RHODE ISLAND COLLEGE
INSTITUTIONAL BIOSAFETY COMMITTEE

Biological Safety Manual

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The Rhode Island College IBC wishes to thank and acknowledge the following information sources for content and reporting formats:

Woods Hole Oceanographic Institution, Environmental Health and Safety Office

U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories

U.S. Department of Health and Human Services, National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

LIST OF ABBREVIATIONS

ABSA	American Biological Safety Association
ABL	Animal Biosafety Level
APHIS	USDA Animal and Plant Health Inspection Service
BL or BSL	Biosafety Level
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BPRF	Biological Project Registration Form
BSC	Biosafety Cabinet
BSO	Biological Safety Officer
CDC	Centers for Disease Control and Prevention
CITI	Collaborative Institutional Training Initiative
Decon	Decontamination
DEM	Rhode Island Department of Environmental Management
DGR	Dangerous goods regulations
DHHS	US Department of Health and Human Services
DOT	Department of Transportation
HBV	Hepatitis B virus
HEPA	High Efficiency Particulate Air
HIV	Human immunodeficiency virus
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICAO	International Civil Aviation Organization
MSDS	Material Safety Data Sheet
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
OBA	Office of Biotechnology Activities
OSHA	Occupational Safety and Health Administration
PI	Principal Investigator
PPE	Personal Protective Equipment
RAC	Recombinant DNA Advisory Committee
recDNA or rDNA	Recombinant DNA
RG	Risk Group
USDA	United States Department of Agriculture
WHO	World Health Organization

1.1 PURPOSE, SCOPE, DEFINITIONS, AND RESPONSIBILITIES

1.2 PURPOSE

The purpose of the **Rhode Island College Biological Safety Manual** is to define policies and procedures that will minimize risks to personnel, facilities, and the environment resulting from the use of biological agents at Rhode Island College. The work practices, procedures and policies specified in this manual are based on current regulatory requirements and accepted safety and well-established microbiological practices. Implementation of these measures will reduce the likelihood that an incident involving a biological agent will occur, and will ensure that Rhode Island College is in compliance with local, state, and federal regulations. Laboratory microbiological work usually involves exposure not only to biological hazards, but to chemical and radiological hazards as well. Accordingly, this manual should be used in conjunction with RIC Chemical Hygiene Plan, the *Laboratory-specific Standard Operating Procedures* and the *School Chemistry Laboratory Safety Guide*, National Institute for Occupational Safety and Health (NIOSH) Publication No. 2007-107, as appropriate. Radiological hazards are not included in this manual because Rhode Island College does hold a radioisotope use permit.

1.3 SCOPE

This manual applies to all Rhode Island College activities involving biological agents, recombinant DNA, human or non-human primate tissues, fluids, unfixed bacterial or eukaryotic cells, transgenic plants or animals, work with animals known to be reservoirs of zoonotic diseases, and use of Select Agents.

1.4 DEFINITIONS & DESCRIPTIONS

BIOLOGICAL AGENTS

Biological agents include all infectious microorganisms (e.g., bacteria, fungi, eukaryotic parasites, prions, and viruses) that can cause disease in humans, or significant environmental or agricultural impact, and the toxins derived from such organisms. Using the precautionary principle, some research materials should be treated as though they contain biological agents (e.g., unfixed human and non-human primate cells and tissues). In general, these are Risk Group 2 and above. Risk Group 3 and Risk Group 4 activities are not authorized at Rhode Island College.

BIOHAZARDOUS WASTE

This includes waste materials that are potentially contaminated with biological agents, recombinant or synthetic nucleic acids, pathological material, select agents, and any regulated biological waste items as defined by Rhode Island Regulation DEM-DAH-MW-01-92.

BIOSAFETY LEVEL (BL)

Biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity Biosafety Level 4 provides the most stringent containment conditions; Biosafety Level 1 provides the least stringent containment conditions. Biosafety Level 3 and Biosafety Level 4 activities are not authorized at Rhode Island College.

BIOTECHNOLOGY BY-PRODUCT EFFLUENTS

Any discarded preparations, liquids, cultures, contaminated solutions made from living tissues and microorganisms and their products including genetically altered living microorganisms and their products.

CONTAMINATED ANIMAL WASTE

Contaminated carcasses, body parts, body fluids, blood or bedding from animals (a) known to be infected with zoonotic diseases listed R.I.G.L. Title 4, Chapter 4, Section 4-4-3, Reportable Animal and Zoonotic Diseases in Rhode Island; or (b) Inoculated with infectious agents for purposes including, but not limited to, research activities, the production of biologicals, and pharmaceutical testing.

CULTURES AND STOCKS OF INFECTIOUS AGENTS

These include all discarded cultures and stocks of infectious biological agents and their associated biologicals, including culture dishes used to transfer, inoculate, and mix cultures. Using the precautionary principle, some cultures should be treated as though they contain infectious agents (e.g., unfixed human and non-human primate cell lines).

INFECTIOUS AGENT

Any organism (such as a virus, bacteria, or other biological agent) that is capable of being communicated by invasion and multiplication in body tissues and capable of causing disease or adverse health impacts in humans.

PATHOLOGICAL WASTE

Human anatomical parts, organs, tissues and body fluids removed and discarded during research, surgery, autopsy, or other medical procedures; specimens of body fluids and their containers; and discarded material saturated with body fluids other than urine. Using the precautionary principle, some research materials should be treated as pathological waste (e.g., unfixed human and non-human primate tissues and cell lines).

RECOMBINANT OR SYNTHETIC NUCLEIC ACIDS

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

RISK GROUP 1, 2, 3, AND 4 AGENTS

Risk group levels are designated to biohazardous agents based on their association with human disease, and the resulting severity of the disease, according to the U.S. Department of Health and Human Services publications, *Biosafety in Microbiological and Biomedical Laboratories*, and the *NIH Guidelines for Research Involving Recombinant Nucleic Acid Molecules*.

SELECT AGENTS AND TOXINS/OVERLAP SELECT AGENTS

Specific biological agents and toxins are considered to be a severe threat to public health and safety as bioterrorism agents, as identified by CDC. "Overlap" select agents and toxins regulated by both USDA's Animal and Plant Health Inspection Service and the CDC.

SHARPS

Discarded research or medical items that may cause puncture wounds or cuts, including, but not limited to: All needles, syringes, lancets, pen needles, Pasteur pipettes, scalpel blades, disposable razors, and broken glassware that is potentially contaminated with biological agents.

ZOONOTIC DISEASES

Zoonotic diseases are diseases caused by infectious agents that can be transmitted between (or are shared by) animals and humans.

1.5 RESPONSIBILITIES

The responsibility for biosafety at Rhode Island College is a community effort requiring the direct involvement of the Rhode Island College Institutional Biosafety Committee, Principal Investigators (PIs), laboratory workers, student researchers, Physical Plant, and College Security and Safety Department.

1.5.1 INSTITUTIONAL BIOSAFETY COMMITTEE

The Institutional Biosafety Committee (IBC) has been established as specified in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and CDC Guidelines for Biosafety in Microbiological and Biomedical Laboratories. The IBC oversees research involving recombinant or synthetic nucleic acids, and develops policies and provides expertise to reduce risks to faculty, staff, and students working with hazardous biological materials, biological toxins, and recombinant nucleic acids technology. The IBC reviews research protocols to determine whether a Principal Investigator (PI) who administers, handles or uses biological agents employs containment, decontamination, and disposal procedures in compliance with federal, state and local regulations, and

ensures workers and students are properly trained in good microbiological techniques and safe handling practices. The IBC has broad authority in oversight of any research involving potentially hazardous biological agents, including recombinant and synthetic nucleic acid research. The IBC Chair and the Committee may block the use of these materials if there is any possibility of the activities could impact health of people or the environment.

IBC composition, standards, and procedures follow the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules Sections I-E-2 through IV-B-2-b-(9). The IBC is comprised of at least five members with expertise in recombinant and synthetic nucleic acid technology and hazards of biological agents, and the capability to assess any potential risk of research activities to public health or the environment; at least three members are RIC Faculty and two members are not affiliated with RIC, as stipulated by the NIH Guidelines. A member of the RIC Administration also serves as the Institutional Official on the IBC. Non-committee faculty or staff with special expertise may be asked to advise the IBC, as appropriate. IBC meetings are open to the public. The IBC must meet at least annually and the meeting minutes shall be recorded and retained.

The IBC provides resources, training guidance, forms and templates at the IBC website (www.ric.edu/biosafety). Researchers can access this site to complete forms to register their research involving recombinant or synthetic nucleic acid molecules, cell/tissue culture experiments, and materials wit the potential to harbor biological agents. Once received, the IBC reviews research registration forms to ensure procedures are in place to protect faculty, staff, and students from biological materials. Research of this nature cannot begin until the IBC has approved the project.

Responsibilities of the IBC include:

- Developing biosafety policies applicable to Rhode Island College activities, including work practices, biohazardous waste, and medical surveillance of personnel, if applicable.
- Reviewing and approving proposed research in accordance with CDC/NIH/OSHA guidelines.
- Setting required containment levels for research projects. Generally, the biosafety levels (BLs) established by the CDC and NIH will be used as the level of containment; however, the IBC can increase or decrease the level of containment according to the specific circumstances of the project.
- Working with the Rhode Island College Security and Safety Department and Physical Plant to recommend and review design specifications and criteria for existing and new containment facilities.
- Evaluate the public health and environmental risks associated with all biohazard material treatment and disposal methods, including disposal of these wastes according to provisions of the Rules and Regulations for Use of Wastewater Facilities Within the Narragansett Bay Commission District and Rhode Island Regulation DEM-DAH-MW-01-92.
- As necessary, make recommendations to management and changes to biohazardous waste management procedures.
- Ensuring that biosafety cabinets used with BL2 materials are inspected annually.
- Providing technical advice to PIs on biosafety and containment protocols.
- Developing emergency response plans for accidental spills and personnel contamination
- Investigating significant violations of RIC biosafety procedures or policies, and significant accidents or illnesses involving Biological Agents. If appropriate, the IBC will recommend corrective action to the proper College officials.

1.5.2 BIOSAFETY OFFICER

A Biosafety Officer (BSO) must be appointed and be a member of the IBC if the institution conducts recombinant or synthetic nucleic acid research at large scale (>10 L cultures) or uses materials requiring high containment (BL3 or BL4). Currently, researchers at RIC do not fall under these categories of use.

1.5.3 PRINCIPAL INVESTIGATORS (PIS) AND SUPERVISORS

Principal Investigators (PIs) and supervisors are responsible for the health and safety of all personnel in their laboratory. Specific responsibilities of the PI include:

- Notifying the IBC and obtaining prior IBC approval for work involving biohazardous material as specified in this manual (see Section 2) for ongoing and new research projects by completing and submitting a Biological Project Registration Form (Biosafety website).
- Compliance with Emergency Planning and Community Right-To-Know Act (EPCRA) by ensuring that specific laboratory hazards are effectively communicated to laboratory personnel, and that controls are in place to minimize risks associated with these hazards. Proper signage is mandatory to comply with the federal laws, which may include a listing of hazardous substances posted on the entry door, and NFPA diamond symbols or approved pictograms.
- Maintaining inventory records, MSDS sheets, and location of biological and chemical hazards.
- Developing laboratory-specific standard operating procedures that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory and provide the personal protective equipment appropriate to the procedures.
- Ensuring that engineering controls are available, are in good working order, and are used appropriately to minimize exposure to biological agents. This includes monthly (minimum) testing and flushing of eye washing stations.
- All participants in research activities must be trained by full-time Rhode Island College faculty (usually, the PI) who is experienced, aware of the risks, and best practices associated with experimental protocols. Alternatively, participants may receive training by an approved institution or facility. This required **pre-approval** from the IBC.
- Faculty, students, and staff must satisfactorily complete the CITI training in responsible conduct of research modules. In addition, those working with biological agents and other biohazards must complete the biosafety module. PIs must follow up with ORGA to ensure the modules are completed within a month of the individual initiating research activities for the responsible conduct of research modules and **prior to** initiating research for individuals required to complete the biosafety module.
- All training **must be documented** and records maintained for all laboratory personnel receiving general laboratory safety, biosafety, and lab-specific training on the hazards, procedures, use of personal protective equipment, and practices relevant to the laboratory activities. The PI must ensure that those working in the laboratory understand and employ proper handling associated with the hazards. These records must be kept for a minimum of 3 years following the last day the individual worked with potentially biohazardous materials.
- Notifying the IBC of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or a release to the environment.
- Proper disposal of biological agents in accordance with methods outlined in this manual and good practices in the field.
- Ensuring that periodic assessments of the laboratory are conducted to verify and maintain compliance with this manual.

1.5.4 LABORATORY WORKERS AND RESEARCH STUDENTS

Laboratory workers and research students are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers and students are responsible for the health and safety of themselves and others. An incident caused by one laboratory worker can have widespread effects on others. Specific responsibilities include:

- Following all required procedures.
- Using accepted good laboratory practices to minimize exposures to biological agents and to prevent other incidents, such as fires, explosions, and chemical exposures.
- Attending biosafety and other laboratory safety training as required.
- Promptly reporting unsafe laboratory conditions to the PI, IBC, or other responsible party.

- Utilizing appropriate control measures properly, such as biological safety cabinets and personal protective equipment (PPE) to prevent exposure to biological agents, and contamination of personnel and facilities.

1.5.5 PHYSICAL PLANT, OTHER RIC FACULTY/STAFF, OUTSIDE CONTRACTORS

- Annually certifies Biological Safety Cabinets (BSCs) to applicable standards with professional inspection and maintenance.
- Maintains autoclaves.
- Works with the IBC to develop design specifications and criteria for existing and new containment facilities.
- Maintains compliance with Article 5 of the Rules and Regulations for Use of Wastewater Facilities Within the Narragansett Bay Commission District for all campus buildings where biotechnology by-product effluents could be sink disposed.

2.1 APPROVAL OF RESEARCH PROJECTS

2.2 PROJECT REGISTRATION

For each research project involving biological agents, recombinant DNA, human or non-human primate tissues, fluids, unfixed bacterial or eukaryotic cells, transgenic plants or animals, work with animals known to be reservoirs of zoonotic diseases, and use of Select Agents, the PI is required to submit a Biological Project Registration Form to the IBC for review and approval prior to obtaining the materials and commencement of activities involving these materials. The IBC will review BPRFs within 30 days of receipt. BPRFs are approved for three years, but are to be renewed each year. Changes involving additional biological agents, significant procedural changes, or modifications that increase the risk of the project must be approved by the IBC before the changes are made. PIs wanting to modify a current BPRF are required to submit a revised BPRF to the IBC Chair. The PI is required to notify the IBC Chair (by e- mail) when a project is completed or is no longer active.

2.3 BIOLOGICAL AGENTS

All work involving biological agents must be reviewed by the IBC for adherence to NIH/CDC biosafety guidance published in the latest edition of Biosafety in Microbiological and Biomedical Laboratories, the latest edition of NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules, applicable regulations, as well as Rhode Island College policies and current biosafety practice. Links are located on the RIC Biosafety website to the American Biological Safety Association (ABSA) Risk Group and Biosafety Level definitions and tables. The *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules* provides definitions for Risk Groups 1-4. The *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* provides definitions for Biosafety Levels 1-4. The general definitions are:

- **BIOSAFETY LEVEL 1 (BL1) AND ANIMAL BIOSAFETY LEVEL 1 (ABL1)**
  Organisms in this category are not known to cause disease in healthy human adults. IBC prior approval is required for all BL1 work. Prior notification can refer to a previously filed "generic" Biological Project Registration Form describing the procedures to be used.
- **BIOSAFETY LEVEL 2 (BL2) AND ANIMAL BIOSAFETY LEVEL 2 (ABL2)**
  All work involving biological agents classified as BL2 must be reviewed by the IBC. Containment levels, facility requirements, and work practices will generally follow NIH/CDC guidance; however, the IBC can modify these requirements as appropriate.
- **BIOSAFETY LEVEL 4 (BL3) AND ANIMAL BIOSAFETY LEVEL 3 (ABL3)**
  Projects involving BL3 organisms are currently **prohibited** at Rhode Island College.
- **BIOSAFETY LEVEL 4 (BL4) AND ANIMAL BIOSAFETY LEVEL 4 (ABL4)**
  Projects involving BL4 organisms are currently **prohibited** at Rhode Island College.

2.4 HUMAN, NON-HUMAN PRIMATE MATERIAL, AND OTHER CELL CULTURE

All projects using unfixed or live human and non-human primate tissues, fluids, and cells, or cells at the BL2 require a full review and approval from the IBC **before** any of these materials are brought onto the RIC campus. Principal Investigators must submit a Biological Project Registration Form (BPRF) to the IBC in order to initiate the approval process. Please consult the RIC Bloodborne Pathogen Exposure Control Plan for more information.

2.5 Use of BL2 Designated Laboratories and Resources

Use of BL2 facilities (laboratories) and/or resources used for BL2 materials (cryogenic storage) must be approved by the IBC. The IBC requires users to be trained in BL2 practices and proper use of these facilities. This is for the safety of all users, research resources, and materials.

2.6 RECOMBINANT AND SYNTHETIC NUCLEIC ACIDS

As a condition of funding from the National Institutes of Health (NIH), all research at Rhode Island College involving recombinant DNA must be conducted in accordance with the most current version of *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules*. PIs are required to make an initial determination of the required biological and physical containment required. The approval level required for the proposed research is dependent on the NIH category to which the work corresponds. **Prior approval by the IBC is required for all proposed experiments involving recombinant or synthetic nucleic acids, including those exempt from NIH Guidelines.** Principal Investigators must submit a Biological Project Registration Form (BPRF) (as described in section 2.0) to the IBC in order to initiate a request for approval. The request for approval can refer to a previously filed "generic" BPRF describing the procedures to be used. The following paragraphs summarize experiments covered by the NIH Guidelines.

EXPERIMENTS REQUIRING IBC APPROVAL, RAC REVIEW, AND NIH APPROVAL

Experiments involving the deliberate transfer of a drug resistance trait to microorganisms that do not acquire the trait naturally, where such acquisition could compromise the use of the drug to control disease in humans, veterinary medicine, or agriculture are included in this category. These experiments are considered "Major Action" and require review by the Recombinant DNA Advisory Committee (RAC) at NIH, and specific approval by NIH, prior to initiation. Additional information on the Office of Biotechnology Activities (OBA) and the RAC is available at the NIH web site. Approval by the IBC is required prior to initiation of the experiments.

EXPERIMENTS REQUIRING IBC AND NIH APPROVAL

Experiments in this category include the cloning of genes encoding toxic molecules with an LD_{50} for vertebrates less than or equal to 100 ng/kg body weight. This includes microbial toxins such as botulinum toxins, tetanus toxins, and diphtheria toxin. These experiments cannot be initiated without submission of relevant information on the proposed experiment to the OBA. IBC approval is required prior to initiation of the experiments.

EXPERIMENTS REQUIRING IBC AND RAC APPROVAL, WITH NIH REGISTRATION

These experiments involve the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into humans (human gene transfer). Prior to initiation of laboratory work, these experiments must be approved by the IBC, the RAC, and be registered with the OBA.

EXPERIMENTS REQUIRING IBC APPROVAL (NIH SECTION III-D)

This category includes whole animal or plant experiments, as well as experiments involving DNA from Risk Group 2, 3, or 4 agents. These experiments must be approved by the IBC prior to initiation. Note that Risk Group 3 and 4 are not authorized at RIC.

EXPERIMENTS USING RISK GROUP 1 AGENTS

Experiments in this category are low risk and can be conducted using BL1 containment. Examples include experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes. IBC notification is required prior to or at the time of initiation of the experiments.

NIH EXEMPT EXPERIMENTS

The recombinant DNA (recDNA) experiments listed below may be considered by NIH Guidelines to be "exempt". Although these experiments may be exempt from NIH Guidelines, the PI **must** submit a BPRF to the IBC. If applicable, PI can refer to a previously filed BPRF describing the procedures to be used. The IBC will review the project to verify that it is exempt. The following recombinant or synthetic nucleic acid molecules are exempt from the *NIH Guidelines* and registration with the

Institutional Biosafety Committee is not required; however, other federal and state standards of biosafety may still apply to such research (for example, the Centers for Disease Control and Prevention (CDC)/NIH publication Biosafety in Microbiological and Biomedical Laboratories):

- Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.
- Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.
- Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.
- Those that consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means.
- Those that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.
- Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

3.1 NON-COMPLIANCE

Non-compliance with the *NIH Guidelines* is a serious matter. Egregious violations can lead to consequences for the individual PI and Rhode Island College, which can range from suspension to termination of federal funding. This not only jeopardizes the research of the non-compliant PI, but also may compromise the research and eligibility to receive funding by other RIC faculty.

3.2 ALLEGATIONS

Any allegations of non-compliance or unsafe working conditions shall be made to the IBC Chair, to any member of the IBC, the Office of Research and Grants (ORGA), or to the Office of Vice President for Academic Affairs. In all instances, allegations shall be immediately forwarded to the IBC Chair. The IBC Chair is responsible for investigation and resolution of all allegations of non-compliance. The allegations and resulting investigations will remain confidential to the extent possible.

3.3 INVESTIGATION AND REVIEW PROCESS

The IBC Chair will appoint a subcommittee to investigate the allegation. The subcommittee will inform all persons involved in the investigation of the purpose and the manner in which it will be conducted. The subcommittee, in its investigation, will examine all documents and procedures relating to the allegation and will interview individuals who are named in the allegation and others who may have knowledge of the circumstances surrounding the allegation and determine if there is a basis in fact to support the allegation. The subcommittee will report its findings to the full IBC for the final determinations.

3.4 IBC DETERMINATION

At a convened meeting, the IBC will discuss the subcommittee report and determine if there is a consensus that the allegation of non-compliance is substantiated and, if so, the seriousness of the incident. All persons involved in the allegation of non-compliance will be given the opportunity to appear to respond to the allegation and/or findings. After all persons who have appeared to respond have left, the report and recommendations will be further discussed and voted upon. The IBC will inform all parties involved, including the submitter of the allegations, if known, of the committee's findings.

3.5 POSSIBLE OUTCOMES

The IBC has the authority to address non-compliance with the NIH Guidelines, the BMBL, RIC policies and procedures and other legal requirements. Findings of non-compliance may result in one or more of the following actions:

- Suspension of use of recombinant and synthetic nucleic acid molecules and/or biohazardous materials agents or toxins.
- Termination of approval for use of recombinant and synthetic nucleic acid molecules and/or biohazardous materials agents or toxins.
- Confiscation of the recombinant and synthetic nucleic acid molecules and/or biohazardous materials agents or toxins.
- Destruction of the recombinant and synthetic nucleic acid molecules and/or biohazardous materials agents or toxins.
- Any other action, including suspension, limitation, or termination of NIH funds as noted above, necessary to protect RIC Faculty, staff, and students, and the community, including restricting access to the laboratory in order to suspend activities.

4.1 BIOSAFETY REGULATIONS AND GUIDELINES

4.2 FEDERAL AGENCIES

The following federal agencies either regulate or provide guidelines covering the use of biological agents. A summary of these regulations and guidelines is provided below. Links to applicable web sites are found on the Rhode Island College IBC web site (http://www.ric.edu/biosafety). Refer to the American Biological Safety Association (ABSA) Risk Group Table for assistance with biosafety hazard level and classification.

- Centers for Disease Controls and Prevention (CDC) and the National Institutes of Health (NIH): *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety and is the basis for this manual.
- National Institutes of Health (NIH): *Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules* (NIH Guidelines). This document provides guidelines for constructing and handling recombinant and synthetic nucleic acid molecules, and organisms containing such material. Although these guidelines are not subject to regulatory enforcement, institutions that receive any NIH funding for this research are required to comply with these guidelines as a condition of funding. This document requires that each institution establish an Institutional Biosafety Committee with the authority to approve proposed research using the NIH Guidelines as a *minimum* standard.
- Occupational Safety and Health Administration (OSHA): *Bloodborne Pathogens*. This regulation covers occupational exposure to human blood and other potentially infectious material, including unfixed human tissue and cells. OSHA specifies a combination of engineering controls, work practices, and training to reduce the risk of infection. Personnel who work with HIV or Hepatitis B in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens
- Centers for Disease Control and Prevention (CDC): *Possession, Use, and Transfer of Select Agents and Toxins* and USDA Animal and Plant Health Inspection Service (APHIS): *Agricultural Bioterrorism Protection Act of 2002: Possession, Use, and Transfer of Biological Agents and Toxins*. These regulations require institutions that possess, use, or transfer certain biological agents and toxins ("Select Agents") to be registered and approved by the CDC and/or APHIS. Individual Rhode Island College laboratories that possess, use, or transfer any of these agents must be included on the Rhode Island College institutional registration. This regulation requires that laboratories comply with the BMBL (see above) and the OSHA Laboratory Standard.
- A specific CDC/APHIS transfer form that requires the signature of the Rhode Island College Responsible Official and serves to document the chain of custody must accompany each transfer of a Select Agent. Detailed safety and security procedures must be developed and maintained by the affected laboratories before select agent work starts. Background checks are required for persons seeking access to select agents. See Section 12 of this manual for additional information.

5.1 BIOSAFETY PRINCIPLES

5.2 CONTAINMENT

Laboratory biosafety practices are based on the principle of containment of biological agents to prevent exposure to laboratory workers and the outside environment. Primary containment protects the laboratory workers and the immediate laboratory environment from exposure to biological agents. Primary containment is achieved through good microbiological technique and the use of safety equipment and personal protective equipment. Secondary containment protects the environment outside the laboratory, and is provided by facility design and operational procedures.

5.3 LABORATORY PRACTICE AND TECHNIQUE

The use of good microbiological technique is the most important element of containment. Personnel working with biological agents must be aware of hazards, and must be trained to safely handle and dispose of these materials. Although we are all responsible for our own safety, the Principal Investigator has ultimate responsibility for ensuring that persons working in their laboratory are adequately trained.

The Rhode Island College Biosafety Manual has been developed to provide general policies and procedures when working with biological agents. Each individual laboratory must supplement this manual with laboratory specific procedures and training that will minimize the specific risks present in the laboratory.

SAFETY EQUIPMENT

Safety equipment includes safety centrifuge cups, containment devices, and other engineered controls designed to minimize exposure to biological agents. Biological safety cabinets (BSCs) are important safety equipment for protection of personnel and the laboratory environment. Safety equipment is most effective at minimizing exposure when it is used correctly and properly maintained.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Depending on the exposure hazards, personal protective equipment may include a combination of safety eyewear, lab coats, protective suits, gloves, face shields and other items.

FACILITY DESIGN

Facility design and security features include physical separation of laboratories from public access, specially designed ventilation systems (to prevent airborne biological agents from migrating outside the laboratory), and autoclaves. These design features protect personnel working outside the immediate laboratory, as well the outside environment.

BIOSAFETY LEVELS

The CDC/NIH has developed four biosafety levels that describe laboratory practices and techniques, safety equipment, and facility design features recommended for work with biological agents. Descriptions of the biosafety levels, as well as assigned biosafety levels for specific biological agents, are contained in the CDC/NIH document, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

5.4 ROUTES OF TRANSMISSION

SKIN AND MUCOUS MEMBRANE CONTACT

Low energy procedures such as decanting of liquids, pipetting, removal of screw caps, vortex mixing, streaking agar plates, inoculation of animals, can result in the generation of infectious

droplets, as well as result in direct contact with infectious material. This includes eye contact as a route of exposure.

INGESTION

Mouth pipetting presents a high risk for ingestion of infectious material. Splashing of material into the mouth, and indirect oral exposure through touching the mouth with contaminated hands, and eating and drinking in the lab can also result in ingestion of infectious material.

PERCUTANEOUS INOCULATION/INJECTION

Use of syringes and needles are considered the greatest risk of exposure through inoculation. Inoculation can also occur as a result of cuts and scratches from contaminated items, and animal bites.

INHALATION

Many procedures have the potential for generation of aerosols, including: sonication, centrifugation, "blowing out" of pipettes, heating inoculating loops, working with volatile materials, and changing litter in animal cages. Aerosols present an increased risk for inhalation exposure. Procedures involving inhalation exposure hazards must be evaluated by Physical Plant staff involved in environmental health and safety or the contracted agency prior to beginning the activities.

6.1 LABORATORY BIOSAFETY PRACTICES

6.2 GENERAL LABORATORY PRACTICES

The biosafety practices listed below are recommended by the National Academy of Sciences. Although these practices may be considered "common sense" and overly simplistic by experienced laboratory personnel, strict adherence to these basic principles will greatly reduce the likelihood of laboratory acquired infections.

- Do not mouth pipette
- Manipulate infectious fluids carefully to avoid spills and the production of aerosols
- Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives - dispose of sharps in leak- and puncture-proof containers
- Use lab coats, gloves, safety eye wear, and other personal protective equipment
- Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents
- Decontaminate work surfaces before and after use, and immediately after spills
- Do not eat, drink, store foods, or smoke in the laboratory
- Uncontaminated laboratory glassware and broken glass in rigid containers (separate from other waste) that will prevent cuts and punctures to personnel. Containers should be labeled "broken glass."

6.3 BIOLOGICAL HAZARD INFORMATION

Laboratory workers must be knowledgeable of the hazards associated with the biological agents present in the laboratory, and have hazard information available to them. The following are some sources of hazard information:

- CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) has descriptions of biosafety levels and recommended biosafety practices for specific biological
- The Canadian Laboratory Centre for Disease Control (LCDC) maintains Material Safety Data Sheets for microbial agents (see link on IBC web site).
- RIC Chemical Hygiene Plan and Biosafety Manual – available on the IBC website.

6.4 LAB-SPECIFIC PROCEDURES

As this manual covers general biosafety topics, PIs/supervisors should develop lab-specific procedures that address, as necessary, procedures that may require more detail instruction and/or require special precautions: Safe manipulation of specific organisms, specific exposure control methods, disinfection procedures, security, waste management, etc. The lab-specific procedures should not duplicate the general procedures contained in this manual or the CDC/NIH documents.

6.5 SECURITY AND INVENTORY OF BIOLOGICAL AGENTS

Each PI is responsible for ensuring that his or her laboratory implements sufficient security measures and procedures to prevent unauthorized access to biological agents. Select Agents (see Section 12) and other higher risk microorganisms and toxins must be adequately secured and the PI must maintain an inventory with sufficient detail to enable identification of missing materials. All BL2 and RG2 materials must be inventoried and cataloged. PIs are responsible for maintaining an updated inventory of these materials.

6.6 PREVENTION OF AEROSOLS AND DROPLETS

Uncontrolled aerosols and droplets increase the risk of exposure. Control methods include, but are not limited to:

BIOLOGICAL SAFETY CABINETS
Procedures involving infectious material should be performed inside a biological safety cabinet (BSC) whenever possible.

PIPETTING
Do not mouth pipette! Always use a mechanical pipetting device. Pipettes should be drained gently with the tip against the inner wall of the receiving vessel and liquid should not be forcibly expelled from the pipette.

BLENDING
Use a safety blender that has leak proof bearings and a secure lid.

CENTRIFUGATION
The potential for contamination and infection is high if liquid and aerosol are released during centrifugation. Sealed centrifuge buckets, or safety cups should be used to prevent release of liquid and aerosol. If sealed buckets or safety cups are not obtainable, it is recommended that the centrifuge chamber be evacuated before the centrifuge is opened. Some centrifuges have an available access port that will allow evacuation of the chamber using a vacuum pump (use an in- line disinfectant trap and/or HEPA filter to protect the pump from contamination) and tubing attached to a port. Ultracentrifuges operate under vacuum and should contain an in-line HEPA filter between the chamber and the vacuum pump.

INOCULATING LOOPS
Flaming inoculating loops can result in spatter and release of aerosols and droplets. Use of an electric micro-incinerator or similar device can reduce this risk.

USE OF ABSORBENT MATERIALS
Work surfaces should be covered with absorbent paper or "diaper" sheets to collect splashes and drips, and minimize the spread of contamination. The absorbent paper should be changed as necessary.

6.7 PERSONAL PROTECTIVE EQUIPMENT
Although not a substitute for use of BSCs and good laboratory practices, personal protective equipment (PPE) is considered a primary barrier to infectious agents and proper use will reduce the likelihood of infection. PPE is most effective when used to supplement other control methods such as biological safety cabinets, safety centrifuge cups, and other containment devices.

LABORATORY COATS
Laboratory coats protect street clothes against chemical and biological spills, and provide additional body protection. Laboratory coats made of 100% cotton are flame resistant and nonreactive to many chemicals. The wearing of lab coats is considered to be standard microbiological practice for BL1 and BL2 laboratories. It is good laboratory practice to remove lab coats or gowns before leaving the laboratory to minimize the spread of contamination outside the laboratory. Lab coats should be left in the laboratory and must not be taken home for washing.

GLOVES
Gloves are available that provide protection against a variety of hazards, including infectious agents, chemicals, and radioactive material. Unfortunately, there is no single glove type that provides adequate protection for all hazards. Standard latex examination type gloves provide protection against microbiological hazards, including human blood and body fluids. Latex gloves do not

generally provide adequate protection against liquid chemicals; additionally, many people develop latex allergies as a result of wearing latex gloves. Nitrile gloves provide protection against microbiological hazards, but without the latex allergy hazard. Contamination control requires that gloves be removed prior to exiting a BSC or touching non-contaminated laboratory areas and equipment (such as clean areas, phones, computers, door knobs, etc.). Always check gloves for pinholes prior to use and wash hands after removing gloves.

EYE AND FACE PROTECTION

Safety glasses, goggles, and face shields provide protection against chemical reagents and disinfectants. Additionally, they also prevent infection that can result from the splashing of pathogenic organisms in the eye. Microbial infection can occur as a result of splashes to the eye. Goggles with indirect venting provide a good barrier against such splashes. A face shield can be worn in addition to goggles (face shields do not provide adequate eye protection by themselves) to provide protection against splashes to the face and mouth.

RESPIRATORY PROTECTION

Certain laboratory and clinical situations require respiratory protection to prevent inhalation of infectious agents. Regulations, as well as good safety practice, require that personnel be medically evaluated, specifically trained, and fit tested prior to wearing respiratory protective equipment. Contact IBC if respiratory protective equipment is required or if there are questions about the respiratory protection program.

6.8 STORAGE AND LABELING OF BIOLOGICAL AGENTS

Biological agents level 2 (BL2) must be stored using double containment. Both the primary and secondary containers must be durable and leak proof so as to prevent accidental exposure. Primary containers must be clearly labeled as to the identity of the agent and should include the universal biohazard symbol, as physical space on the container permits. Freezers, refrigerators, and other storage areas must also be labeled with the biohazard symbol. Waste and contaminated equipment or other objects to be decontaminated must also be labeled with the biohazard symbol.

6.9 BIOHAZARD LABELS AND SIGNS

Signs must be posted at or on the access doors indicating that biological agents are used within the room. Storage locations and BSCs may also need to be posted with warning labels. The sign must include the universal biohazard symbol, the name of the agent(s) present, any specific entry requirements (such as personal protective equipment or immunization), and the name and telephone number of the PI and/or other responsible person(s). This information should be indicated on the RIC Space Hazard Placard.

7.1 SAFETY TRAINING

7.2 GENERAL BIOSAFETY TRAINING

The following personnel are required to receive general biosafety training:

- Personnel working with Risk Group 2 biological agents and/or in Biosafety Level 2*
- Personnel working in BL2 designated laboratories or with other resources used for BL2 research*
- Personnel working with recombinant DNA
- Personnel working with select agents.

*Note that the IBC has the authority to elevate the level of containment from BL1 or RG1 to BL2 or RG2 for certain activities deemed to be safer to the entire faculty, staff, and student body if performed with BL2 or RG2 containment.

7.3 BLOODBORNE PATHOGENS TRAINING

Laboratory workers who are potentially exposed to human blood and body fluids, unfixed human tissue, or human cell lines that are not sourced from a commercial vendor with the appropriate testing for pathogens are within the scope of the OSHA Bloodborne Pathogens Standard. It is the position of the Centers for Disease Control and Prevention (CDC) and OSHA that all cell lines of human origin are considered potentially infected with bloodborne pathogens, and that these materials should be handled using a minimum of BL2 containment and procedures (see Section 9). Consequently, RIC personnel who work with human cell lines are required to participate in the Bloodborne Pathogens Program and receive annual training; this training can be conducted through CITI. See Appendix 1 for the full 'Bloodborne Exposure Pathogen Control Plan'.

7.4 HIV/HBV LABORATORY TRAINING

Personnel who work in research laboratories that culture, produce, or otherwise perform microbiological manipulation of human immunodeficiency virus (HIV) or hepatitis B virus (HBV) must receive additional training beyond the standard bloodborne pathogen training. Prior to working with HIV or HBV, laboratory workers must demonstrate proficiency in standard microbiological techniques, and in the practices and techniques specific to the laboratory. Personnel that have no experience with human pathogens must be trained by the PI/supervisor before working with HIV or HBV. Workers are permitted to handle infectious agents only after demonstrating proficiency to the satisfaction of the cognizant PI or supervisor. The IBC must approve of the training program for faculty, staff, and students conducting research with these materials.

7.5 TRAINING IN THE PACKAGING AND SHIPPING OF INFECTIOUS AGENTS

All personnel that prepare and/or ship hazardous materials (including infectious substances, biological substances, and any samples shipped on dry ice) must be properly trained in accordance with U.S. DOT regulations. See Section 13 for additional information.

7.6 LABORATORY SPECIFIC TRAINING

Principal Investigators' laboratories are required to develop specific training for the particular agents and procedures that personnel will perform in that laboratory. This training should be specific to the hazards in the laboratory and to each person's laboratory duties. Each person in the laboratory must understand the hazards associated with laboratory operations, how to prevent exposures to biological and chemical agents. This laboratory specific training should not duplicate the general biosafety training, but should supplement it. Training records must be updated and maintained by each PI on annually or when new personnel join the laboratory. These records must be retained for at least 3 years following the last day of the student or staff. Ongoing training is required as new hazards and procedures are introduced into the laboratory.

8.1 DECONTAMINATION AND DISINFECTION

8.2 INTRODUCTION

Disinfection (also known as decontamination) of materials and equipment that are contaminated with infectious agents is routinely performed in microbiological laboratories. Decontamination is a vital component of microbiological safety practice and serves to protect laboratory personnel (as well as others) from infection. Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of cultured organisms. The PI/supervisor is responsible for maintaining decontamination and disinfection supplies.

8.3 CHEMICAL DISINFECTION

Decontamination of work surfaces, equipment, biological safety cabinets, and other inanimate objects using antimicrobial agents is referred to as disinfection. Several chemical agents are used as disinfectants. There are hazards associated with all of these chemical disinfectants and the material safety data sheet (MSDS) should be reviewed for required controls before using the product. Additional information for some of the common chemical disinfectants is summarized below (TABLE 1).

8.4 AUTOCLAVING

Autoclaving uses saturated steam under pressure (approximately 15 psi) to achieve a temperature in the autoclave of at least 121 °C (250 °F). Autoclaving can be used to destroy vegetative bacteria, bacterial spores, and viruses. When decontaminating biohazardous waste, it is recommended that the temperature in the waste reach a minimum of 115 °C for a minimum of 20 minutes. The total processing time required to meet these conditions depends on several loading factors (see below); however, it is recommended that a minimum autoclave cycle of one hour be used when decontaminating waste. There are three factors that in combination determine the effectiveness ofautoclaving:

- **Temperature** Autoclave uses steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121 °C. Although the autoclave chamber may reach 121 °C, this does not necessarily mean that the interior of the load will reach this temperature.
- **Time** A minimum autoclave cycle time of twenty minutes at a chamber temperature of 121°C is commonly recommended for sterilization of clean items (time begins when the chamber has reached 121°C, not when the autoclave cycle is initiated). However, the total processing time required to achieve decontamination depends on several loading factors, including the load container (heat transfer properties), the amount of water added to the load, and the weight of the load. For increased loads, an increased cycle time will be required to ensure effective decontamination.
- **Contact** Steam saturation is essential for maximum heat transfer. Steam must contact all areas of the load. Autoclave bags and other containers should be left partially open (or otherwise permit entry of steam) to ensure adequate contact. Studies have shown that adding water to the interior of the bag improves the time-temperature profile of the autoclave cycle, increasing the sterilization efficiency of the autoclave.

DRY HEAT

Dry heat is less effective than moist heat (autoclaving), requiring higher temperature and longer contact time. Nevertheless, dry heat is preferable to moist heat for decontamination of anhydrous materials and closed containers. This is due to the fact that the moisture component of the steam used in an autoclave will not effectively penetrate anhydrous materials and closed containers.
The highest dry heat equivalent temperature that these materials will reach in an autoclave is 121

°C. The highest temperature that material will reach in a dry-heat oven will be the actual temperature inside the oven. A temperature of 160-180 °C for 3-4 hours is recommended for decontamination of waste using a dry heat oven.

TABLE 1. DECONTAMINATION CHEMICALS AND TARGET AGENTS

Summary of Chemical Disinfectants Disinfectant	Use Parameters	Effective Against					Important Characteristics	Potential Application
		Vegetative cells	Lipophilic viruses	Tubercle bacilli	Hydrophillic viruses	Bacterial spores		
Alcohol (ethyl, isopropyl)	conc.: 70-85% Contact time: 10-30 min.						eye irritant, toxic, flammable, inactivated by organic matter	surfaces – (work & equipment)
Chlorine Compounds	conc.: 0.05-0.5% (commercial bleach ≥5%) Contact time: 10-30 min.	+	+	+	+	±	may leave residue; corrosive; skin, eye & respiratory irritant; inactivated by organic matter; makeup at least weekly; DO NOT autoclave	spills, equipment surfaces, instruments, glassware, water baths
Quaternary Ammonium Compounds	conc.: 0.1-2% Contact time: 10-30 min.	+	+				toxic, inactivated by organic matter	surfaces (work & equip.), BSCs, floor maintenance, glassware, instruments
Phenolic Compounds	conc.: 0.2-3% Contact time: 10-30 min.	+	+	+	±		leaves residue; corrosive, skin, eye & respiratory irritant; toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, floors, spills, glassware, instruments, water baths
Iodophor Compounds (Wescodyne)	conc.: 0.47% Contact time: 10-30 min.	+	+	+	±		leaves residue; corrosive, skin & eye irritant; toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, glassware, water baths
Formaldehyde (Formalin)	conc.: 4-8% Contact time: 10-30 min.	+	+	+	+	±	leaves residue; skin, eye & respiratory irritant; toxic (carcinogen)	less effective than other disinfectants but can be used for equipment surfaces, glassware, instruments
Glutaraldehyde	conc.: 2% Contact time: 10-600 min.	+	+	+	+	+	leaves residue; skin, eye & respiratory irritant; toxic	equipment surfaces, glassware, instruments

9.1 LABORATORY VENTILATION FOR BIOSAFETY

9.2 LABORATORY CHEMICAL ("FUME") HOODS

Traditional laboratory chemical (or fume) hoods are designed to capture and control chemical vapors and pull them away from the worker. Although the flow of air provides protection to the user, chemical hoods do not provide protection for the product (the desired organism being manipulated). Unless a High Efficiency Particulate Air (HEPA) filter is added, chemical hoods do not provide protection against release of viable organisms to the environment. The airflow within a chemical hood is often somewhat turbulent, which can potentially result in exposure of the user to the organisms being used. In short, a chemical hood is not a biological safety cabinet, and generally does not provide product protection or environmental protection.

9.3 HORIZONTAL LAMINAR FLOW CLEAN BENCH

With horizontal laminar flow clean benches, HEPA filtered air flows horizontally across the workspace directly toward the user. These clean benches provide product protection and were originally designed to provide a particulate free environment. Clean benches do provide product protection against microbial contamination, but they do not provide personal protection or environmental protection. In fact, the horizontal flow of air will blow biological agents directly toward the user and into the laboratory. Clean benches are not a biological safety cabinet, and they should not be used with any hazardous materials (biological, chemical, or radiological) requiring containment for protection of personnel or the environment. Clean benches are acceptable for tissue culture work only with cell lines considered to represent low risk (BL1 agents) to laboratory workers (including immunocompromised individuals who may frequent the lab). Human cell lines and nonhuman primate cell lines are generally considered to be BL2 agents and would not be suitable for use in a clean bench.

9.4 BIOLOGICAL SAFETY CABINETS

There are three classes of biological safety cabinets (BSCs), class I, II, and III. Class II BSCs are subdivided into type A and type B cabinets. All BSCs provide personnel and environmental protection, with Class II BSCs also providing product protection. Personnel protection is achieved by inward airflow through the front of the cabinet; product protection is achieved by downward HEPA filtered airflow from the top of the cabinet; and environmental protection is achieved by HEPA filtration of exhaust air. In general, hazardous chemicals should not be used in a BSC, unless it has been designed for that purpose (e.g., BSC exhaust air is not recirculated, electrical systems are properly protected and rated for flammable atmosphere, etc).

CLASS I BSC

Class I BSCs are similar to chemical hoods in that inflow air enters the front of that cabinet, flows across the work area, exits at the rear of the cabinet, and is exhausted outdoors. The primary difference is that chemical hoods usually do not have any filtration mechanism to prevent contaminants from being released to the outside (unless a filter or scrubber is added), whereas all air exhausted from a Class I BSC must pass through a HEPA filter before being exhausted outdoors. The inflow of air into a Class I BSC provides personnel protection, and HEPA filtration of the exhaust air provides environmental protection; however, Class I BSCs do not provide product protection. Class I BSCs are suitable for work involving BL1 and BL2 agents when product protection is not required.

CLASS II TYPE A BSCS

Type A cabinets have a minimum airflow of 75 feet per minute (fpm), and recirculate approximately 70% of the air as HEPA filtered downflow air. Some Type A cabinets have potentially contaminated air plenums that are under positive pressure. Any breach of the positively pressured plenum or ducting would result in loss of containment and possible release of material. Although all air is HEPA filtered before it is exhausted, Type A cabinets can be exhausted directly into the room. Type A cabinets are suitable for BL1 and BL2 agents. Recirculation of air within the cabinet and discharge of exhaust air directly into the room preclude the use of Type A cabinets for volatile chemicals or volatile radionuclides. As of the writing of this Biosafety Manual, all BSCs in Fogarty Life Science are Class II A type cabinets.

CLASS II TYPE B BSCS

All Type B cabinets differ from Type A cabinets in three important design features: 1) all potentially contaminated plenums are under negative pressure, 2) exhaust air is discharged directly to the outside rather than to the room, and 3) they have a higher minimum inflow velocity of 100 fpm.

Type B1 BSCs

Type B1 cabinets are designed such that small quantities of carcinogens and volatile radionuclides required for microbiological work can be handled safely. To prevent buildup of these chemicals within the cabinet, downflow air is "split", with a portion directed to the front of the cabinet and a portion directed to the back of the cabinet where it is exhausted directly to the outside without recirculation. Volatile chemicals should be handled in the direct exhaust (rear) portion of the cabinet to prevent recirculation. Approximately 30% of outgoing air is recirculated as HEPA filtered downflow air. Type B1 cabinets are suitable for BL1 and BL2 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies if the work is performed in the direct exhaust (rear) portion of the BSC.

Type B2 BSCs

These cabinets are referred to as "total exhaust cabinets" because all inflow and downflow air passes through the cabinet only once (without any recirculation), and then is directly exhausted to the outside. Since there is no recirculation of air within the cabinet, downflow air must be drawn in from the room (at the top of the cabinet) and then HEPA filtered prior to entering the cabinet. Type B2 cabinets are suitable for BL1 and BL2 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies.

Type B3 BSCs

Type B3 cabinets are a modified Type A BSC that has no potentially contaminated plenums under positive pressure, and which is exhausted directly to the outside. Type B3 cabinets are suitable for BL 1, 2, or 3 agents and minute quantities of volatile toxic chemicals or tracer amounts of volatile radionuclides.

CLASS III BSCS

Class III BSCs are of a glove-box design (gas-tight absolute containment) that provides the highest level of personnel protection, as well as product and environmental protection. Both supply and exhaust air are HEPA filtered. These cabinets should be maintained under a minimum negative pressure of 0.5" w.g., or as per manufacturer's recommendations. Exhaust air is discharged to the outdoors through double HEPA filters (or HEPA and incineration). Class III cabinets provide the highest level of containment and can be used for work involving any infectious agent; however, they are most appropriate for work involving BL 4 agents.

9.5 CERTIFICATION OF BSCS

Commercial BSCs are tested by the cabinet manufacturer in accordance with National Sanitation Foundation (NSF) criteria. Cabinets that meet the NSF criteria for performance characteristics including biological containment, ventilation, cabinet leakage, and HEPA filter leakage are NSF certified. Field certification of BSCs is also required to ensure that the cabinet still performs as it did when it obtained NSF certification at the factory. Field certification is required by the National Institutes of Health (NIH) under the following circumstances: 1) upon installation of a new BSC, 2) annually thereafter, 3) after repair or maintenance is performed, and 4) after the BSC is relocated.

NSF standard 49 provides criteria for construction of BSCs, testing by manufacturers (including biological containment testing), and field certification. NSF has also established a certification program for field certifiers to ensure a minimum level of competency and professionalism. It is recommended that NSF field certifiers be used for field certification of BSCs. In general, field certification tests include:

1. Primary Tests (BSC performance):
   a. Inflow test
   b. Downflow test
   c. Smoke pattern test
   d. HEPA filter leakage

e. Cabinet leakage (when BSC is newly installed, relocated, or maintenance has been performed that involved removal of access panels)

2. Additional tests (worker comfort and safety): performed at discretion of certifier
   a. Noise
   b. Vibration
   c. Lighting
   d. Electrical leakage, polarity, and ground circuit resistance

9.6 GUIDELINES FOR USE OF BIOLOGICAL SAFETY CABINETS

The installation and use of a BSC is an indication that safe work practices are needed to prevent contamination and infection. Modern BSCs are extensively engineered and provide excellent containment of microorganisms; however, they are not substitutes for good work practices and can only serve to complement a safe worker. Below are general recommendations for BSC use. (The BSC user's manual should be reviewed for specific instructions.) The following instructions are to be posted on or near the BSC.

Before Use

- Turn off UV light and position sash to appropriate height
- Turn on fan at least 5 min before starting
- Check flow with gauge or hold a tissue to ensure flow is inward
- Do not bring bacterial cultures into BSC
- Good microbiological techniques should always be used; reduce splatter and aerosol production
- Don appropriate personal protective equipment (coat, gloves)
- Disinfect work surface with 70% ethanol
- Assemble all supplies in BSC as far back as practical, do not obstruct grille, segregate clean items from contaminated
- Minimize movement of hands in the BSC and avoid moving materials in and out of BSC during use
- Discard or decontaminate pipettes inside BSC
- Discard decontaminated sharps in proper receptacle
- Avoid use of flame unless necessary

After Use

- Allow BSC to run for 5 minutes after completing work
- Disinfect and clean all work surfaces, tubing, vacuum flasks, reservoirs, pipettes with 70% ethanol
- Return culture tools to proper location
- Waste medium is not to be stored in the BSC
- Dispose of contaminated media properly (see CDC decon. protocols)
- Do not store materials inside the BSC
- Turn off blower and close sash
- Turn on UV light
- Autoclave biohazard waste; do not leave a full box
- Wash hands with soap and water before leaving culture room
- Close and lock culture facility before leaving

10.1 HUMAN TISSUE AND CELL CULTURE

10.2 WORKING WITH HUMAN TISSUES AND CELLS

All unfixed human tissue and cells are to be assumed to be infectious (the concept of "Universal Precautions") and must be handled using Biosafety Level 2 (BL2) practices and procedures. Persons who are exposed to unfixed human tissues are considered to have potential exposure to bloodborne pathogens such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV), and must be included in the RIC Bloodborne Pathogens Program. Commercially available cell lines that have been characterized to be free of recognized bloodborne pathogens are exempt from the OSHA Bloodborne Pathogens regulations. The Institutional Biosafety Committee will make the final determination on exemption of cell lines based on evidence provided in the Biological Project Registration Form that is submitted to the IBC.

10.3 TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Spongiform encephalopathies (Creutzfeldt-Jakob, Kuru, and related agents) are fatal prion diseases that have been demonstrated in the brain and spinal cord of infected persons. These agents are resistant to conventional inactivation procedures including chemicals (formalin, alcohol), boiling, dry heat, and irradiation, and these agents can be present in fixed tissue from infected persons. Although nerve tissue (brain, spinal cord) is usually more infectious, all tissues from humans and animals infected with these agents should be considered potentially hazardous. Laboratory-associated infections have not been demonstrated; however, it is prudent to consider nerve tissue (even fixed tissue) potentially infectious. BL2 or higher containment and practices are recommended for all activities utilizing known or potentially infectious tissues and fluids from naturally infected humans and from experimentally infected animals.

10.4 CELL CULTURE

Human or animal pathogens may be associated with cell or organ cultures. Cell cultures known (or suspected) to contain an etiologic agent or an oncogenic virus are classified at the same biosafety level as that recommended for the agent. The following cell cultures and tissues require BL2 containment and procedures:

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

11.1 BIOHAZARDOUS SPILL RESPONSE

11.2 PREPLANNING FOR BIOHAZARDOUS SPILL CLEANUP

The PI is responsible for providing spill response materials and planning. All spills of biological materials and agents do not represent the same risk to personnel and the environment, making each spill somewhat unique. Nevertheless, preplanning of spill response will lower the risk of cleaning up a spill and will increase the likelihood that the spill is handled appropriately. Laboratory supervisors should prepare their laboratory for typical spill scenarios expected in the laboratory, including appropriate disinfection and decontamination materials (see Section 7). Laboratory workers should be informed of the hazards of the biological agents used in the laboratory, the risk associated with these agents during spill scenarios, how to safely cleanup the agents, and how to properly dispose of cleanup materials by the lab supervisor.

Each laboratory area should have spill cleanup materials available to respond to the largest spill anticipated for that area. It is recommended that as a minimum, the following spill cleanup materials be available in the laboratory:

- Gloves - thick chemical resistant gloves or double pair of thin, nitrile gloves recommended
- Safety Goggles - face shield is strongly recommended to avoid splashes to the nose and mouth
- Lab coat or smock to protect clothing and body
- Absorbent pads
- Disinfectant appropriate for the agents used in the laboratory (Section 7)
- Forceps or other devices to pick up contaminated material (especially sharps)
- Sharps disposal container
- Autoclavable biohazard bags

Commercial chemical spill kits may not be adequate for the response to a biological spill. Additional items needed for the cleanup of biohazardous agents should be maintained in the laboratory.

11.3 BIOHAZARDOUS SPILL CLEANUP PROCEDURES

There are several factors that must be considered when assessing the risk that a spill represents. These factors include:

- Volume and concentration of the spilled material
- The infectious dose of the spilled material and routes of exposure
- Location of the spill
- Degree of aerosolization of the agent resulting from the spill
- Susceptibility of the spilled material to disinfection
- Nature of the affected surface(s) and its ability to "hide" organisms from disinfection
- Immune status of immediate personnel

As with any spill scenario (biological or chemical), the safety of personnel is the most important consideration. Cleanup is to begin only after it is determined that the personnel who will clean the spill have appropriate knowledge, training, and equipment. The following are general biohazardous spill cleanup procedures that are appropriate for most spill scenarios; however, the appropriate response to any spill is based on an assessment of the risk associated with that particular situation.

11.4 BIOHAZARDOUS SPILLS INSIDE BIOLOGICAL SAFETY CABINETS

1. Wear laboratory coat (disposable recommended), safety glasses, and gloves (appropriate for the biological agent and the chemical disinfectant) during cleanup.
2. Allow the biological safety cabinet to run continually during cleanup.
3. Surround the affected spill area with absorbent material to prevent spread of the spill.
4. Apply disinfectant appropriate for the biological agent, and allow a minimum of 20 minutes contact time

(or as directed by manufacturer's instructions). Alcohol or other flammable liquids in a non-vented biosafety cabinet are not recommended.

5. Wipe up spill with disposable cloth or towel soaked with disinfectant.
6. Wipe the walls and work surface of the BSC, and any equipment in the cabinet with a disinfectant-soaked cloth.
7. Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
8. Allow non-autoclavable items to have a minimum of 20 minutes contact time with disinfectant (or as directed by manufacturer's instructions) before removing from the BSC.
9. Remove protective clothing and place in a biohazard waste bag for autoclaving.
10. Thoroughly wash hands, forearms, and face with soap and water.
11. Allow BSC to run for a minimum of 10 minutes before resuming work in the cabinet or shutting the cabinet off.

11.5 Biohazardous Spills In the Laboratory, Outside the Biological Safety Cabinet

1. If a BL1 agent (or less than 100 ml of a BL2 agent) is spilled, proceed to step 4.
2. If the spill involves greater than 100 ml of a BL2 agent, immediately evacuate all personnel from the affected area. Wait for aerosol to settle (usually a minimum of 30 minutes) before entering the spill area. Exception: If the laboratory is not under negative pressure, cleanup should begin as soon as possible to minimize the spread of aerosols.
3. Notify Rhode Island College Security and Safety (x 8201) as soon as possible for assistance with the cleanup.
4. Remove any contaminated clothing and place in a biohazard waste bag for autoclaving, and wash all areas affected by skin contact with soap and water. Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.
5. Wear a long-sleeved gown or lab coat (disposable recommended), shoe covers, safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant).
6. Place absorbent pads over the spill (to absorb liquid), then place a second layer of disinfectant- soaked absorbent pads over the spill.
7. Pour additional disinfectant around the spill, being careful to minimize aerosolization, and work from the periphery toward the center, ensuring thorough contact of the spill with the disinfectant. Disinfect all items in the spill area.
8. Allow a minimum of 20 minutes contact time (or as directed by manufacturer's directions) with the disinfectant.
9. Wipe down all equipment, tools, etc. with disinfectant.
10. Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.
11. Remove protective clothing and place in a biohazard waste bag for autoclaving.

11.6 Biohazardous Spills Inside a Centrifuge

1. Clear the area of all personnel and allow aerosol to settle (usually a minimum of 30 minutes) before re-entering the area.
2. Wear a laboratory coat (disposable recommended), safety glasses, and gloves during cleanup.
3. Transfer the rotor and buckets to a biological safety cabinet for cleanup.
4. Using an appropriate disinfectant, thoroughly disinfect the inside of the centrifuge, and the rotor and buckets.
5. Discard cleanup materials and protective clothing as biohazardous waste.
6. Thoroughly wash hands, forearms, and face with soap and water.

11.7 Biohazardous Spills Outside the Laboratory During Transport

1. Immediately clear the area of all personnel and secure the area.
2. Cleanup should be initiated as soon as possible to prevent spread of aerosol. Attempt cleanup only if appropriate cleanup materials and protective clothing are available.
3. Notify Rhode Island College Security and Safety (x 8201) as soon as possible for assistance with the

cleanup. Since it is impossible to prevent aerosolization when a spill occurs outside of the laboratory, the primary emphasis when transporting biological agents is on spill prevention. All biological agents shall be transported from the laboratory inside an unbreakable, well-sealed, primary container containing absorbent material that is contained inside of a second unbreakable, well-sealed, secondary container. Both the primary and secondary containers must be labeled with the universal biohazard symbol and the identity of the agent.

12.1 BIOHAZARDOUS WASTE

12.2 INTRODUCTION

Biohazardous waste includes, but is not limited to waste materials that are derived from or have been in contact with cultures and stocks of infectious agents (BL2 and RG2 or above), human pathological wastes, animal carcasses and body parts contaminated with biohazard agents, and human blood or blood products. PIs must understand risks involved in using their research materials. Proper handling and disposal of biohazardous waste is necessary to prevent infection of personnel (laboratory workers, custodians, laboratory visitors, etc.) and release to the environment. OSHA and the State of Rhode Island require that biohazardous waste be properly labeled, stored, and disposed.

12.3 WASTE MINIMIZATION

The PI or Lab Supervisor has responsibility to provide guidelines and stress the importance of:

- Small sample size
- Avoiding generation of mixed waste: Biohazardous waste and hazardous chemical waste
- Minimize the amount of biohazardous waste and/or hazardous chemical waste that requires commercial disposal

12.4 LABELING AND STORAGE OF BIOHAZARDOUS WASTE

At a minimum, all biohazardous waste must be labeled with the universal biohazard symbol. If the waste is a mixed waste (e.g., radioactive and/or hazardous), additional labeling is required.

12.5 HANDLING AND DISPOSAL OF BIOHAZARDOUS WASTE

All biohazardous waste must be decontaminated before disposal. Autoclaving or use of approved chemical disinfectants may be used for decontamination. See below for procedures.

Sharps

Sharps include all syringes, lancets, scalpels and other similar medical instruments (whether contaminated or not), as well as contaminated Pasteur pipettes, broken glass and other instruments or materials that can cut or puncture personnel. Sharps must be collected in rigid containers that are leak proof and resistant to puncture from the sharps. Sharps containers must be designed so that sharps can be safely introduced into the container but not easily retrieved. Containers should be red or orange in color and labeled with the universal biohazard symbol. When the sharps container is approximately 3/4 full, autoclave the container or request a waste pickup. In order to dispose of autoclaved infectious waste in the normal trash, the Rhode Island solid waste regulations require that all infectious waste must be treated (i.e. disinfected or autoclaved) to make it non-infectious. Contaminated laboratory glassware and broken glass can be decontaminated using a chemical disinfectant and then reused or disposed of as uncontaminated broken glass (see below).

Solid Biohazardous Waste

Solid biohazardous waste includes microbial agents, tissue culture, and contaminated material (such as petri dishes, pipettes, gloves, towels, etc.). These materials can be decontaminated with an approved chemical disinfectant or autoclaved. These materials must be collected in autoclavable biohazard bags that are labeled with the universal biohazard symbol (the bag or the symbol must be red or orange in color). Biohazardous waste must be either autoclaved prior to disposal in the normal trash, or sent to a commercial infectious waste disposal company. A visual steam sterilization indicator (such as autoclave strips or tape) must be included on every biohazard bag. After autoclaving, the waste is considered non-infectious and can be disposed of as ordinary trash; however, it is recommended that the autoclaved bag be placed inside an opaque bag prior to disposal.

Liquid Biohazardous Waste

Liquid biohazardous waste includes all blood and liquid waste from humans or animals, and all other liquid biohazardous waste (such as microbial cultures). Collect liquid waste in closeable, rigid plastic, leak proof containers labeled with the universal biohazard symbol. Small quantities of waste treated bleach or other approved disinfectants can be disposed of by flushing directly to the sanitary sewer. Before treating liquid biohazardous wastes with hazardous chemicals that could create hazardous waste, contact the Rhode Island College Security and Safety (x 8201) Office for assistance.

Animal Carcasses, Body Parts, and Tissue

Non-infectious carcasses are to be placed in an opaque plastic bag and the bag taped shut with duct tape. Store non-preserved, non-infectious carcasses in a freezer or cold storage area prior to disposal. Animals sacrificed by ether euthanasia can be an explosion hazard upon incineration of the carcass; therefore, ether euthanasia of animals is not normally permitted, and must be pre-approved by the IBC. Infectious carcasses are to be collected in a red, biohazard bag and taped shut. Secure limbs and sharp protrusions so they will not puncture the bag. Twist the open end of the bag, fold the end over and tape securely. Infectious carcasses must be sterilized in a steam autoclave as they are generated. Carcasses may be placed in a storage area only after being rendered non-infectious. Infectious carcasses that are not autoclaved on-site will be disposed of through a commercial infectious waste disposal company.

Mixed Waste

All mixed waste (biohazardous waste in a hazardous chemical) must be managed through the Rhode Island College Security and Safety (x 8201).

Decontamination Procedures

To ensure that biohazardous waste is properly decontaminated during autoclaving, the following procedures should be followed:

- Biohazardous waste treated with disinfectant agents should not be autoclaved as some of these chemicals can generate toxic gases.
- Infectious waste must be treated in an autoclave for a minimum of 30 minutes at 121 °C (250 °F); however, the total processing time required to decontaminate infectious waste depends on the specific loading factors (container type, water content, quantity, etc.). A total processing time of 60 minutes is recommended for gravity displacement autoclaves and 10 minutes for vacuum-type autoclaves (132 °C).
- Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Since steam will not penetrate a sealed plastic autoclave bag, bags containing dry loads must not be tightly sealed (rubber band closures will allow bags to "breath") or adequate amounts of water must be added to the load. Consult the manufacturer's instructions for sterilizing materials inside plastic autoclave bags. Liquid waste and fresh animal carcass waste may be autoclaved inside a tightly sealed bag.
- All autoclaved waste must include a steam sterilization indicator (autoclave tape is available).
- Steam autoclaves used to treat infectious waste must operate at a minimum temperature of 121°C. The operating temperature of the autoclave must be verified for each run by maintaining a record of the temperature either as a chart or paper tape recording or a manual recording in a logbook.
- For autoclaves used for biohazardous waste (RG2/BL2 or above), the autoclave monitor or the PI must confirm on a monthly basis that adequate sterilization conditions are being met through the use of ampoules containing heat resistant spores (*Bacillus stearothermophilus*) placed in the center of an autoclave load. In conjunction with the *B. stearothermophilus* testing, Diack Sterilization Monitors will be used to insure that the temperature of the

autoclave reaches 250°F/121°C. The latter monitors consist of a glass ampoule containing a plastic that melts if the temperature reaches at least 250°F/121°C. See the testing procedure below.

12.6 AUTOCLAVE QUALITY ASSURANCE PROGRAM

Autoclaving is an accepted procedure for decontamination of certain biohazardous waste. Biological cultures and stocks, contaminated solid waste, liquid waste, and small animal carcass waste can be sterilized through autoclaving. After sterilization in a steam autoclave, these materials are considered non-infectious. Except for animal carcasses, this bagged waste can then be disposed of as ordinary trash; however, it is recommended that autoclave bags containing sterilized waste be placed in an opaque trash bag prior to disposal. Materials that contain hazardous chemicals or radioisotopes are not to be autoclaved (contact Rhode Island College Security and Safety (x 8201) for assistance).

Monthly Spore Testing Procedure

1. Place ampoule of *B. stearothermophilus* spores and a Diack sterilization monitor in the center of an autoclave load.
2. Process the load under normal operating procedures.
3. Examine the Diack ampule. If the material within the Diack monitor failed to melt, the temperature failed to reach 121 °C. In this case, the autoclave is not to be used to treat infectious waste until it has been repaired and passes retesting. In the interim, tag the autoclave as "Not Approved for Infectious Waste."
4. Incubate the autoclaved *B. stearothermophilus* ampoule and a non-autoclaved, control ampoule according to the manufacturer's instructions (normally 55-60 °C for 24-48 hours).
5. If a color change occurs, the sterilization process was unsuccessful. Discontinue use of the autoclave until it is repaired and passes retesting. Tag the autoclave as "Not approved for Infectious Waste" until the autoclave passes retesting.
6. Indicate test results on Autoclave QC Log (see below) and retain for at least one year.

AUTOCLAVE QC LOG

Year: _____
Autoclave Location: _____
Manufacturer: _____
Model: _____
Serial Number: _____
Autoclave Testing Instructions:

1. Perform autoclave QC tests monthly.
2. Place a *B. stearothermophilus* spore ampoule and a Diack monitor in the center of an autoclave load.
3. Process the load under normal operating procedures.
4. Observe the Diack monitor. If the material has not melted, the autoclave failed to reach 121 °C and the autoclave is not to be used to treat infectious waste until it has been repaired and passes retesting.
5. Incubate the autoclaved *B. stearothermophilus* spore ampoule and a non-autoclaved, control ampoule according to the manufacturer's instructions (normally 55-60 °C for 24-48 hours).
6. If a color change occurs, the sterilization process was unsuccessful. Discontinue use of the autoclave (for infectious waste) until it is repaired and passes retesting.
7. Record testing results on Autoclave QC Log and retain for at least one year.

TABLE 2. AUTOCLAVE QC LOG

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

13.1 CDC/USDA SELECT AGENTS

13.2 FEDERAL JURISDICTION

The Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) have identified specific biological agents and toxins that are considered to be a severe threat to public health and safety as bioterrorism agents. These materials are referred to as select agents by the CDC; and high consequence livestock pathogens and toxins, and listed plant pathogens by the USDA; and their transfer, possession, use, and disposal are strictly regulated. Since the list of select agents may be revised, it is recommended that the CDC Select Agent Program web site and the USDA Agriculture Bioterrorism Protection Act web site be checked before acquiring pathogenic agents and biological toxins. The regulations associated with select agents are very complex and strict, and there are significant monetary fines and criminal penalties associated with non-compliance. Investigators must review and understand the select agent regulations and their responsibilities prior to acquiring or working with, any select agent.

13.3 SELECT AGENTS AT RIC

Any investigator that plans to work with a Select Agent must contact the IBC several months before procuring the material.

TABLE 3. REGULATORY THRESHOLD QUANTITY REQUIRING CDC OR USDA CERTIFICATE OF REGISTRATION

Toxin	Regulatory Threshold Quantity
Abrin	100 mg
Conotoxins	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Tetrodotoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Botulinum neurotoxins	0.5 mg
Clostridium perfringens epsilon toxin	100 mg
Shigatoxin	100 mg
Staphylococcal enterotoxins	5 mg
T-2 toxin	1000 mg

14.1 PACKAGING AND SHIPPING INFECTIOUS AGENTS

14.2 INTRODUCTION

The International Civil Aviation Organization (ICAO) is the entity within the United Nations that governs all international civil aviation matters. The ICAO Technical Instructions for the Safe Transport of Dangerous Goods by Air are the regulations that govern the shipping of dangerous goods. These technical instructions have been incorporated into US law and are an acceptable method of transport in the US (49 CFR 171.11). Packaging and shipping biological materials involves certain risks with numerous potential liabilities. The International Air Transport Association (IATA), Dangerous Goods Regulations (DGR), latest edition, is the worldwide gold standard for shipping. The IATA regulations apply to all air transport, both domestic and international flights. By following IATA DGR you ensure that your package will also meet U.S Department of Transportation requirements for ground transport. All responsibilities for packaging and shipment of these agents have been assigned to the shipper. An overview of the packaging and shipping regulations is provided below. As these regulations may change, the shipper should refer to the applicable regulations for any changes.

14.3 DEFINITIONS

Dangerous Goods – articles or substances which are capable of posing significant risk to health, safety, property or the environment when transported by surface or air.

Diagnostic Specimens – articles or substances that are shipped for routine screening tests for the purpose of diagnosis. There must be a relatively low probability that infectious substances are present. If the article or substance is being shipped for testing or diagnosis of an infectious substance (e.g. HIV, Hepatitis B, Cytomegalovirus, Hantavirus) then the substance must be shipped as an infectious agent.

Infectious Substances – substances known to contain, or reasonably expected to contain, pathogens.

Pathogens – microorganisms or recombinant microorganisms that are known to, or reasonably expected to, cause infectious disease in humans or animals.

14.4 TRAINING REQUIREMENTS

Those involved in the packaging and shipping of diagnostic specimens or infectious substances must undergo training every two (2) years or when activities change. It is the responsibility of the department to assure training is completed. The shipper is under obligation to receive further qualification when shipping hazardous materials of a class or division where current training is insufficient.

14.5 SHIPMENT CLASSIFICATIONS

DIAGNOSTIC SPECIMENS:

Diagnostic specimens may be shipped utilizing IATA packaging instructions 650. The manufacturer will identify compliance with package specifications on the outer shipping container. Orientation labels may be used. Specimen containers are to be placed in the cushioning material, which is then placed into the primary leak-proof container. Absorbent material, sufficient to absorb the quantity of the shipment, must be placed next to the primary container. The specimens and an inventory of the contents are then placed into the outer container.

INFECTIOUS SUBSTANCES

IATA regulations for infectious substances apply to World Health Organization (WHO) risk groups 2, 3, and 4. Infectious substances must be shipped utilizing IATA Packaging Instructions 602. The outer shipping container must be marked in compliance with these packaging instructions. Orientation labels must be used.

TABLE 4. RISK GROUP DEFINITIONS FOR SHIPPING INFECTIOUS SUBSTANCES

Risk Grou	Definition
2	Moderate individual risk, low community risk. These pathogens can cause disease in animal or humans but are unlikely to do so. Effective treatment and preventative measures are available to prevent widespread infection.
3	High individual risk, low community risk. These pathogens usually cause serious disease in animals or humans but do not ordinarily spread from one organism to another. Effective treatment and preventative measures are available.
4	High individual and community risk. These pathogens usually cause serious human or animal disease that is easily transmitted. Treatment and preventative measures are generally not available.

For Infectious Substances IATA Packaging Instructions 602 apply. Three containment levels are used to package Infectious Agents for shipment. The three levels ensure containment of the agent and include:

1) Primary – plastic, metal or glass leak-proof container, containing the product. Enough absorbent material must be placed inside this container to prevent leakage of the contents.
2) Secondary - plastic, metal or glass leak-proof container, containing the primary container and sufficient absorbent material for the entire quantity of the product.
3) Tertiary – contains secondary container, description of contents, dry ice or other preserving material, and any packaging material required to prevent shifting of contents.

14.6 PRESERVATIVES

When preservatives are shipped with a specimen or agent, the preservative must be declared on the Shipper's Declaration (see below). The hazard class label that applies to the preservative, and the quantity of the preservative, must also be on the outer package. Additional information may be found in the IATA Dangerous Goods Regulations, latest edition.

TABLE 5. HAZARD CLASSIFICATION FOR SHIPPING WITH PRESERVATIVES OR CRYOGENIC AGENTS

Preservative	Hazard Class	UN Code
Dry Ice*	9	1845
Formalin, Formaldehyde Solution	8	2209
Nitrogen, Refrigerated Liquid	2.2	1977

Appendix 1

Bloodborne Pathogen Exposure Control Plan

Rhode Island College
Health Services

Revised
January 5, 2015

Reviewed
January 2017

Developed in accordance with the OSHA Bloodborne Pathogens Standard, 29 CFR 1910.1030

PURPOSE:
The purpose of this exposure control plan is to eliminate or minimize employee occupational exposure to human blood or other infectious body fluids. Other potentially infectious body fluids include: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, and any body fluid visible contaminated with blood.

SCOPE:
This Standard applies to all College Health Services personnel who, during the course of their employment, may come into contact with human blood or potentially infectious bodily fluids.

ENGINEERING AND WORK PRACTICE CONTROLS:
Universal precautions will be observed by all employees in order to prevent contact with blood or other potentially infectious materials. All blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual.

Engineering and work practice controls will be utilized to eliminate or minimize exposure to bloodborne pathogens.

1. Employees must wash their hands or other skin with soap and water, or flush mucous membranes with water, as soon as possible following an exposure incident (such as a splash of blood to the eyes or an accidental needle stick).

2. Employees must wash their hands immediately (or as soon as feasible) after removal of gloves or other personal protective equipment (PPE).

3. Needles shall be disposed of in labeled sharps containers.

    a. Needles should never be recapped.

    b. Needles may be moved or picked up only by using a mechanical device or tool (forceps, pliers, broom and dust pan).

4. Breaking or shearing of needles is prohibited.

5. No eating, drinking, smoking, applying cosmetics or lip balm, or handling contact lenses is allowed in a work area where there is a reasonable likelihood of occupational exposure.

6. No food or drinks shall be kept in refrigerators, freezers, cabinets, shelves, or on counter tops or bench tops where blood or other potentially infectious materials are present.

7. Employees must perform all procedures involving blood or other potentially infectious materials in such a manner as to minimize splashing, spraying, splattering, and generation of droplets of these substances.

HOUSEKEEPING:

Decontamination will be accomplished by utilizing the following materials:

a. EPA-registered disinfectants
b. 10% (minimum) solution of chlorine bleach

- All contaminated work surfaces, tools, objects, etc. will be decontaminated immediately or as soon as feasible after any spill of blood or other potentially infectious materials. The bleach solution or disinfectant must be left in contact with contaminated work surfaces, tools, objects, or potentially infectious materials for at least 10 minutes before cleaning.

- Equipment that may become contaminated with blood or other potentially infectious materials will be examined and decontaminated before servicing or use.

- Broken glassware will not be picked up directly with the hands. Sweep or brush material into a dustpan.

- Known or suspected contaminated sharps shall be discarded immediately or as soon as feasible in containers that are closable, puncture-resistant, leak-proof on sides and bottom, and marked with an appropriate biohazard label.

- When containers of contaminated sharps are being moved the containers shall be closed immediately before removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

OTHER REGULATED WASTE:

Other regulated waste shall be placed in red bags and containers that are closable, constructed to contain all contents and prevent leakage of fluids during handling, storage, transportation or shipping.

The waste must be labeled and closed before removal to prevent spillage or protrusion of contents during handling, storage, or transport.

Incineration of biohazardous waste shall be handled by a biological waste destructor (Triumverate). This shall be coordinated through the Physical Plant.

PERSONAL PROTECTIVE EQUIPMENT (PPE):

Where occupational exposure remains after institution of engineering and work controls, personal protective equipment (PPE) shall also be utilized and is provided to Health Services staff at no cost to them.

The types of PPE available to employees are as follows:

- Gloves
- Masks
- Eye protection
- Disposable gowns

All PPE will be chosen based on the anticipated exposure to blood or other potentially infectious materials. The protective equipment will be considered appropriate only if it does not permit blood or other potentially infectious materials to pass through or reach the employee's clothing, skin, eyes, mouth, or mucous membranes under normal conditions of use and for the duration of time for which the protective equipment

will be used.

Employees must:

- Utilize protective equipment in occupational exposure situations.
- Wear gloves when it is reasonably anticipated that there may be hand contact with blood or other potentially infectious material (OPIM), and when handling or touching contaminated items or surfaces; replace gloves if torn, punctured or contaminated, or if their ability to function as a barrier is compromised.
- Wear appropriate face and eye protection when splashes, sprays, spatters, or droplets of blood or OPIM pose a hazard to the eye, nose, or mouth.
- Utility gloves may be decontaminated for reuse if their integrity is not compromised; discard utility gloves if they show signs of cracking, peeling, tearing, puncturing, or deterioration.
- Never wash or decontaminate disposable gloves for reuse.
- Wash hands immediately or as soon as feasible after removing gloves or other PPE.
- Remove garments that become penetrated by blood or OPIM immediately or as soon as feasible in such a way as to avoid contact with the outer surface.
- Remove all personal protective equipment before leaving the work area.
- Place all garments in the appropriate designated area or container for storage or disposal.

HEPATITIS B VACCINE:

The hepatitis B vaccination series is available at no cost after initial employee training and within 10 days of initial assignment to Health Services. Vaccination is encouraged unless: 1) documentation exists that the employee has previously received the series; 2) antibody testing reveals that the employee is immune; or 3) medical evaluation shows that vaccination is contraindicated.

If the employee initially declines Hepatitis B vaccination, but at a later date decides to accept the vaccination, the vaccination shall then be made available at no cost.

All employees who decline the Hepatitis B vaccination offered shall sign the OSHA-required waiver indicating their refusal.

POST-EXPOSURE EVALUATION AND FOLLOW-UP:

All exposure incidents shall be reported, investigated, and documented. When the employee incurs an exposure incident, it shall be reported immediately to their supervisor.

If an employee of Health Services incurs a needlestick or sharps injury or is exposed to the blood or body fluid of a patient during the course of their job, the following steps should immediately be followed:

- Wash needlesticks and cuts with soap and water
- Flush splashes to the nose, mouth, or skin with water
- Irrigate eyes with clean water, saline, or sterile irrigants
- Report incident to Director of Health Services
- Immediately seek medical treatment. Staff are referred to Roger Williams Medical Center Emergency Room at 825 Chalkstone Ave., Providence, RI. for an evaluation for post exposure prophylaxis (PEP) will occur.

Following a report of an exposure incident, the exposed employee shall go to the Student Health Center for a confidential medical evaluation and follow-up, including at least the following elements:

1. Documentation of the route(s) of exposure.

2. A description of the circumstances under which the exposure occurred.

3. The identification and documentation of the source individual. (The identification is not required if the employer can establish that identification is impossible or prohibited by state or local law.)

4. The collection and testing of the source individual's blood for HBV and HIV serological status.

5. Counseling.

7. Evaluation of any reported illness.

The Healthcare professional evaluating an employee will be provided with the following information:

1. A copy of this plan.
2. A copy of the OSHA Bloodborne Pathogen regulations (29 CFR 1910.1030)
3. Documentation of the route(s) of exposure.
4. A description of the circumstances under which the exposure occurred.
5. Results of the source individual's blood testing, if available.
6. All medical records applicable to treatment of the employee, including vaccination status.

The employee will receive a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation.

The healthcare professional's written opinion for Hepatitis B vaccination is limited to the following: (1) whether the employee needs Hepatitis B vaccination; (2) whether the employee has received such a vaccination. The healthcare professional's written opinion for post-exposure evaluation and follow-up is limited to the following information:

1. That the employee was informed of the results of the evaluation.
2. That the employee was informed about any medical conditions resulting from exposure to blood or other infectious materials that require further evaluation or treatment.

All other findings or diagnoses will remain confidential and will not be in a written report.

All medical evaluations shall be made by or under the supervision of a licensed physician or by or under the supervision of another licensed healthcare professional. All laboratory tests must be conducted by an accredited laboratory at no cost to the employee. All medical records will be kept in accordance with 29 CFR 1910.1020.

TRAINING:

All high-risk employees shall participate in a training program. Training will occur before assignment to a task where occupational exposure may take place and at least annually thereafter. Additional training will be provided when changes such as modification of tasks or procedures affect the employee's occupational exposure.

Any employee who is exposed to infectious materials shall receive training, even if the employee was allowed to receive the HBV vaccine after exposure.

The training program will include at least the following elements:

1. An accessible copy of the regulatory text of 29 CFR 1910.1030 and an explanation of its contents.

2. A general explanation of the epidemiology and symptoms of bloodborne diseases.

3. An explanation of the modes of transmission of bloodborne pathogens.

4. An explanation of the employer's exposure control plan and the means by which the employee can obtain a copy of the written plan.

5. An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood or other potentially infectious materials.

6. An explanation of the use and limitations of methods that will prevent or reduce exposure, including appropriate engineering controls, work practices, and personal protective equipment.

7. Information on the types, proper use, location, removal, handling, decontamination, and disposal of personal protective equipment.

8. An explanation of the basis for selection of personal protective equipment.

Hepatitis B Vaccine Declination

I understand that due to my occupational exposure to blood or other infectious materials that I may be at risk of acquiring Hepatitis B virus infection. I have been given the opportunity to be vaccinated with the Hepatitis B vaccine at no charge to myself. However, I decline the Hepatitis B vaccination at this time. I understand that by declining this vaccine, I continue to be at risk of acquiring Hepatitis B, a serious disease. If in the future I continue to have occupational exposure to blood or other potentially infectious materials and I want the Hepatitis B vaccine, I can receive the vaccine series at no charge to me.

(print name)	(title)	(date)

(signature)