

Biosafety Manual  
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## TABLE OF CONTENTS

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<b>CONTACT INFORMATION AND USEFUL WEBSITES .....</b>	<b>1</b>
CONTACT INFORMATION .....	1
WEBSITES .....	1
<b>1.0 INTRODUCTION TO BIOSAFETY .....</b>	<b>2</b>
1.1 BACKGROUND .....	2
1.2 REGULATIONS .....	2
1.3 PARTNERS INSTITUTIONAL BIOSAFETY COMMITTEE (PIBC) .....	4
1.4 RESPONSIBILITIES .....	5
<b>2.0 HAZARD ANALYSIS/RISK ASSESSMENT .....</b>	<b>7</b>
2.1 LIMITED INFORMATION .....	7
2.2 BIOLOGICAL EXPRESSION SYSTEMS .....	7
2.3 GENETICALLY MODIFIED MICROORGANISMS .....	8
<b>3.0 PRINCIPLES OF BIOSAFETY .....</b>	<b>9</b>
3.1 BIOSAFETY LEVELS 1 AND 2 .....	9
3.2 BIOSAFETY LEVEL 2+ .....	10
3.3 BIOSAFETY LEVEL 3 .....	12
<b>4.0 LABORATORY PRACTICES .....</b>	<b>13</b>
4.1 PERSONAL PROTECTIVE EQUIPMENT .....	13
4.2 BIOLOGICAL SAFETY CABINETS .....	14
4.3 DISPOSAL OF BIOLOGICAL WASTE .....	16
4.4 SHARPS MANAGEMENT .....	18
4.5 DISINFECTION AND DECONTAMINATION .....	20
4.6 AUTOCLAVING PROCEDURES .....	20
4.7 SPILL MANAGEMENT .....	22
<b>5.0 IMMUNIZATIONS AND MEDICAL RESTRICTIONS .....</b>	<b>24</b>
5.1 HEPATITIS B VACCINE .....	24
5.2 INFLUENZA VACCINE .....	24
5.3 REPRODUCTIVE HEALTH .....	24
5.4 OTHER MEDICAL RESTRICTIONS .....	25
<b>6.0 BIOSAFETY TRAINING INFORMATION .....</b>	<b>26</b>
<b>7.0 SHIPPING AND RECEIVING PROCEDURES FOR BIOLOGICAL SPECIMENS .....</b>	<b>27</b>
<b>8.0 GENERAL LABORATORY SAFETY AND BIOLOGICAL SAFETY INSPECTIONS .....</b>	<b>29</b>
<b>9.0 WORKING WITH LABORATORY ANIMALS .....</b>	<b>30</b>
9.1 INTRODUCTION .....	30
9.2 ALLERGIES .....	30
9.3 ZOOSES .....	31
9.4 BITES AND SCRATCHES .....	32
9.5 RESPONSIBILITIES .....	33

## LIST OF APPENDICES

**Appendix A**     Laboratory Biosafety Level Criteria

## TABLE OF CONTENTS (CONTINUED)

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### LIST OF TABLES

<b>Table 3.1</b>	Biosafety Level Classifications for Biological Agents
<b>Table 3.2</b>	Summary of Biosafety Level Criteria for BL1, BL2, and ABL2
<b>Table 4.1</b>	Biological Safety Cabinet Characteristics
<b>Table 4.2</b>	Summary of Disinfectants and Their Uses

### LIST OF FIGURES

<b>Figure 4.1</b>	Class II, Type B1 Biological Safety Cabinet (classic design)
<b>Figure 4.2</b>	Universal Biohazard Symbol
<b>Figure 4.3</b>	Sharps Container

### LIST OF ABBREVIATIONS AND ACRONYMS

<b>ABL</b>	animal biosafety level
<b>BBP</b>	bloodborne pathogens
<b>BMBL</b>	Biosafety in Microbiological and Biomedical Laboratories
<b>BSC</b>	biological safety cabinet
<b>BL</b>	biosafety level
<b>BSO</b>	Biosafety Officer
<b>CDC</b>	Centers for Disease Control and Prevention
<b>CFR</b>	Code of Federal Regulations
<b>CMR</b>	Code of Massachusetts Regulations
<b>DNA</b>	deoxyribonucleic acid
<b>DOT</b>	U.S. Department of Transportation
<b>EH&amp;S</b>	Environmental Health and Safety
<b>EPA</b>	U.S. Environmental Protection Agency
<b>FDA</b>	U.S. Food and Drug Administration
<b>GMMO</b>	genetically modified microorganism
<b>HBV</b>	hepatitis B virus
<b>CCM</b>	Center for Comparative Medicine
<b>HCV</b>	hepatitis C virus
<b>HEPA</b>	high efficiency particulate air
<b>HIV</b>	human immunodeficiency virus
<b>IACUC</b>	Institutional Animal Care and Use Committee
<b>IATA</b>	International Air Transport Association
<b>IBC</b>	Institutional Biosafety Committee
<b>LAA</b>	laboratory animal allergies
<b>MGH</b>	Massachusetts General Hospital
<b>NIH</b>	National Institutes of Health
<b>OSHA</b>	U.S. Occupational Safety and Health Administration
<b>PHS</b>	U.S. Public Health Service
<b>PI</b>	principal investigator
<b>PIBC</b>	Partners Institutional Biosafety Committee
<b>PPE</b>	personal protective equipment
<b>Ragon</b>	The Ragon Institute
<b>rDNA</b>	recombinant DNA
<b>USDA</b>	U.S. Department of Agriculture
<b>°C</b>	degrees Celsius

## CONTACT INFORMATION AND USEFUL WEBSITES

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### CONTACT INFORMATION

Name/Title	Phone Number	E-mail
Anthony Troiano, Biosafety Officer	781-707-8438	atroiano@eheinc.com
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Alexandria Real Estate Management Office (8:30 am – 5:00 pm)	617-661-6962	
Security at Tech Square (after hours)	617-577-9177	
MGH Occupational Health Department at 165 Charles River Plaza, Suite 404, Boston	617-732-8501	

### WEBSITES

Department	Contact For
Partners Institutional Biosafety Committee (PIBC)	<a href="https://partnershealthcare.sharepoint.com/sites/phrmDepartments/poc/pibc/Pages/Key-Contacts.aspx">https://partnershealthcare.sharepoint.com/sites/phrmDepartments/poc/pibc/Pages/Key-Contacts.aspx</a>
National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA and Synthetic Nucleic Acid Molecules (NIH Guidelines)	<a href="http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines">http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines</a>
NIH/Centers for Disease Control and Prevention (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL)	<a href="http://www.cdc.gov/biosafety/publications/index.htm">http://www.cdc.gov/biosafety/publications/index.htm</a>
OSHA Bloodborne Pathogen Standard	<a href="http://www.osha.gov/SLTC/bloodbornepathogens/index.html">http://www.osha.gov/SLTC/bloodbornepathogens/index.html</a>

## 1.0 INTRODUCTION TO BIOSAFETY

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### 1.1 BACKGROUND

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

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

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

The first is the *NIH Guidelines for Research Involving Recombinant DNA and Synthetic Nucleic Acid Molecules* (<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>). 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/bmb15/BMBL.pdf>); the most recent edition was published in 2009. These two publications classify work with biological agents into four distinct biosafety levels (BLs). Each of these levels is matched with progressively restrictive practices and laboratory design features that reduce health risks from exposures to potentially hazardous biological agents. These levels are further discussed in Section 3.

### 1.2 REGULATIONS

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

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

### 1.2.1 Federal

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

The use of Universal Precautions is a key element of any BBP program and must be followed at all times when working with human materials. Universal Precautions means that all samples are treated as potentially infectious, regardless of origin. For example, blood from any source, including HIV-seronegative control donors, must be handled as potentially infectious. Employees are trained in Universal Precautions techniques at orientation and on an annual basis. This training is offered through the Partners Massachusetts General Hospital (MGH) HealthStream online training program.

Safe practices for studies involving the use of rDNA are governed by the NIH Guidelines (<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>). It is Ragon policy that all laboratories comply with these Guidelines, which is also a City of Cambridge requirement.

### 1.2.2 Commonwealth of Massachusetts

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

### 1.2.3 City of Cambridge

All rDNA work conducted in the City of Cambridge is subject to the Cambridge Recombinant DNA Technology Ordinance. In general, the requirements set forth in the city ordinance agree with NIH and CDC guidelines. Further information can be found at

<http://www.cambridgepublichealth.org/services/regulatory-activities/rdna/overview.php>.

## 1.3 PARTNERS INSTITUTIONAL BIOSAFETY COMMITTEE (PIBC)

The PIBC serves as the Institutional Biosafety Committee (IBC) for Partners HealthCare System (PHS) member institutions engaged in biological research. These institutions include Massachusetts General Hospital, Brigham and Women's Hospital, Partners Research Building, McLean Hospital, and the Ragon Institute. The PIBC conducts specific review and oversight of biological research activities in compliance with the following guidelines and regulations:

- NIH—[\*NIH Guideline for Research Involving Recombinant and Synthetic Nucleic Acid Molecules \(NIH Guidelines\)\*](#)
- U.S. Occupational Safety and Health Administration—[OSHA Bloodborne Pathogen Standard 1910.1030](#)
- Massachusetts Department of Public Health—[Medical Waste Regulation](#)
- Cambridge Public Health Department—[Laboratory Biosafety Regulations](#)

Additional information on the responsibilities of the PIBC can be found on their website <https://partnershealthcare.sharepoint.com/sites/phrmDepartments/poc/pibc/Pages/Key-Contacts.aspx>

### 1.3.1 Registering Research Projects with PIBC

All biological research involving the use of recombinant and synthetic nucleic acid molecules, biological agents, human and nonhuman primate materials, and biological toxins must be registered with the PIBC. Projects are registered using the Insight Research Portal eIBC Module: <https://insight.partners.org/login/login.asp?CTAuthMode=BASIC>. To gain access to this system, email the PIBC office at [PIBC@partners.org](mailto:PIBC@partners.org).

Each completed registration will be reviewed by the Ragon Institute Biosafety Officer (BSO), who may request additional information or clarification from the submitter or Principal Investigator (PI). Once the BSO review is complete, the submission is sent to the PIBC office where it will be reviewed to determine if the research can be administratively approved. If the research falls under Sections III-A through III-E of the NIH Guidelines, it must be reviewed at a convened meeting of the PIBC. Additionally, research with biological agents is typically reviewed at a convened meeting. For additional details on the review process, please reference the PIBC website.

## 1.4 RESPONSIBILITIES

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

### 1.4.1 Principal Investigator

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

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

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

Additional responsibilities of the PI when working with recombinant or synthetic nucleic acid molecules are located in the NIH Guidelines. Failure to comply with the NIH Guidelines by one PI could affect all NIH-funded projects at Ragon; therefore, compliance is absolutely mandatory.

### 1.4.2 Laboratory Staff Responsibilities

Laboratory staff members are responsible for following the Ragon Institute health and safety policies and the procedures and instructions from their PIs and BSO. They need to comply with



all NIH, CDC and OSHA requirements, 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 PIBC Policies and Procedures, the BSO is the primary intermediary between investigators and the PIBC. BSO responsibilities include:

- Managing the biosafety program and implementation of PIBC policies and procedures at Ragon.
- Assisting laboratories in conforming to pertinent regulatory guidelines and PIBC policies by providing training, facility inspection, and communication of program requirements.
- Making annual inspections of laboratory containment, procedures, records, and equipment for laboratories working at BL1, ABL1, BL2, ABL2, BL2+, BL3, and ABL3
- Screening research protocols proposed by PIs and submitting to PIBC for approval. The BSO will determine whether more information is necessary and, if so, will communicate this need to the PI. Once the revised application is complete, the BSO completes a risk assessment for the PIBC summarizing the salient characteristics of the study and recommending an appropriate biosafety level to reviewers and/or the full committee.
- Reporting to the PIBC on the program status.

In addition, the BSO is responsible for:

- Providing advice on safe methods for new procedures.
- Recommending emergency response procedures in the event of an infectious spill or an exposure to a biological material.
- Obtaining any necessary permits such as CDC and U.S. Department of Agriculture (USDA) import permits.
- 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 PIBC, IACUC, and researchers.

## 2.0 HAZARD ANALYSIS/RISK ASSESSMENT

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

- Pathogenicity and infectious dose of the biological material
- Consideration of the outcome of an exposure
- Natural route of exposure
- Other routes of exposure (e.g., parenteral, airborne, ingestion)
- 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 (e.g., concentration, sonication, aerosolization, centrifugation)
- 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 must be used:

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

### 2.2 BIOLOGICAL EXPRESSION SYSTEMS

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

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

## 2.3 GENETICALLY MODIFIED MICROORGANISMS

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

- Arising directly from the inserted gene (donor organism):
  - Toxins
  - Cytokines
  - Hormones
  - Gene expression regulators
  - Virulence factors or enhancers
  - Oncogenic gene sequences
  - Antibiotic resistance
  - Allergens
  
- Associated with the recipient/host
  - Susceptibility of the host
  - Pathogenicity of the host strain, including virulence, infectivity, and toxin production
  - Modification of the host range (i.e., tropism)
  - Recipient immune status
  - Consequences of exposure
  
- Arising from the alteration of existing pathogenic 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 BL2+ are summarized in Table 3.1.

Table 3.1 Biosafety Level Classifications for Biological Agents		
Biosafety Level	Risk Group	Examples
BL1	Individual risk: LOW Community risk: LOW	<i>Escherichia coli</i> K12 (lab strain)* Adeno-associated viruses*
BL2	Individual risk: MODERATE Community risk: LOW	<i>Streptococcus</i> <i>Staphylococcus</i> Adenoviruses* Most retroviral and lentiviral vectors*
ABL2	Individual risk: MODERATE Community risk: LOW	Influenza virus (non-USDA regulated)* Human immunodeficiency virus* LCMV*
BL2+	Individual risk: MODERATE Community risk: LOW	Human immunodeficiency virus* Viral vectors with oncogenic inserts* USDA-regulated Influenza virus*
BL3	Individual risk: HIGH Community risk: MODERATE	<i>Mycobacterium tuberculosis</i> * West Nile virus* Chikungunya virus* Powassan virus* Japanese encephalitis virus*
BL4 <sup>1</sup>	Individual risk: HIGH Community risk: HIGH	Ebola virus
<sup>1</sup> Biosafety Level 4 work is not permitted within the City of Cambridge. * Approved for use at the Ragon Institute by the Partners Institutional Biosafety Committee.		

Appendix A contains specific information drawn from the BMBL concerning BL1, BL2, and BL3.

Laboratory work at Ragon is conducted using BL1, BL2, BL2+, and BL3 containment and procedures. Separate manuals and standard operating procedures (SOPs) exist for the BL3 laboratory and are not included in this Biosafety Manual.

#### 3.1 BIOSAFETY LEVELS 1 AND 2

BL1 is applicable to work involving well-characterized agents not known to consistently cause disease in healthy adult humans; these agents present minimal potential health hazards to laboratory personnel and the surrounding community. BL2 is recommended for work involving agents that present moderate potential health hazards to laboratory personnel and the surrounding

community. BL2 includes all of the practices and procedures of BL1 and then builds upon these guidelines. Table 3.2 provides a brief summary of the biosafety level criteria for BL1 and BL2.

<b>Table 3.2 Summary of Biosafety Level Criteria for BL1, BL2, and ABL2</b>				
<b>Biosafety Level</b>	<b>Agents</b>	<b>Practices</b>	<b>Safety Equipment (Primary Barriers)</b>	<b>Facilities (Secondary Barriers)</b>
BL1	Not known to consistently cause disease in healthy adults.	Standard Microbiological Practices.	Personal protective equipment (PPE) includes laboratory coats; gloves; eye protection as needed.	Open bench top. Sink required.
ABL1	Not known to consistently cause disease in healthy adults.	Standard animal care and management practices, including appropriate medical surveillance programs.	As required for normal care of each species. PPE: laboratory coats and gloves; eye / face protection as needed.	Standard animal facility: No recirculation of exhaust air. Directional airflow recommended. Hand washing sink is available.
BL2	Associated with human disease. Potential hazards from percutaneous injury, ingestion, and mucous membrane exposure.	BL1 practices plus: Limited access. Biohazard signs. PPE. Sharps precautions. Biosafety manual that defines any biological waste decontamination policies.	Primary barriers include Class I or II biosafety cabinets or other physical containment devices for all manipulations of agents that cause splashes or aerosols of infectious materials. PPE includes laboratory coats; gloves; face protection as needed.	BL1 plus: Method of disinfection (i.e., chemical or autoclave) must be available.
ABL2	Agents associated with human disease. Hazard: percutaneous injury, ingestion, mucous membrane exposure.	ABL practices plus: Limited access. Biohazard warning signs. "Sharps" precautions. Biosafety manual. Decontamination of all infectious wastes and animal cages prior to washing.	ABL1 equipment plus primary barriers: Containment equipment appropriate for animal species.  PPE: laboratory coats, gloves, face, eye and respiratory protection, as needed.	ABL1 plus: Autoclave available. Handwashing sink available. Mechanical cage washer recommended. Negative airflow into animal and procedure rooms recommended.
ABL	animal biosafety level			
BL	biosafety level			

### 3.2 BIOSAFETY LEVEL 2+

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

The Ragon Institute contains several BL2+ laboratories. Specific Standard Operating Procedures (SOPs) for BL2+ are reviewed as a part of new employee orientation and are posted on the Ragon Connect site.

BL2+ is used when working with infectious agents that may cause serious illness, but that do not have a documented aerosol route of exposure. This containment level 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.

Biological agents that may require BL2+ conditions include HIV and viral vectors expressing oncogenes and toxins. BL2+ is defined as the use of BL2 practices plus selected BL3 practices such as:

- The BL2+ laboratory is self-contained. If BL2+ materials must be transported outside of the BL2+ laboratory, they must be kept in sealed secondary containment.
- Strict needle and sharps precautions must be observed. Plastic is substituted for glass whenever possible.
- All work must be done in a biosafety cabinet.
- Sealed rotors and centrifuge safety cups must be used when centrifuging BL2+ materials and the rotors/cups must be opened inside a BSC within the BL2+ laboratory.
- Vacuum lines must be protected with high efficiency particulate air (HEPA) filters.
- Gloves, closed-front gowns, and safety glasses must be worn.
- Solid and liquid waste materials must be autoclaved prior to disposal, unless they contain material(s) that may cause a hazardous situation when autoclaved (e.g., bleach, phenolics, radioactive isotopes or materials that release formaldehyde gas from formaldehyde containing waste streams). The initial risk assessment conducted by the BSO will include recommendations for managing laboratory waste streams. Any changes to laboratory protocols that could change the hazards associated with this waste must be reported to the BSO, and an additional risk assessment will be conducted.

### 3.3 BIOSAFETY LEVEL 3

The Ragon Institute maintains a Biosafety Level 3 containment laboratory on the ninth floor of the 400 Technology Square building to provide a facility for the safe conduct of biomedical research involving the use of *Mycobacterium tuberculosis*, HIV, West Nile virus, Chikungunya virus, Powassan virus, Deer Tick virus, and Japanese Encephalitis virus. Special containment and handling procedures are required because these agents may cause serious human disease (lung and extrapulmonary tuberculosis in the case of *Mycobacterium tuberculosis*) and have the potential for respiratory transmission. Specialized equipment includes a pass-through autoclave for treating biohazardous wastes, Class II B2 biosafety cabinets, and a negatively pressurized suite. All laboratory air is exhausted through HEPA filters. The laboratory also contains an animal room at ABL3 for work with mice infected with *Mycobacterium tuberculosis*.

The requirements and procedures for work in this laboratory are provided in a separate document which describes the organization of the facility, security, requirements for staff training, the standard operating procedures (SOPs) and emergency procedures designed to maintain and protect the barrier, while providing biocontainment. This document, the BSL3 Operations and Safety Manual, may be obtained from the BSO or the BL3 Laboratory Manager.

## 4.0 LABORATORY PRACTICES

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### 4.1 PERSONAL PROTECTIVE EQUIPMENT

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

- Gloves
- Laboratory coats or gowns (impervious)
- Face shields/masks
- Safety glasses with side shields
- Prescription safety glasses with side shields
- Goggles
- Hoods
- Sleeve covers
- Shoe covers
- Respiratory protection (through the Ragon Institute Respiratory Protection Program for BL3)
- 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 manipulating these materials in such a manner that droplets could form and/or materials splashes could occur, or if the agent in use can be easily transmitted through ocular exposure. Laboratory personnel should wear other PPE (apron, face shield, mask, etc.) as needed or required to prevent potentially infectious materials from reaching their clothes, skin, eyes, mouth, or other mucous membranes. PPE must be removed prior to leaving the work area and placed in designated areas. PPE must be treated as medical waste when discarded. If PPE is not disposable, PPE shall be cleaned with disinfectant before and after use.

For the Ragon BL1 laboratories, a laboratory coat, gloves and safety glasses must be worn when handling biological materials. For the BL2 laboratories, a disposable solid gown, gloves and eye protection must be worn when working in the laboratory. For the BL2+ laboratories, a disposable solid front gown, double gloves and eye protection must be worn when working in the laboratory. Persons who enter the laboratory when work is in progress must adhere to these PPE requirements. If no work is in progress, personnel may enter with gloves only.

Certain agents such as USDA-regulated influenza strains are subject to more stringent PPE policies, including change of clothing and footwear. These are addressed in Ragon-specific SOPs.

Contact the BSO if you have any questions on selection, use and disposal of PPE.

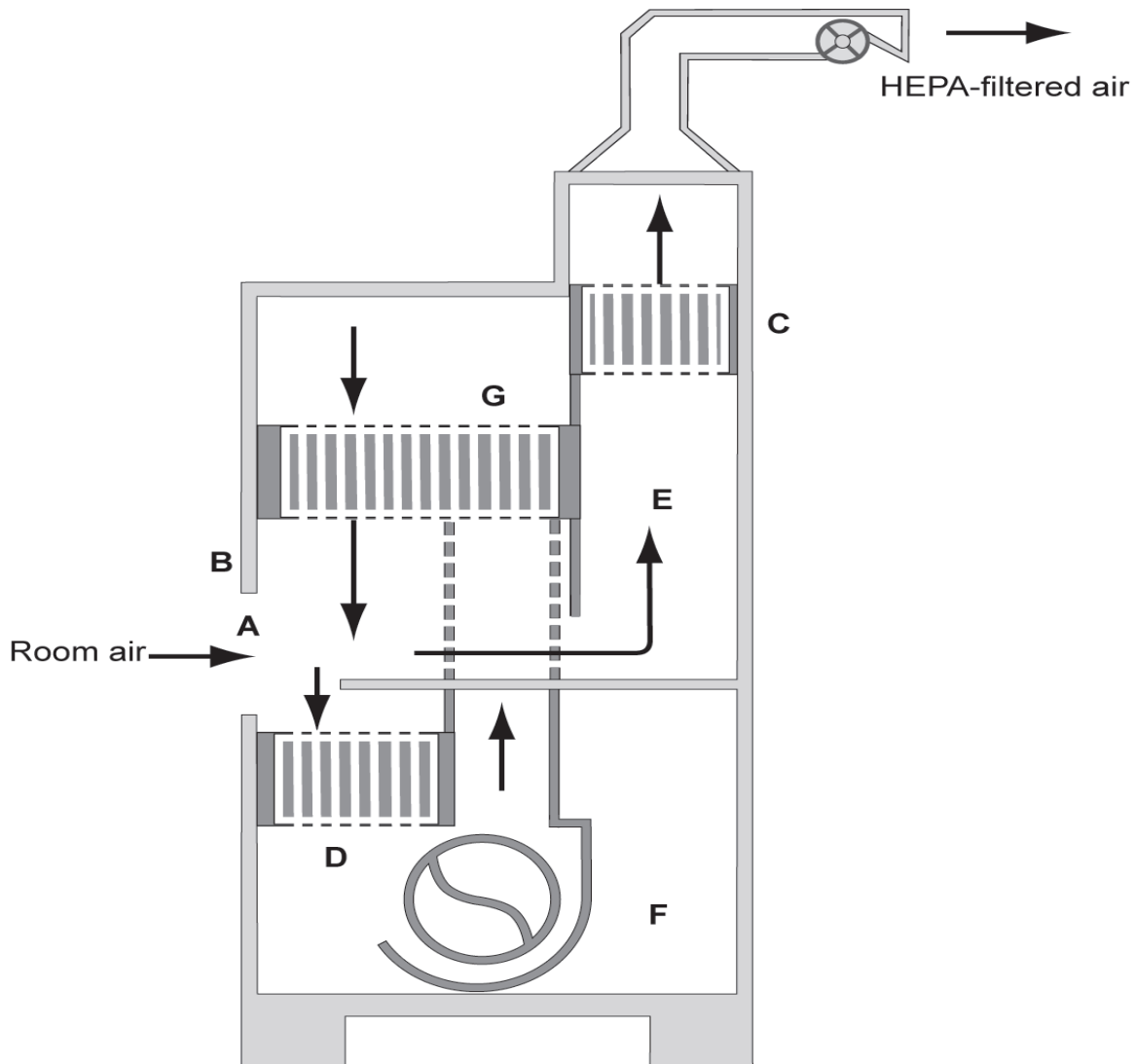


## 4.2 BIOLOGICAL SAFETY CABINETS

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

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

Table 4.1 Biological Safety Cabinet Characteristics <sup>1</sup>				
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 <sup>2</sup> )
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 <sup>2</sup> )
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 <sup>2</sup> )
NSF National Sanitation Foundation ft/min feet per minute				
<sup>1</sup> Information from Baker Labs. <sup>2</sup> Under no circumstances should the chemical concentration approach the lower explosion limits of the compound.				



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

**Figure 4.1** The Class II, Type B1 Biological Safety Cabinet (classic design)

(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air.

*Note:* The cabinet exhaust needs to be hard connected to the building exhaust system.

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

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

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

## 4.3 DISPOSAL OF BIOLOGICAL WASTE

### 4.3.1 Biological Waste

Biological waste may be disposed of in a variety of ways:

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

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

Potentially infectious solid waste and solid waste containing recombinant or synthetic nucleic acid molecules must be disposed of in designated biological waste boxes. Each box is labeled with the universal biohazard symbol (Figure 4.2). Cardboard boxes must be lined with two red plastic bags to reduce the likelihood of leakage. Hard plastic boxes (gray bins) must be lined with at least one red plastic bag.

When a biological waste box is three-quarters (3/4) full, the bags must be individually sealed. Cardboard boxes must be sealed with two-inch-wide tape. Hard plastic boxes must have the lid flaps closed. **Do not overfill the boxes.** Boxes that leak any liquid or that weigh more than 55 pounds will not be removed for disposal by the vendor.

Once the box is taped or closed, it may be moved to a Biohazardous Waste storage room on the seventh, eighth, and ninth floors. The vendor, Stericycle, picks up the closed boxes at least twice per week, except on holiday weeks when the volume may be lighter.



Figure 4.2 Universal Biohazard Symbol

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

**Note:** If bleach is not an adequate disinfectant for the biological agent in use, a U.S. Environmental Protection Agency (EPA)-approved disinfectant must be used. Ensure the proper contact time is met prior to disposal. Contact the BSO for advice. For example, D-125 disinfectant is also used in the BL2+ laboratories.

Prior to sink disposal, the pH of the disinfected solution must be checked to ensure that it is within the permissible pH range under the Massachusetts Water Resources Authority (MWRA) discharge permit (5.5 – 12.0 standard units). If it is within this range, then sink disposal should be done while the water is running in order to minimize possible plumbing damage due to the corrosive effects of the disinfectants. Autoclaving solutions containing bleach is **forbidden** due to the potential for production of toxic chlorine gas and damage to the autoclave. D-125 solutions may be autoclaved.

#### 4.3.2 Biological/Radionuclide Waste

There are three steps in disposing of potentially infectious radioactive waste:

- Disinfect the waste using an approved method.
- Check disinfected waste for radioactivity.
- If waste is radioactive, discard as radioactive.

Prior to disinfection of radioactive waste, consider the following issues:

- Autoclaving radioactive material is forbidden.
- If chlorine bleach is used for disinfection of material labeled with I<sup>125</sup>, radioactivity, gases, and iodine may be released. Do not disinfect iodinated compounds or cells with chlorine bleach.

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

**Liquid waste** should be treated with a 1:10 dilution of household bleach for at least 30 minutes. Add concentrated bleach to the waste liquid until a final dilution of 1:10 is achieved. Evaluate the liquid waste for presence of radioactivity. Contact the MGH Radiation Safety Officer if help

is needed in determining the best method for measuring radioactivity in liquids. If the levels are below Massachusetts Department of Public Health Radiation Control Program sink disposal limits, it may be possible to dispose of the waste in a designated sink. Record the disposal. If the radioactivity exceeds the permissible sink disposal limits, contact the MGH Radiation Safety Officer for further guidance.

### 4.3.3 Biological/Chemical Waste

The approach for managing biological waste containing hazardous or potentially hazardous chemicals is similar to radioactive biological waste. Disinfect the infectious material with chemical disinfectant and dispose of as chemical waste. Select chemical disinfectants carefully because some disinfectants can react with other chemicals. Check with the Ragon EH&S Office if you have any questions.

## 4.4 SHARPS MANAGEMENT

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

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

- Minimize use of needles and syringes.
- Wear puncture-resistant glove liners under disposable gloves.
- Do not bend, shear, or break needles.
- Do not recap needles.
- Do not remove needles from syringes.
- Throw away the entire syringe-needle combination in the sharps container.
- Be careful during cleanup; some sharp items may be hidden in the waste materials.
- If you do stick yourself, encourage the wound to bleed for a few minutes, wash the area, and then get medical attention immediately. Report the incident to your supervisor and the BSO.

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

#### 4.4.1 Sharps Disposal

To protect yourself and others from injury from sharps, place all needles, Pasteur pipettes, syringes, suture needles, scalpels, and razor blades into standard sharps containers. Large volumetric/serological pipettes, or other items that can puncture biohazardous red bags should be disposed of in Sharps Boxes. **Please do not dispose of sharps that may contain mercury or other metals in sharps containers. Contact EH&S for proper disposal.** 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 and should not contain any non-sharps waste. All filled disposable sharps containers need to be placed into a Stericycle biological waste box.



Figure 4.3 Sharps Container

#### 4.4.2 Broken Glassware Disposal

Place clean broken glassware as well as contaminated broken glassware into the sharps containers.

#### 4.4.3 Pasteur Pipettes Disposal

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

#### 4.5 DISINFECTION AND DECONTAMINATION

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

Disinfectant	Final Concentration	Effective On	Ineffective On
Sodium Hypochlorite Bleach: e.g., Clorox™*	1:10 freshly prepared	Bacteria, some spores, viruses, TB†, HIV	Some spores
Chlorine Dioxide: e.g., Clidox®-S*	*1:18:1~ (disinfection) or *1:3:1~ (sterilizing solution)	Bacteria, spores, viruses, TB	
Alcohols (ethanol, isopropanol)	70%	Bacteria, most viruses	Spores, TB
Quaternary Ammonium Compounds: e.g., D-125®*	Follow manufacturer's directions for dilutions	Bacteria, spores, viruses, HIV	
Phenolic Compounds; e.g., Vesphene®*	Follow manufacturer's directions for dilutions	Bacteria, viruses, TB, HIV	Spores
TB      tuberculosis HIV     human immunodeficiency virus  *      The use of brand names does not imply a recommendation. †      Use 1/5 dilution. ~      Please check the manufacturer's directions for specific dilutions.			

#### 4.6 AUTOCLAVING PROCEDURES

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

As a result, anything that does not come in contact with steam inside the autoclave may not be adequately decontaminated. The potential for inadequate decontamination becomes a greater

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

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

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

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

- Do not overfill containers. Leave the top third as empty expansion space.
- Only remove bags full of contaminated materials from secondary container, when ready to autoclave. Place bags inside plastic or metal trays when autoclaving. Once autoclaving is complete, place bags in biological waste boxes (i.e., cardboard boxes or gray bins) for disposal.
- 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.

#### 4.6.1 Autoclave Testing and Validation

Massachusetts regulation 105 CMR 480 requires autoclaves used for decontaminating biological waste must be tested quarterly to ensure that they are operating properly and killing the biological organisms in each autoclave load. The preferred method to check your autoclave is to test it with a commercial spore test system. This system uses ampoules containing a bacterial species called *Bacillus stearothermophilus* that is tolerant to high temperatures and a color indicator solution. The ampoules are autoclaved under realistic conditions, such as in the middle of a bag of waste, and then incubated for two days at 56 °C. If the spores grow, a color change will occur indicating inadequate sterilization in the autoclave. If there is no growth, no color change occurs and the autoclaving procedure is adequate. It is important to note that autoclave tape indicates only that a critical temperature was reached; it **does not** indicate the length of time at the desired temperature or whether steam was present.



The BSO is available to assist laboratory personnel with the periodic autoclave validation and is a resource for questions on proper autoclave procedures.

#### 4.7 SPILL MANAGEMENT

The following procedures are recommended for the management of small spills of blood, bodily fluids, or other potentially infectious materials. If a large volume of a biological material is spilled, or if equipment (centrifuge/homogenizer/biosafety cabinet) malfunctions while processing biological materials, call the Ragon BSO for immediate consultation to implement appropriate measures to contain the spill.

- **Wear gloves and proper protective clothing.** Heavyweight, puncture-resistant, utility gloves are recommended to be worn over disposable latex or nitrile gloves. If the spill contains broken glass or other objects, these should be removed and discarded without contact with the hands. Rigid sheets of cardboard used as a "pusher" and "receiver" may be used to handle such objects and should be discarded with the objects into an appropriate biohazard container. If the spill is large and/or there is a potential of contaminating the worker's shoes, water-impermeable shoe covers should be worn.
- **Absorb the spill.** Because most disinfectants are less active, or even ineffective in the presence of high concentrations of protein that are found in blood and serum, the bulk of the spilled liquid should be absorbed prior to disinfection. Absorb the spilled material with disposable absorbent material (e.g., paper towels, gauze pads, or tissue paper wipes). If the spill is large, granular absorbent material may be used to absorb the liquid. Absorbent granular material, such as an Isolyzer, containing a chemical that releases chlorine upon wetting is commercially available. The efficacy of such material for disinfection is not known and, therefore, should not be relied upon to disinfect a spill. After absorption of the liquid, all contaminated materials should be discarded as biological waste.
- **Clean the spill site** of all visible spilled material using an aqueous detergent solution. Any household detergent may be used. The intent is to dilute the spilled material, lyse red blood cells, and further remove proteins from the contaminated area. Absorb the bulk of liquid prior to disinfection to prevent dilution of the disinfectant. The use of a disinfectant detergent is not necessary.
- **Disinfect the spill site** using an appropriate intermediate to high-level disinfectant, such as a freshly prepared dilution of household bleach (see Table 3.1). Carefully flood the spill site or wipe down the spill site with disposable towels soaked in disinfectant to make the site "glistening wet."

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

- **Rinse the spill site** with water to remove any noxious chemicals or odors. Dry the spill site to prevent slipping.
- **Dispose** all disposable materials used to decontaminate the spill into a biological waste container. Handle the material in the same manner as other infectious waste.

#### 4.7.1 Management of Small Spills

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

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

#### 4.7.2 Management of Large Spills

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

- If the spill occurs in a BSC, close the sash and leave the BSC running.
- Keep people out of the area to prevent spread of the contamination. Put up a warning sign.
- Remove any contaminated clothing and put it into a biohazard bag for decontamination later.
- Wash hands and exposed skin thoroughly.
- Contact the Ragon BSO.
- Complete an incident report form.

## 5.0 IMMUNIZATIONS AND MEDICAL RESTRICTIONS

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Certain biological materials require personnel working with them to receive immunizations or participate in medical surveillance programs. Each project that is registered with the Partners Institutional Biosafety Committee (PIBC) will be reviewed to determine if any medical surveillance, immunizations or restrictions are required. The MGH Occupational Health Department will provide any necessary medical surveillance and immunizations, and serves as a resource for employees who have questions or concerns.

### 5.1 HEPATITIS B VACCINE

Under the OSHA BBP Standard, hepatitis B vaccine is recommended for all employees working with human blood, body fluids, or tissues. Employees will be offered Hepatitis B vaccine during their initial visit at the MGH Occupational Health Department. Those employees declining vaccination will be asked to sign the OSHA declination form indicating that hepatitis B vaccine has been offered and refused. Any questions should be directed to the MGH Occupational Health Department at 617-732-8501.

### 5.2 INFLUENZA VACCINE

All projects involving influenza that are registered with the PIBC require that project personnel be offered the seasonal influenza virus vaccine. Employees will be offered the vaccine by contacting the MGH Occupational Health Department. Any questions should be directed to the MGH Occupational Health Department at 617-732-8501.

### 5.3 REPRODUCTIVE HEALTH

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:

- Zika virus
- HIV

This list is not all inclusive. Please contact the MGH Occupational Health Department for further information.

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

- Influenza virus

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

- Radiation exposure can also cause fetal damage.

#### **5.4 OTHER MEDICAL RESTRICTIONS**

Some health conditions that may warrant special precautions when working in a laboratory. Examples of some conditions that might warrant special precautions are HIV infection, immunosuppressive conditions, or drug therapy that suppresses the immune system.

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.

Therefore, anyone who has any of the above-mentioned conditions should 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 laboratory so that they may obtain appropriate counseling and guidance.

## 6.0 BIOSAFETY TRAINING INFORMATION

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Biological safety training is provided for Ragon Institute laboratory staff through the online HealthStream program. A module within HealthStream is assigned to personnel registered on a Partners Institutional Biosafety Committee (PIBC)-registered project: MGH Ragon Biosafety and Bloodborne Pathogen Training.

Supplemental general safety, laboratory safety and biosafety training is provided by the Ragon EH&S Office and the BSO. Contact the Ragon EH&S Office or the BSO for further information.

## 7.0 SHIPPING AND RECEIVING PROCEDURES FOR BIOLOGICAL SPECIMENS

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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 shipping training through an online training program administered by the MGH Safety Office. 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 Ragon BSO.

The required type of packaging, labeling, and documentation depend on the biohazardous material being shipped, how it is being shipped, and where it is being shipped. Specific packaging requirements for various biological agents should be reviewed by the principal investigator to ensure compliance with all regulatory requirements. Please be aware that anyone who ships restricted items improperly and without authorization may be subjected to fines and/or incarceration.

For more information of DOT Research and Special Programs Administration Office of Hazardous Materials Safety regulations (49 CFR 100-185) please refer to <http://phmsa.dot.gov/hazmat>; for more information about shipping packaging materials, go to the Saf-T-Pak® website <http://www.saftpak.com>.

**Adhere to the Ragon Institute Standard Operating Procedure (SOP) for shipping. The SOP can be found on the Ragon Connect site.**

Before shipping or receiving biological material, contact the BSO to determine if any permits are required and the appropriate classification of the material for shipping purposes. Certain agents and materials potentially or known to be infected with specific agents require CDC and/or USDA import permits prior to receipt of the agent or material at the Ragon Institute. These permits can take many weeks to obtain and may require an inspection by CDC or USDA, so plan accordingly.

## **8.0 GENERAL LABORATORY SAFETY AND BIOLOGICAL SAFETY INSPECTIONS**

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Laboratory and biosafety inspections are typically unannounced. The BSO or his/her designee will review any non-compliant conditions observed, and make recommendations for improvement.

Weekly biosafety inspections of the Ragon BL2+ laboratories are conducted by the BSO or his/her designee. Results are documented and communicated to the appropriate personnel for follow-up and closure.

The BL1 laboratories are inspected on a semi-annual basis.

The BL3 laboratory is inspected at least yearly.



## 9.0 WORKING WITH LABORATORY ANIMALS

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### 9.1 INTRODUCTION

Working with animals in a laboratory setting can present risks from infections and injuries to all personnel. Personnel working with laboratory animals must be aware of the potential risks and implement measures to prevent injury or illnesses related to laboratory animal use. The purpose of this section is to communicate the risks involved with laboratory animal use and the protective procedures in place in the MGH animal facility in the basement of 400 Tech Square.

All handling and use of animals must be conducted safely, humanely, and in compliance with all institutional and federal regulations. The basement Animal Facility at Ragon is managed by the MGH Center for Comparative Medicine (CCM). All work must comply with CCM requirements and procedures. Any hazardous or non-compliant behavior or work conditions regarding the use of animals needs to be reported to the Animal Resources Manager, Attending Veterinarian/Director, or the MGH Institutional Animal Care and Use Committee (IACUC).

Prior to commencing any work in animals that utilize BL2 or higher materials, it is **mandatory** that all Investigators contact the Animal Resources Manager and the BSO to review the project plan. Note that only room 040B in the 400 Technology Square Animal Facility is in the jurisdiction of the Ragon Institute, although other rooms are utilized by Ragon personnel. Specific MGH CCM procedures exist and must be followed by all Ragon personnel. In addition, procedures exist for room 040F when USDA-regulated influenza strains are in use.

### 9.2 ALLERGIES

Allergic reactions to animals are among the most common adverse health effects associated with the care and use of animals in biomedical research.<sup>1,2</sup> 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.

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<sup>1</sup> Wolfe TL and Bush RK, 2001, The Science and Pervasiveness of Laboratory Animal Allergy, *ILAR Journal*, 42:1 – 3.

<sup>2</sup> National Research Council, 1997, *Occupational Health and Safety in the Care and Use of Research Animals*, Washington, DC: National Academy Press.

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.<sup>3</sup> Promptly report any suspicious clinical symptoms to the MGH Occupational Health Department.

Guidelines for working with animals are summarized below.

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

### 9.3 ZONOSSES

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

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<sup>3</sup> Bush RK, 2001, Assessment and Treatment of Laboratory Animal Allergy, *ILAR Journal*, 42:55 – 64.

### 9.3.1 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 and hantavirus. Animals brought into the vivarium must be free from these diseases and the Animal Health Monitoring program evaluates for these agents on a regular basis.

### 9.3.2 Rats

No known risk of zoonotic diseases results from typical exposure to the microbial flora of laboratory-reared rats. All rats 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 must be free from hantavirus and the Animal Health Monitoring program evaluates for the virus on a regular basis.

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

## 9.4 BITES AND SCRATCHES

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

Injured and sick animals and certain strains of mice and rats may display unusually high levels of aggression towards one another and towards humans. When working with these animals, even experienced handlers must exercise caution. Diseases such as rat-bite fever are transmitted through bites and scratches. All bite wounds and scratches should receive immediate first aid; an evaluation for more extensive medical care may be needed. Please report all bites and scratches, and seek proper medical care, through the MGH Occupational Health Department.

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<sup>4</sup> National Research Council, 1997, previously cited.

## **9.5 RESPONSIBILITIES**

### **9.5.1 Institutional Animal Care and Use Committee (IACUC)**

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

### **9.5.2 Principal Investigator**

The PI is responsible for ensuring that research is conducted in accordance with Ragon policies and safe laboratory practices. The PI is responsible for completing all appropriate hazardous agent protocols (radiation, chemical and biological hazards) and required MGH IACUC and PIBC registrations 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 MGH CCM can be contacted for assistance.

### **9.5.3 Laboratory Staff**

Laboratory staff members are responsible for conducting all animal work in a safe and humane manner in accordance with Ragon and CCM Animal Facility 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 Ragon policies.

## **APPENDIX A**

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### **LABORATORY BIOSAFETY LEVEL CRITERIA**

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

## **Section IV—Laboratory Biosafety Level Criteria**

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

### **Biosafety Level 1**

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

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

#### ***A. Standard Microbiological Practices***

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

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
  7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
  8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
  9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
  10. An effective integrated pest management program is required. (See Appendix G.)<sup>1</sup>
  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.*

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<sup>1</sup> Refer to Appendix G, Integrated Pest Management (IPM), of the BMBL, 5<sup>th</sup> Edition.

### ***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. (See Appendix G.)<sup>2</sup>
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

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<sup>2</sup> Refer to Appendix G, Integrated Pest Management (IPM), of the BMBL, 5<sup>th</sup> Edition.

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 (PPE), 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 PPE.

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. (See Appendix G.)<sup>3</sup>
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.

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<sup>3</sup> Refer to Appendix G, Integrated Pest Management (IPM), of the BMBL, 5<sup>th</sup> Edition.

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. 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 PPE 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 are 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:
  - c. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - d. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

- e. 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.

## **Animal Biosafety Level 2**

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created. Appropriate PPE must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered. The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

### ***A. Standard Microbiological Practices***

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies. Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures. Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. The supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided 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. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, PPE requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room. Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities: ABSL-2 69 Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.1,3,4.
6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals. Gloves and

PPE should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. 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 of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:
  - a. The use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
  - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
  - 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; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
  - e. Use of equipment with sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or manipulated.
14. An effective integrated pest management program is required. (See Appendix G.)

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

### ***B. Special Practices***

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a base line serum sample should be stored. Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities: ABSL-2 71.
2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of PPE and other containment devices must be used. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.
3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse. A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.
4. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

### ***C. Safety Equipment (Primary Barriers and PPE)***

1. Properly maintained BSCs, PPE (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.
2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Gowns, uniforms, laboratory coats and PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the biological safety cabinet 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 should also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with NHPs should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.
4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities: ABSL-2 73. Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary. Gloves must

not be worn outside the animal rooms. Gloves and PPE should be removed in a manner that prevents transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

#### ***D. Laboratory Facilities (Secondary Barriers)***

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning. Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted

exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they 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. Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities: ABSL-3 75 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 directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance. All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.
14. Emergency eyewash and shower are readily available; location is determined by risk assessment.