facilities are available for laboratory staff members and used properly. They must also arrange for appropriate employee training regarding the safe use of potentially hazardous biological agents and require that individuals handling BBPs 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 responsibilities of the PI above, when research involves the use of rDNA, the PI agrees to maintain full compliance with the *NIH Guidelines*. Under Section IV-B-7 of the *NIH Guidelines*, the PI has a number of specific responsibilities. In particular, **the Principal Investigator must** (among other tasks):

- Ensure that no research is conducted with biological materials prior to approval by COMS.
- Obtain COMS approval for rDNA research prior to initiation (Sections III-1, III-B, III-C, and III-D of the *NIH Guidelines*).
- Determine whether experiments are covered by Section III-E of the *NIH Guidelines* (IBC Notice Simultaneous with Initiation).
- Ensure that reporting requirements are fulfilled and be held accountable for any reporting lapses.

- Report any significant problems, violations of the *NIH Guidelines*, or any research-related accidents, illnesses, or potential exposures to the Wyss Institute Biosafety Officer, COMS, NIH/OBA, and the City of Boston Public Health.
- Be adequately trained in good microbiological techniques.
- Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents. This instruction should be specific to the agents and materials used in the research project. The Wyss Institute EH&S Office recommends documentation of this training for compliance purposes.
- Make available to all laboratory staff protocols that describe the potential biohazards and the precautions to be taken with the agents to be used.
- Comply with shipping requirements for rDNA molecules (Appendix H of the *NIH Guidelines*).

Additional responsibilities of the PI when working with rDNA are located in the *NIH Guidelines*

(http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm#_Toc7261589)

and the COMS Policy Manual

(<http://www.hms.harvard.edu/orsp/coms/BiosafetyResources/HarvardResources.htm>).

Failure to comply with the *NIH Guidelines* by one PI could affect all NIH-funded projects at the Wyss Institute; therefore, compliance is absolutely mandatory.

1.4.2 Laboratory Staff Responsibilities

Laboratory staff members are responsible for following the Wyss Institute health and safety policies and the procedures outlined in this Biosafety Manual and instructions from their PIs and the BSO. They need to comply with the NIH/OBA, CDC, OSHA, MADPH, and the City of Boston regulations, use safe laboratory practices, and inform the PI, laboratory supervisor, or BSO regarding any potentially hazardous situations or conditions.

1.4.3 Biosafety Officer

Per Harvard's COMS Policies and Procedures Manual, the BSO is the primary intermediary between investigators and COMS. The BSO responsibilities include:

- Managing the biosafety program and implementation of COMS policies and procedures at the Wyss Institute. This includes annual updating of records for all laboratories registered with COMS. Records of infectious agents, recombinant DNA activities and Select Agents must be kept current.
- Assisting laboratories in conforming to pertinent regulatory guidelines and COMS policies by providing training, facility inspection, and communication of program requirements.
- Conducting annual inspections of laboratory containment, procedures, records, and equipment for laboratories using Biosafety Level (BL) 2, Animal Biosafety Level 2 (BL2N), and Biosafety Level 2 with stipulations (a.k.a. BL2+) practices and procedures, and Select Agents, to ensure that laboratory standards are rigorously fulfilled. Less frequent inspections of BL1 laboratories are necessary.
- Screening research protocol applications proposed by PIs and submitting them to COMS for approval. The BSO will determine whether or not more information is necessary and, if so, will communicate this need to the PI. Once the revised application is complete, the BSO writes a memorandum to COMS summarizing the salient characteristics of the study, listing any COMS precedents, and recommending whether the study should be administratively approved or if it requires review and approval from the full COMS committee.
- Reporting to COMS on the program status.

In addition, the BSO is responsible for:

- Preparing Biosafety Officer Memorandum to Principal Investigators explaining the requirements associated with their COMS recommendations.

- 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 COMS, IACUC, Institutional Review Board (IRB), and researchers.
- Under Section IV-B-3 of the *NIH Guidelines*, conducting a Hazard Analysis/Risk Assessment when a PI proposes to work with a biological material. Refer to Section 2 for more information.
- Providing technical advice to PIs and COMS on research safety procedures.
- Developing emergency response plans for handling spills and personnel contamination and for investigating accidents involving rDNA research.
- Reporting to COMS and the Wyss Institute any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the BSO becomes aware, unless the BSO determines that a report has already been filed by the PI.
- Providing advice on laboratory security

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 of the biological material and infectious dose
- 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 is insufficient to perform a risk assessment. For these situations, the following conservative approach should be used:

- Universal precautions should always be followed, and barrier protections applied (Gloves, gowns, eye protection), regardless of the origin of the samples.
- Biosafety level 2 should 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 BSO should consider the following:

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

2.3 GENETICALLY MODIFIED MICROORGANISMS

When a PI is proposing to work with genetically modified microorganisms (GMMO), the BSO should consider the characteristics of donor and recipient/host organisms. In addition, s/he should 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
 - Recipient immune status
 - Consequences of exposure
- Arising from the alteration of existing pathogenic traits
 - 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 the Harvard designation BL2 with stipulations (a.k.a. BL2+) that has been established by COMS, are summarized in Table 3.1.

Biosafety Level	Risk Group	Examples
BL1	Individual risk: LOW Community risk: LOW	<i>Escherichia coli</i> Adeno-associated viruses
BL2	Individual risk: MODERATE Community risk: LOW	<i>Streptococcus</i> <i>Staphylococcus</i> Hepatitis B and C viruses Adenoviruses Most retroviruses and lentiviruses
BL2+ ¹	Individual risk: MODERATE Community risk: LOW	Retroviruses and lentiviruses that express toxic genes Prions
BL3	Individual risk: HIGH Community risk: MODERATE	Human immunodeficiency virus (HIV) types 1 and 2 West Nile virus
BL4	Individual risk: HIGH Community risk: HIGH	Ebola virus

¹ This classification has been established by the Committee on Microbiological Safety and is not included in the *Biosafety in Microbiological and Biomedical Laboratories*.

Appendix A contains specific information drawn from the BMBL concerning BL1, BL2, and BL3, which applies to BL2+ (also referred to as BL2 with Stipulations).

3.1 BIOSAFETY LEVELS 1 AND 2

The majority of laboratory work at the Wyss Institute 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 3.2 provides a brief summary of the biosafety level criteria for BL1 and BL2. Refer to Appendix A for details regarding Standard Microbiological Practices, Primary Barriers, and Secondary Barriers.

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 (refer to Appendix A)	PPE includes laboratory coats; gloves; eye protection as needed	<ul style="list-style-type: none"> • Doors for access control • Sink for hand washing • Work surfaces, floors, benches, and furniture should be impervious to moisture, easily cleaned/disinfected, and resistant to heat and chemicals.
BL2	Associated with human disease. Potential hazards from percutaneous injury, ingestion, and mucous membrane exposure.	BL1 practices plus: <ul style="list-style-type: none"> • Limited access • Biohazard signs • PPE • Disposal or proper cleaning of PPE • Sharps precautions • Biosafety manual that defines any biological waste decontamination policies 	<ul style="list-style-type: none"> • 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; eye and face protection, as needed 	BL1 plus: <ul style="list-style-type: none"> • Self-closing, lockable doors • Properly-installed biosafety cabinets (refer to BMBL) • In-line vacuum filters • Readily accessible eyewash station. • A method for decontaminating all laboratory wastes should be available in the facility (e.g. autoclave, chemical disinfection, incineration, or other validated decontamination method).
PPE personal protective equipment				

3.2 BIOSAFETY LEVEL 2+ (BL2 WITH STIPULATIONS)

BL2 with stipulations (a.k.a. BL2+) work is performed in a BL2 facility used in conjunction with BL3 procedures and work practices with the appropriate safety equipment (safety centrifuge cups, biosafety cabinets, disposable labware, etc.). This BL2+ containment level affords a greater margin of safety for personnel for instances when BL3 containment is not necessary.

BL2+ involves infectious agents that do not have a documented aerosol route of exposure. This containment level is also 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.

The most common example of biological agents that require BL2+ conditions are lentiviral and retroviral vectors that express an oncogene or silence a tumor suppressor gene. BL2+ conditions are defined as the use of BL2 practices plus the following practices:

- The BL2+ laboratory must be self-contained. If a centrifuge cannot be located in the laboratory, then rotors and centrifuge cups must be opened inside a biosafety cabinet within the laboratory.
- Strict needle and sharps precautions must be observed.
- All work must be done in a biosafety cabinet.
- Vacuum lines must be protected with filters.
- Gloves must be worn for handling cultures.
- Before beginning *in vitro* work with the vectors, the PI must provide the COMS with a protocol for testing the viral vector preparations for replication competence. The sensitivity of the assay must be indicated.

- Vector preparations must be tested for the presence of replication-competent virus. Testing result records must be maintained and will be subject to periodic evaluation.
- Before beginning any animal experiments under this registration, the COMS must be provided with a protocol for testing whether virus is shed from treated animals.
- Treated animals must be tested for viral shedding.
- Waste materials may be required to be autoclaved prior to disposal depending on the risk assessment conducted by BSO.

Further personal protective equipment, protocol, training, or engineering requirements may be stipulated by COMS. A training video on COMS-approved BL2+ laboratory practices is available on the following website link:

<http://mycourses.med.harvard.edu/MediaPlayer/Player.aspx?v=%7bA673BE45-C2E3-4FCE-AE11-9A623C9A09DF%7d>

4.0 LABORATORY PRACTICES

4.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) is an essential element laboratory safety, and must be provided to all staff members by their respective institutions free of charge. PPE provided to staff members includes, but is not limited to:

- Gloves
- Disposable or reusable laboratory coats (impervious to liquids)
- Side shields (for glasses)
- Face shields
- Surgical masks
- Safety glasses
- Prescription safety glasses
- Goggles
- Hoods
- Shoe covers
- Respiratory protection (e.g., N95 respirator)
- Other site-specific PPE

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 these materials could potentially be splashed in the face. Laboratory personnel shall wear other personal protective equipment (apron, face shield, surgical mask, N95 respirator, 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 or laundered by an outside vendor by placing it into designated biohazard bags provided by the vendor.

NOTE: If a particulate respirator (e.g., N95 respirator) is required as part of your PPE, you must be medically cleared to wear one and fit tested prior to use.

Contact the Wyss Institute's EH&S Office for additional information.

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 standard microbiological practices, BSCs can protect both laboratory personnel and the environment. Although many may think that the principle function of BSCs is to protect cells and cultures from contamination by bacteria and fungi, **their primary purpose should be to protect the laboratory workers from exposures to potentially infectious agents.**

BSCs are designated as Class I, II, or III based on specific airflow patterns within the BSC and on the locations of high efficiency particulate air (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 Class II, A2 BSC.

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)

Table 4.1 Continued

NSF National Sanitation Foundation
ft/min feet per minute

¹ Information from Baker Labs.

² Under no circumstances should the chemical concentration approach the lower explosion limits of the compound.

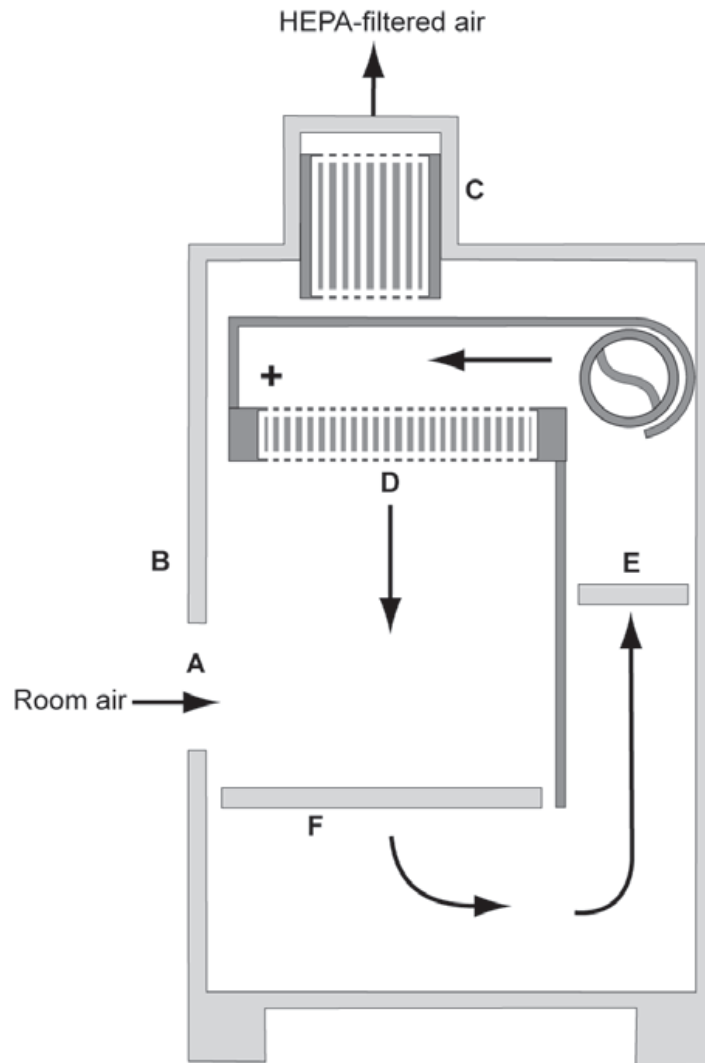


Figure 4.1 Tabletop Model of a Class II, Type A2 Biosafety Cabinet

(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. The Class II Type A2 BSC is not equivalent to what was formerly called a Class II Type B3 unless it is connected to the laboratory exhaust system. Note: The A2 BSC should be canopy connected to the exhaust system. (Figure taken from *Biosafety in Microbiological and Biomedical Laboratories*, Fifth Edition, 2009.)

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.
- Bunsen burner use will cause airflow disruptions and damage to the HEPA filter, and should be avoided. Refer to the Bunsen Burners Policies developed for the Wyss Institute by contacting the Wyss Institute EH&S Office.
- Certification must be performed annually.

BSCs are required to be tested and certified annually by technicians accredited by the National Sanitation Foundation (NSF International). Additionally, BSCs will be certified when they are first 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 EH&E staff at 617-432-7222 or 800-825-5343 or an EH&E employee when s/he is on-site Tuesdays for additional assistance with BSC certifications.

NOTE: 800-825-5343 is the phone number for the main switchboard for EH&E, which is the company supplying biosafety support. Please specify the employee when the receptionist answers the phone. For biosafety emergencies, please call HUPD at 617-432-1212 and specify the type of emergency.

4.3 DISPOSAL OF BIOLOGICAL WASTE

4.3.1 Biological Waste

Biological waste may be disposed of in three ways: designated biological waste box, chemical disinfection, and steam sterilization/autoclave. Appropriate disinfection procedures will be chosen and utilized in accordance with both the PI and the BSO in order to ensure adequate decontamination of biological wastes.

Infectious and potentially infectious waste and waste containing rDNA may only be disposed of in designated biological waste boxes (Figure 4.2). Each box is labeled with the universal biohazard symbol (Figure 4.3) 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 should be individually sealed with tape

and the box itself sealed with two-inch tape. Do not overfill the boxes. Boxes that leak any liquid or that exceed 55 pounds will not be moved or removed for disposal.

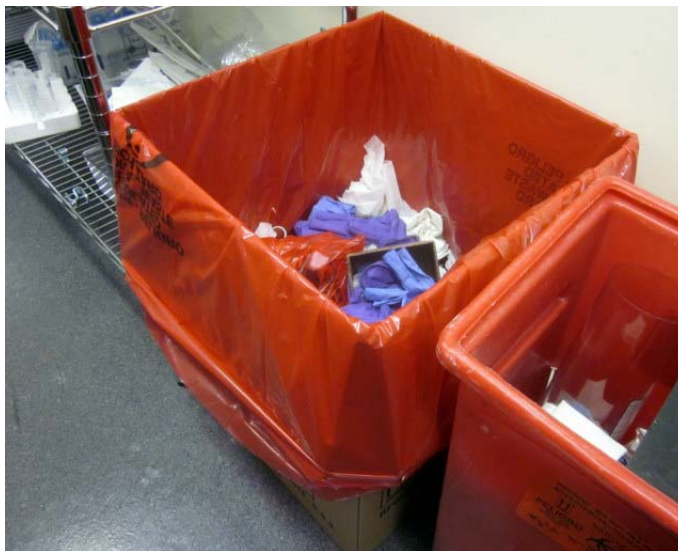


Figure 4.2 Biological Waste Box



Figure 4.3 Universal Biohazard Symbol

Liquid biological waste 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 for bleach.

NOTE: If bleach is not an adequate disinfectant for the biological agent in use, an U.S. Environmental Protection Agency (EPA) approved disinfectant must be used and the disinfection protocol must be approved by COMS. Ensure the proper contact time prior to disposal.

Before disposing of the treated solution down the sink, check the pH to ensure 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 the permissible range, then disposal of the treated solution in the sink should be done with running tap water to minimize possible plumbing damage due to the corrosive effects of the disinfectants. Autoclaving solutions containing bleach is **not permitted** due to the potential for production of toxic chlorine gas.

4.3.2 Biological/Radionuclide Waste

Refer to the radioactive waste section of Harvard University's Radiation Safety Manual for guidance, or contact Harvard Radiation Safety at 617-496-3979.

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. Check with EH&E staff at 617-432-7222 or 800-825-5343, or when an EH&E employee is on-site Tuesdays if there are any questions.

NOTE: 800-825-5343 is the phone number for the main switchboard for EH&E, which is the company supplying biosafety support. Please specify the employee when the person answers the phone. For biosafety emergencies, please call HUPD at 617-432-1212 and specify the type of emergency.

4.4 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. Sharps must be disposed of separately from all other waste streams and sharps containers cannot be disposed of with other biological waste.

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.
- Throw away the entire syringe-needle combination.
- Be careful during cleanup; some sharp items may be hidden in the waste materials.
- If a needle stick occurs, encourage the wound to bleed for a few minutes, 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/index.html>. Laboratories at the Wyss Institute are required to evaluate the use of safety needles whenever possible, and if feasible, select safety needles for use. Please refer to Section 9 of this document, which is the Wyss Institute Exposure Control Plan, for details.

4.4.1 Sharps Disposal

To prevent 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 the biological waste red bags should be disposed of in Sharps Boxes. Sharps containers must be red, fluorescent orange or orange-red leakproof, rigid, puncture-resistant, shatterproof containers 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.



Figure 4.3 Sharps Container

4.4.2 Broken and Clean Glassware Disposal

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

4.4.3 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 broken glassware boxes. 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 bleaches: e.g., Clorox™*	1:10	Bacteria, some spores, viruses, TB†, HIV	Some spores
Chlorine dioxide: e.g., Clidox®-S*	1:5:1 or 1:18:1	Bacteria, spores, viruses, TB	
Alcohols (ethanol, isopropanol)	70%	Bacteria, most viruses	Spores, TB
Quaternary ammonium compounds: e.g., Quatricide®*	Ready to use	Bacteria, spores, viruses, TB	
TB tuberculosis HIV human immunodeficiency virus * The use of brand names does not imply a recommendation. † Use 1/5 dilution			

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. It is the steam that kills.

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 1 hour to reach sterilizing temperatures throughout its contents.

Massachusetts regulations 105 CMR 480 requires that if an autoclave is used 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 MADPH website:

http://www.mass.gov/Eeohhs2/docs/dph/environmental/sanitation/105cmr480_medical_waste_on_site_log.pdf.

4.6.1 Autoclave Testing and Validation

Autoclaves should be tested quarterly and validated to insure that they are operating properly and killing the biological organisms in each autoclave load. The preferred method for autoclave validation 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. Frequent validation is not necessary, unless required by regulatory authorities in special circumstances. Using an established autoclave test procedure, quarterly checks with a biological indicator are usually adequate to assure proper autoclave function and to detect gradual deterioration of operation. 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.

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 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.

Massachusetts regulations 105 CMR 480 requires that if an autoclave is used for the treatment of infectious waste, quarterly efficiency testing, maintenance, and parametric monitor calibration must be performed.

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 1.0×10^4 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.

4.7 SPILL MANAGEMENT

4.7.1 Management of Small Spills (spills <10 ml or that can be absorbed using two paper towels)

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 BSC.

- 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 BSC, keep the cabinet turned on.
- 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.
- Remove PPE and place disposable PPE into a biological waste box. Reusable PPE should be cleaned with the proper disinfectant.
- Wash hands and exposed skin areas thoroughly with soap and water.

4.7.2 Management of Large Spills (spills >10 ml)

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 indicating that there was a spill in the BSC, the steps taken to treat/contain the spill, and contact information for a responsibly party.

- Remove any contaminated clothing and put it into a biohazard bag for decontamination later.
- Wash hands and exposed skin thoroughly with soap and water.
- Call HUPD at 617-432-1212 to report the size, location, and composition of the spill.
- Complete an incident report form (Appendix B).

5.0 IMMUNIZATIONS AND MEDICAL RESTRICTIONS

Certain biological materials require personnel working with them to receive immunizations and/or have medical restrictions.

5.1 VACCINIA

If any researcher working at the Wyss Institute wishes to use vaccinia or vaccinia constructs in their research, they must first contact the Wyss Institute EH&S Office and his/her institution's Occupational Safety and Health Department for information about medical restrictions and vaccination.

5.2 HEPATITIS B VACCINE

Under the OSHA BBP Standard, a hepatitis B vaccine is recommended for all employees working with human blood, body fluids, or tissues. Those employees declining vaccination will be asked to sign the OSHA waiver indicating that hepatitis B vaccine has been offered and refused. Any questions should be directed to the home institution's Occupational Health Department where the employee/researcher is primarily affiliated.

5.3 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

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. Please contact the Wyss Institute EH&S Office for further information. Women who become or wish to become pregnant, should inform their obstetrician and gynecologist of any infectious agents and any chemicals that may encountered in their work.

Women who wish to become pregnant, or who become pregnant while working in the BL2+ laboratories, are encouraged to inform their supervisors or PIs, the Wyss Institute EH&S Office, her institution's Occupational Safety and Health Department, and Harvard Radiation Safety. Such employees are urged to discuss exposure issues relating to fetal infection and radiation exposure with their supervisors or PIs. The BSO is available to give advice about precautions which might be necessary to protect the fetus and mother against exposure to infectious materials. Contact the Wyss Institute EH&S Office for precautions when working with hazardous chemicals, and Harvard Radiation Safety for precautions when working with radioisotopes during pregnancy.

5.4 OTHER MEDICAL RESTRICTIONS

Restrictions or recommendations will be made on an individual basis after discussion with either an occupational medicine practitioner or the affected individual's personal physician.

Examples of some conditions that might warrant special precautions are HIV infection, immunosuppressive conditions, or drug therapy that suppresses the immune system. Therefore, anyone who has any of the above-mentioned conditions is required to inform their personal physician and their home institution's Occupational Health Department about any issues that prevent them from being able to work in a BL2+ laboratory.

6.0 LABORATORY SAFETY TRAINING INFORMATION

General laboratory safety information, including biological safety training is provided for all Wyss Institute laboratory staff by the Wyss Institute EH&S Office. Laboratory staff are required to take this training annually. Currently, the Wyss Institute EH&S Office offers the training in both an online format and scheduled in-person trainings. These training options provide, at a minimum, the following topics:

Acrylamide	Formaldehyde
Autoclaves	Gel Electrophoresis
Biosafety	Glutaraldehyde
Bloodborne Pathogens	Hazard Communication
Cell and Tissue Culture	Hazardous Waste
Centrifuge	Laboratory Animal Research
Chemical Hygiene Plan	Laboratory Ergonomics
Compressed Gases	Laboratory Move Policy
DEA Controlled Substances	MWRA Sewer Use Permit
Electrical Safety	Office Ergonomics
Emergency Action Plan	Polymerase Chain Reaction
Ethidium Bromide	Regulated Medical Waste
Ethylene Oxide	Research With biological Agents
Fall Prevention	Shipment of Dangerous Goods
Fire Department	Sustainability
Flammable and Combustible Liquids	Ultraviolet Light

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.

Laboratory staff may receive awareness level training from the Wyss Institute 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. For assistance regarding training and other requirements necessary for the legal shipping of hazardous materials, please contact the Wyss Institute 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 regulation (49 CFR 171 et al.), please see <http://hazmat.dot.gov/67fr-53118.pdf>; for more information about shipping packaging materials contact a courier, a laboratory supplier that sells shipping materials (i.e., Saf-T-Pak[®], Fisher or VWR), or the EH&S Office.

8.0 GENERAL LABORATORY SAFETY AND BIOLOGICAL SAFETY INSPECTIONS

Various federal, state, and local regulations require that laboratory inspections are conducted on an annual basis for all BL2 laboratories and higher, and once every two years for BL1 laboratories. Copies of the inspections forms are available from the Wyss Institute EH&S Office.

Laboratory inspections are typically scheduled beforehand to ensure the visit to the laboratory does not create a disruption; however, the Wyss Institute 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 after 30 days to make certain that all conditions are corrected.

9.0 BLOODBORNE PATHOGENS AND THE EXPOSURE CONTROL PLAN

9.1 BACKGROUND

Laboratory workers who come in contact with human blood or other human bodily fluids are at increased risk for exposures to and infections from certain BBP, such as HIV, HBV, and HCV. Exposures to BBP can occur in a variety of ways. The most common route of exposure is a needle stick injury, but transmission may also occur through contact with mucous membranes or through cuts in the skin.

In response to these health concerns, the federal government issued the OSHA BBP Standard (29 CFR 1910.1030) in December 1991. The primary purpose of the BBP Standard is to eliminate or minimize occupational exposures to blood and other bodily fluids, as well as the risks for developing the infectious diseases associated with them. In addition to HIV and the hepatitis viruses, the BBP Standard covers a wide variety of bloodborne infectious agents that can cause disease. Some of the included agents are simian immunodeficiency virus and the biological agents that cause syphilis, malaria, babesiosis, brucellosis, leptospirosis, relapsing fever, arboviral infections, Creutzfeldt-Jacob disease, and viral hemorrhagic fevers.

Sources of potential exposures to BBP include human blood and a variety of potentially infectious materials (PIMs). The OSHA definition of human blood includes whole blood, blood products, and blood components. PIMs include body fluids, such as saliva, semen, vaginal, cerebrospinal, synovial, pleural, peritoneal, pericardial, amniotic fluids, any body fluid in which visible blood is present, and any unfixed tissue or organ from a human either living or dead. Cell or tissue cultures, organ cultures, or media containing HIV, HBV, or HCV are also included.

OSHA has designated the term “standard precautions” as the approach for controlling against infections from BBP. The concept is that all human blood and certain body fluids are treated as if they contain HIV, HBV, or other BBP. In the laboratory environment, BL2 practices and containment are required for activities involving BBP. BL2 practices are also recommended for work with human cell lines that have been maintained in culture for decades, such as HeLa cells. Experienced researchers may argue that they

have worked with these cells for years without incident. However, even though the potential risks are low, current molecular biology manipulations may unknowingly depress proviral forms of viruses embedded in the DNA of these human cell lines. Therefore, BL2 practices should be implemented to reduce the potential for infections from such agents in these cell lines.

9.2 PURPOSE OF THE EXPOSURE CONTROL PLAN

The BBP Standard requires that an Exposure Control Plan (ECP) be written and implemented and that a copy of the ECP be available to employees. The ECP includes several required elements, policies, and procedures that are designed to eliminate or minimize BBP exposures. The purposes of the plan are to:

- Protect Wyss Institute staff members from the health hazards associated with BBP.
- Coordinate appropriate treatment and counseling should an employee be exposed to BBP.

9.3 EXPOSURE CONTROL PLAN PROGRAM MANAGEMENT

Responsibilities for implementation of the ECP are as follows:

- **Institute PIs:** Institute Principal Investigators are responsible for ensuring that proper work practices, such as standard precautions and use of PPE, are followed on a day-to-day basis.
- Wyss Institute **Staff Members:** All individuals working at the Wyss Institute are responsible for attending the annual training sessions, participating in the orientation program, knowing what tasks they perform that have potential occupational exposures to BBP and employing safe work practices in the performance of their duties.

9.4 EXPOSURE DETERMINATION

The following procedures have been implemented to identify employees that have occupational exposures to BBP. Each staff member is classified as either exposed or unexposed and is informed of their classification by respective supervisors.

1. Job classifications have been identified in which:
 - a. All employees have occupational exposure to BBP.
 - b. Some employees have occupational exposure to BBP.

These classifications are based on the individual's potential for coming in contact with any potentially infectious material and/or their duties as they relate to work in the laboratory. Employees with no exposure are also identified. Department managers or supervisors are responsible for reviewing and modifying their employee's classification as exposed or unexposed based on detailed knowledge of the employee's work responsibilities.

2. Lists of tasks and procedures during which occupational exposure may occur are maintained for employees identified above in 1b.

9.5 METHODS OF COMPLIANCE

PIs are responsible for ensuring the effectiveness of and compliance with the following controls and practices.

9.5.1 Standard Precautions

Standard precautions, also called Universal Precautions, are implemented by Wyss Institute staff members to minimize or prevent contact with PIMs. **REMINDER:** This means that all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other BBPs.

9.5.2 Engineering Controls

One of the key aspects of the Wyss Institute ECP is the use of engineering controls to minimize or eliminate BBP exposures. Staff members use equipment such as hand

washing facilities, sharps disposal containers, leak-proof containers for human blood and tissue samples, and biological safety cabinets, as appropriate. New engineering controls will be evaluated and implemented as they become available.

9.5.3 Work Practice Controls

In addition to engineering controls, Wyss Institute policies require a number of work practice controls to help eliminate or minimize employee exposure to BBP. New work practice controls will be evaluated and implemented as they become available.

- Employees must wash their hands immediately with soap and water, or as soon as feasible, after the removal of gloves or other personal protective equipment.
- Following any contact of body areas with blood or any other infectious materials, employees must wash their hands and any other exposed skin with soap and water as soon as possible. They also must flush exposed mucous membranes with water.
- Contaminated needles and other contaminated sharps are not bent, recapped, or removed unless:
 - It can be demonstrated that no feasible alternative is available.
 - The action is required by specific research procedure.
 - In the two situations above, the recapping or needle removal is accomplished through the use of a medical device or a one-handed technique.
- Contaminated reusable sharps are placed in appropriate containers immediately, or as soon as possible, after use.
- Mouth pipetting/suctioning of blood or other PIMs (OPIMs) is prohibited.
- All procedures involving blood or OPIMs minimize splashing, spraying, or other actions generating droplets of these materials.
- Specimens of blood or OPIMs are handled and stored in designated leak-proof containers that have been labeled appropriately.

9.5.4 Personal Protective Equipment

PPE, as described in Section 4.1, is a primary line of defense against BBP exposures. Wyss Institute staff members must be trained regarding the use of the appropriate PPE for their job classifications and for the activities they perform with BBP. Additional training will be provided by PIs or their designees, e.g., when an employee takes a new position or new job functions are added. BBP training is a component of the safety training provided by the Wyss Institute EH&S Office. BBP training is an annual requirement for laboratory employees working with BBP.

The following procedures are implemented during the handling of BBP:

- A laboratory coat is worn whenever potential exposure is anticipated. Disposable laboratory coats will be placed into biological waste boxes after each use. Reusable laboratory coats will be placed into biohazard bags provided by an outside laundry vendor.
- If any garments are penetrated by blood or other infectious materials, they are removed immediately, or as soon as is feasible.
- All PPE is removed prior to leaving a work area.
- Gloves are worn in the following circumstances:
 - Whenever employees anticipate hand contact with PIMs.
 - When handling or touching contaminated items or surfaces.
- Disposable gloves are replaced as soon as practical after contamination or if they are torn, punctured, or otherwise lose their ability to function as an exposure barrier.
- Utility gloves are decontaminated for reuse unless they are cracked, peeling, torn, or exhibit other signs of deterioration.
- Full-face protection, such as facemasks, face shields, and eye protection, is used whenever splashes or sprays may generate droplets of infectious materials.
- Head covers/hoods and/or shoe covers/boots are used in any instances where gross contamination is anticipated, such as perfusion activities.

9.5.5 Housekeeping

All laboratory surfaces are cleaned with a U.S. Environmental Protection Agency approved germicidal disinfectant. The disinfectant solution is applied in accordance with the manufacturer's recommendations. Laboratory personnel must clean decontaminated equipment and surfaces after contact with blood or other potentially infectious materials after the completion of procedures, immediately (or as soon as feasible) when surfaces are overtly contaminated, after any large spill of blood or infectious materials, or at the end of the work shift if the surface may have been contaminated during that shift.

9.6 VACCINATION PROGRAM AND POST EXPOSURE EVALUATION AND FOLLOW-UP

Staff members must contact the Occupational Health Department at their home Institution for information regarding vaccinations and post exposure follow up, and to obtain information about the HBV vaccination program.

9.6.1 Vaccination Program

The HBV vaccination program is available, at no cost, to all staff members who have occupational exposures to BBP. Those who decline to take part in the vaccination program must sign the "Vaccination Declination Form" and will have the opportunity to be vaccinated at a later date.

9.6.2 Post-Exposure Evaluation and Follow-Up

If an employee is exposed to blood or OPIMs, the following procedures must be performed immediately:

1. Remove contaminated clothing.
2. Wash and flush the affected area vigorously with soap and water for 15 minutes.
3. Report to the institution's Occupational Health Department or the Emergency Department for treatment immediately.
4. Notify the Wyss Institute EH&S Office.
5. Be certain to complete an incident report.

9.6.3 Investigation of Circumstances Surrounding Exposure Incidents

Researchers are responsible for reporting exposure incidents to their laboratory manager or supervisor. The investigation is initiated as soon as possible after the incident is reported and involves gathering the following information:

- When the incident occurred.
 - Date and time.
- Where the incident occurred.
 - Location within the facility.
- What PIMs were involved in the incident.
 - Type of material (blood, specific infectious agent, etc.).
- Source of the material.
- Under what circumstances the incident occurred.
 - Type of work being performed.
- How the incident was caused.
 - Accident.
 - Unusual circumstances, such as equipment malfunction.
- PPE being used at the time of the incident.
- Actions taken as a result of the incident.
 - Employee decontamination.
 - Clean up.
 - Notifications to supervisors.

After this information is gathered and evaluated, a written summary of the incident and its causes is prepared and recommendations are made for avoiding similar incidents in the future.

9.7 TRAINING AND RECORD KEEPING

Staff members with potential occupational exposures to BBP must receive annual training in accordance with the BBP Standard. This training is offered through the Wyss Institute EH&S Office on a frequent basis, and is also offered as part of an on-line training program. PIs and laboratory supervisors are responsible for ensuring that all employees with potential occupational exposures to BBP participate in this training.

10.0 WORKING WITH LABORATORY ANIMALS

10.1 INTRODUCTION

Working with animals in a laboratory setting can present risks from infections and injuries to all personnel. Those 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 and protective procedures in place at the Wyss Institute regarding laboratory animal use.

All use and handling of animals at the Wyss Institute must be conducted safely and humanely, and in compliance with all institutional and federal regulations. The Harvard Center for Comparative Medicine (HCCM) Policy and Procedure Manual (<http://arcm.med.harvard.edu/>) provides details for the required training and the procedures that must be followed when working with laboratory animals. When working with hazardous agents in and around animals, please refer to the applicable Sections in this Manual for specific guidance.

Any unsafe or hazardous behavior or work conditions regarding the use of animals needs to be reported to the Animal Resources Manager, Attending Veterinarian/Director, or the IACUC.

Prior to commencing any work **in animals** that utilize BL2 pathogens, it is **mandatory** that all Investigators contact HCCM to report the pathogen name(s) and the animal facility requested for such use. All Investigators must contact the HCCM Office of the Director at 617-432-1289 and speak directly to Dr. Arthur Lage or Linda Janse.

10.2 ALLERGIES

Allergic reactions to animals are among the most common conditions that adversely affect the health of workers involved in 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 the home institution's Occupational Health Department.

Guidelines for working with animals are summarized as follows.

- Wear required PPE at all times when working with animals.
- Do not wear PPE outside of the animal facility.
- Wear gloves at all times when handling animals.
- Do not distribute animal bedding in the 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 fitted into ventilated racks and that static microisolator cage lids properly fit.
- Do not overpopulate animal cages.
- Work with animals in a ventilated hood or BSC when required and whenever possible.
- Work with animals in well ventilated areas when not working under a hood/cabinet.

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, D.C: National Academy Press.

³ Bush RK. 2001. Assessment and Treatment of Laboratory Animal Allergy. *ILAR Journal* 42:55-64.

- Clean and disinfect all equipment after use.
- Wash hands with soap and water frequently and always after handling animals (even when wearing gloves).
- Avoid touching eyes, nose or mouth 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.

10.3 ZONOSSES

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

10.3.1 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 mice are lymphocytic choriomeningitis virus (LCM) and hantavirus. Animals brought into the Animal Resource Centers are known to be free from these diseases and the Animal Health Monitoring program evaluates for these agents on a regular basis.

10.3.2 Rats

No known risk of zoonotic diseases results from typical exposure to the microbial flora of laboratory-reared rats. All rats at the HCCM have been raised commercially and have a non-pathogenic, well-defined microbial flora. Two diseases of concern when working with rats are hantavirus and rat-bite fever. Animals brought into the Animal Resource Centers 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 this bacterium from their animals.⁴

10.4 BITES AND SCRATCHES

Bites and scratches are hazards associated with all laboratory animals. A thorough understanding of species-specific rodent 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; even experienced animal 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, through respective occupational health services departments.

10.5 RESPONSIBILITIES

10.5.1 Institutional Animal Care and Use Committee

The Harvard IACUC will review all animal care and use protocols to ensure a safe working environment for laboratory personnel. The IACUC will work with HCCM staff to ensure that the animal care and use program complies with current regulations and standards. The IACUC also requires on-line training for occupational health and safety for all Animal Resource Center users.

⁴ National Research Council. 1997.

10.5.2 Principal Investigator

The PI is responsible for ensuring that research is conducted in accordance with Wyss Institute policies and safe laboratory practices. The PI is responsible for completing all appropriate hazardous agent protocols (radiation, chemical and biological hazards) and the IACUC protocol prior to the start of the research. The PI and/or a designee is responsible for obtaining necessary safety equipment and maintaining awareness of safety policies and procedures. The HCCM, the Wyss Institute EH&S Office, or Harvard Radiation Safety can be contacted for assistance.

10.5.3 Laboratory Staff

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

APPENDIX A
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 1 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.
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.
 - 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).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment. A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

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. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination 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. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
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-3 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 must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

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

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls is worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
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. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must 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-3 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 must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is

restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
 - a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
 - b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
 - c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must 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. All windows in the laboratory must be sealed.
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, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with 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 in the laboratory.

9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
 - a. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
 - b. The laboratory exhaust air must not re-circulate to any other area of the building.
 - c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated 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. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
12. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory

isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

APPENDIX B
INCIDENT REPORT FORM



INCIDENT REPORT FORM

Date: _____ Time of Incident: _____

Location of Incident: _____

Name: _____ Phone#: _____

Wyss Institute Affiliation: _____

Person Initiating call to EH&S: _____ Phone #: _____

Brief Characterization of Incident:

Describe the Incident:

Response Summary:

Recommended Corrective/Preventive Action:



Comments:

Wyss Institute EH&S Responder: _____

Date/Time of Resolution: _____

cc: Wyss Institute Operations Director
Wyss Institute Facility Manager

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

Signature of Person Filling Out Report: _____