**27 May 2009**

**WPI Biosafety Manual**

**Introduction to the Manual**

The goal of this manual is to provide policies and procedures that when implemented, will reduce risks to Worcester Polytechnic Institute (WPI) employees, students and the greater community from biological agents, including pathogenic organisms and derived toxins. These policies and procedures reflect current biosafety regulations, as well as generally accepted biosafety practices, and are designed to safeguard personnel, facilities, and the environment without inhibiting research activities. Principal Investigators and laboratory workers are expected to be familiar with the requirements of this Manual, and to apply these requirements in their laboratory operations.

The hazards present in any particular laboratory are rarely limited to biological agents; chemical hazards are almost always of concern, and radiological hazards may also be present. Consequently, biosafety should not be approached separately from other laboratory hazards, but be viewed as just one component of a total laboratory safety program.

Success of the Biosafety Program requires a team effort involving everyone and Environmental Health and Safety. **Principal Investigators are responsible for the health and safety of personnel who work under their supervision and occupy their laboratory space.**

Worcester Polytechnic Institute management endorses this manual and encourages active participation in maintaining high standards of biosafety in WPI laboratories.

**Worcester Polytechnic Institute**

**BIOSAFETY MANUAL**

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**Chapter 1**

**Purpose, Scope, and Responsibilities**

Purpose

The purpose of the Worcester Polytechnic Institute Biosafety Program is to define policies and procedures to minimize risks to personnel, facilities, and the environment resulting from the use of biological agents. The work practices, procedures and policies specified in this manual are based on current laws and guidelines from the city, state and Federal agencies and recognized good biosafety practices. Implementation of these measures will reduce the likelihood of incidents involving biological agents and will fulfill regulatory biosafety requirements. Laboratory microbiological work usually involves exposure not only to biological hazards, but to chemical and radiological hazards as well. Consequently, this manual should be used in conjunction with the site Chemical Hygiene Plan and the Radiation Safety Manual as well as requirements of the Institutional Animal Care and Use Committee (IACUC) and the Institutional Review Board (IRB) when appropriate.

Scope

This manual applies to all activities involving biological agents. All faculty, staff, students, and visitors when working on Worcester Polytechnic Institute sponsored projects or facilities, are included in the scope of this manual.

Biological agents include all infectious microorganisms (bacteria, chlamydia, fungi, parasites, prions, rickettsias, viruses, etc.) that can cause disease in humans, or significant environmental or agricultural impact, and products or toxins derived from such organisms. Additionally, recombinant DNA; human or non-human primate tissues, fluids, cells or cell culture; transgenic plants or animals; and work with animals known to be reservoirs of zoonotic diseases are wholly or partly covered by the procedures and policies in this manual.

Responsibilities

The responsibility for biosafety is a team effort requiring the direct involvement of the Institutional Biosafety Committee, Principal Investigators (PIs), laboratory workers, the Biosafety Officer and Environmental Health and Safety (EH&S).

Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) develops policies and provides leadership with the goal of reducing risks to workers and the community due to biological agents. The IBC is composed of at least five members that collectively represent experience and expertise in a wide range of biosafety areas applicable to planned activities. At least two members of the IBC must be from outside the Worcester Polytechnic Institute community (not otherwise affiliated with WPI).

Non-committee faculty or staff with special expertise may be asked to advise the IBC, as appropriate.

**Current members of the IBC are listed below:**

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*Two community members*

(Contact the IBC Chair for contact information)

Responsibilities of the IBC include:

1. Developing biosafety policies applicable to all activities, including work practices, biohazardous waste, and medical surveillance of personnel.

2. Reviewing and approving new research proposals in accordance with CDC/NIH guidelines.

3. Setting required containment levels for research projects. Generally, the biosafety levels (BSLs) 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.

4. Developing design specifications and criteria for containment facilities.

5. Investigating significant violations of biosafety procedures or policies, and significant accidents or illnesses involving Biological Agents. If appropriate, the IBC will recommend disciplinary action to the proper University officials.

6. Ensure permits and licenses pertaining to rDNA work are obtained and maintained.

Biosafety Officer

The Biosafety Officer (BSO) is responsible for providing guidance on safe handling of biological agents and overall management of the Biosafety program. The BSO is a member of the IBC. Specific responsibilities of the BSO include:

1. Providing technical advice to the IBC and PIs on biosafety protocols.

2. Developing emergency response plans for accidental spills and personnel contamination, and investigating incidents involving biological agents.

3. Making periodic inspections of laboratories to assess biosafety issues.

4. Keeping the IBC informed of pertinent biosafety issues and program status, including the

 biowaste program.

5. Providing general biosafety and Bloodborne Pathogens Safety training for personnel on a regular basis.

Principal Investigators

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

1. Ensuring that specific laboratory hazards are effectively communicated to laboratory personnel, and that controls are in place to minimize risks associated with these hazards.

a. Developing laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory.

b. Ensuring that engineering controls are available, are in good working order, and are used appropriately to minimize exposure to biohazardous agents.

c. Ensuring that appropriate personal protective equipment is available and used by laboratory personnel.

2. Ensuring that all laboratory personnel receive general Biosafety training conducted by the Biosafety Officer, as well as specific training on the hazards, procedures, and practices relevant to the laboratory they are working in. All training must be documented and records maintained.

3. Notifying the IBC and obtaining prior IBC approval for work involving biohazardous material as specified in this manual.

4. Ensuring that laboratory workers are provided immunizations and medical surveillance prior to exposure to biohazardous agents as appropriate (based on current recommendations of the Centers for Disease Control and Prevention, and IBC recommendations).

5. Notifying the BSO of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment.

6. Ensuring that biological agents are disposed of according to regulations and as outlined in this manual.

7. Ensuring that biohazardous materials to be transported are packaged and shipped in accordance with regulations.

8. Ensuring that periodic assessments of the laboratory are conducted.

9. Submitting a “Biowaste SOP” to the IBC Chair once per year, and whenever there are changes in volumes, locations, nature of experiment or organisms used. Find the form in Appendix, page 61.

Laboratory Workers and Project Students

Those working at the laboratory benches are the most important element in developing and maintaining a safe laboratory environment. Project Students can be undergraduate students, graduate students, or volunteers, who conduct research activities in a laboratory. This includes activities that are part of a research project, classroom course work or any other laboratory activity.

Supervision of Project Students is the direct responsibility of a Principal Investigator (PI). The PI is responsible for assuring all Project Students are properly trained, as defined in Chapter 5. Laboratory staff and project students are responsible for their own health and safety, as well as that of their lab mates.

An incident caused by one laboratory worker can have a widespread affect on others. Specific responsibilities include:

1. Following procedures and practices established by the site and the laboratory.

2. Using accepted good laboratory practices to minimize exposures to biological agents, and to avoid other incidents (such as fire, explosion, etc.).

3. Attending biosafety and other laboratory safety training as required.

4. Reporting unsafe laboratory conditions to the PI, EH&S, or other responsible party.

5. Utilizing control measures such as biological safety cabinets and personal protective equipment to prevent exposure to biological agents, and contamination of personnel and facilities.

Custodial staff, Incidental Staff and Visitors

Non-laboratory staff (custodial, tradespersons, administrative, etc.) or visitors are occasionally present in the laboratory. Their conduct is the responsibility of the PI or designated staff member (Supervisors in the case of Custodial and WPI trades) while in spaces that may contain biological agents. WPI lab personnel must discuss the following guidelines with those individuals entering the labs prior to admittance.

Non-Laboratory staff and visitors have the responsibility to:

1. Follow procedures and practices established by the site and the laboratory.

2. Stay in the presence of the PI or designated staff member if not trained to work alone in these spaces.

3. Refrain from touching surfaces or objects unless allowed or trained to do so, and only with specific, case-by-case permission of the PI or designated escort.

4. Leave the laboratory promptly, and if a visitor, escorted by the PI or WPI staff designee.

**Chapter-2**

**Approval of Research Projects**

**Project Approval**

The WPI IBC oversees the use of all biological agents on campus. Depending on the biosafety level (BSL), some projects require prior IBC approval, others require simultaneous IBC approval, while others require a general IBC notification. The table below summarizes various levels of biological research based on the section number in the NIH Guidelines for Research Involving Recombinant DNA Molecules and the various required approvals.

|  |  |  |
| --- | --- | --- |
| **NIH Guidelines Section** | **REQUIRED APPROVALS** | **Examples** |
| **WPI IBC** | **RAC** | **NIH Director** | **NIH ORA** | **IRB** |
| III-A | Prior | Prior | Prior |  |  | Drug resistance transfers |
| III-B | Prior |  |  | Prior |  | Cloning of potent toxins |
| III-C | Prior |  |  | Prior | Prior | Transfer of rDNA into humans |
| III-D | Prior |  |  |  |  | BSL-2, 3, 4 |
| III-E | Simultaneous |  |  |  |  | BSL-1 |
| III-F | Notification |  |  |  |  | Exempt |

**IBC Notification and Compliance**

It is the responsibility of each PI to notify the IBC of any biological work performed on campus. All work involving biological agents must be reviewed by WPI’s Institutional Biosafety Committee (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 DNA Molecules, applicable regulations, as well as WPI policies and current biosafety practice.

Notification to the IBC can be achieved in any of several ways:

1. Review of WPI’s Grant Proposal Coordination Form: WPI’s Office of Research Administration (ORA) requires that each grant proposal submitted to their office for review must be accompanied by a “Proposal Coordination Form”. This form is available at: <http://www.wpi.edu/Images/CMS/ORA/WPI_PCF_Rev.9.23.08.pdf>

On the form, if the PI checks the box marked “Biohazardous Materials”, the ORA automatically forwards a copy of the form to the IBC Chair for review. The IBC Chair solicits additional information from the PI to determine the biosafety level of the proposed research, and to determine whether the proposed research matches the general BSL level approved for that lab.

2. Annual IBC Chair Review of Specific BSL Designations for Each Lab: Each year, the IBC Chair emails all faculty and staff at WPI reminding them that all biological research falls underneath IBC jurisdiction, and if they are performing research with any biological agents they must notify the IBC. This general email is then followed to PI’s known in advance to be handling bioagents to obtain updates on organisms being handled in the lab, and to review the BSL designation for that lab.

3. Non-Funded or Contract Work: Regardless of whether or how the research is funded, it is the responsibility of each PI doing research with any bioagent to notify the IBC.

PIs are required to verify and update approved projects on an annual basis. The purpose of the annual update is to allow the PI to verify continuance of the project, discontinue a project, or amend a project. Significant modifications will require IBC approval. When notifying the IBC, all changes involving biological agents, significant procedural changes, or modifications that increase the risk of the project must be approved by the IBC. The PI is also required to notify IBC Chair when a project is completed or is no longer active.

**General BSL Classifications**

**Exempt Organisms**

Although some biological agents are exempt from NIH guidelines, working with these agents still requires general notification to WPI’s IBC to allow oversight. Examples of such agents include routinely used highly mutated *E. coli* host-vector systems.

**Biosafety Level 1 (BSL1) and Animal Biosafety Level 1 (ABSL1)**

Agents in this category are not known to cause disease in healthy human adults. Simultaneous IBC approval is required for recombinant BSL1 work. Examples of BSL-1 experiments include routine worm, fly and fish transgenics (IIIE2b5), the creation of rodent transgenics with RG-1 genes, and the use of recombinant murine retroviruses, bacteriophages, or non-pathogenic *E. coli* not on the exempt list.

**Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2)**

Agents in this category are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. All work involving biological agents classified as BSL2 must be previously 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. Examples of BSL2 experiments include all work with human cell lines, the use of live vaccinia or adenoviruses encoding routine cloned genes (i.e. non-oncogenes), the use of enteropathic *E. coli*, non-routine worm, fly, and fish transgenics (IIID2a), and the transfer of RG-2 DNAs into non-pathogenic prokaryotes or into lower eukaryotes.

**Biosafety Level 3 (BSL3) and Animal Biosafety Level 3 (ABSL3)**

Agents in this category are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. All work involving biological agents classified as BSL3 must be previously 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. Examples of BSL-3 experiments include using live vaccinia or adenoviruses encoding potent oncogenes, using live lentiviruses, and use of live pathogenic bacteria such as *Yersinia*.

**Biosafety Level 4 (BSL4) and Animal Biosafety Level 4 (ABSL4)**

Agents in this category are likely to cause serious lethal human disease for which preventive or therapeutic interventions are not usually available. Projects involving BSL4 organisms are currently prohibited at WPI.

**Recombinant DNA**

As a condition of funding from the National Institutes of Health (NIH), all research at involving recombinant DNA must be conducted in accordance with the most current version of *NIH Guidelines for Research Involving Recombinant DNA 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. The following paragraphs summarize experiments covered by the NIH Guidelines; refer directly to these guidelines for a more detailed description of experiments and specific requirements.

NIH Section III-A

These experiments require prior approval from the IBC, the NIH Recombinant DNA Advisory Committee (RAC), and the NIH director. These experiments involve 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. These experiments are considered “Major Action”. Additional information on the Office of Biotechnology Activities (OBA) and the RAC is available at http://www4.od.nih.gov/oba/rdna.htm.

NIH Section III-B:

These experiments require prior approval from the IBC and the NIH Office of Biotechnology Assessment (OBA). Experiments in this category include the cloning of genes encoding toxic molecules with an LD50 for vertebrates less than or equal to 100 ng/kg. This includes microbial toxins such as botulinum toxins, tetanus toxins, and diphtheria toxin.

NIH Section III-C:

These experiments require prior approval from the IBC, the WPI Institutional Review Board (IRB), the NIH RAC, and the NIH OBA. These experiments involve the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into humans (human gene transfer).

NIH Section III-D:

These experiments must be approved by the IBC prior to initiation. This category includes non-routine whole animal or whole plant experiments (i.e. experiments not covered by section III-F), as well as experiments involving DNA from Risk Group 2, 3, or 4 agents.

NIH Section III-E:

Notification to the IBC simultaneous with experiment initiation is required for these experiments. Experiments in this category are low risk and can be conducted using BSL1 containment. This category includes experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes, for example:

1. the use of RG-1 agents including murine retroviruses, highly defective viral vectors, bacteriophages, and non-pathogenic *E. coli* not on the excluded list (see below) (III-E1).

2. routine rDNA experiments using whole plants (III-E2)

3. routine worm, fly, and fish transgenics (III-E2b5).

4. the creation of rodent transgenics using RG-1 genes (III-E3).

NIH Section III-F:

Although these experiments are exempt from the NIH Guidelines by NIH policy, the WPI IBC requires IBC general notification of this work. Examples in this category include:

1. rDNA that is not in organisms or viruses (i.e. the routine *in vitro* manipulation of rDNA by restriction digestion and ligation) (III-F1).

2. rDNA consisting entirely of DNA segments from a single non-chromosomal or viral DNA source (III-F2).

3. rDNA consisting entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host or when transferred to another host by well established physiological means (III-F2).

4. rDNA consisting entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria, or plasmids when propagated only in that host (III-F4).

5. rDNA consisting entirely of DNA segments from different species that exchange DNA by known physiological processes (III-F5).

6. rDNA that does not present a significant risk to health or the environment (III-F6).

**Other WPI Oversight Committee Approvals:**

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

Before starting work with live animals, the Institutional Animal Care and Use Committee (IACUC) should be contacted. They are charged with overseeing and evaluating WPI's animal program, procedures, and facilities to insure that it is consistent with all applicable guidelines, recommendations, and regulations.

http://www.wpi.edu/Admin/OSP/IACUC/

***Institutional Review Board (IRB)***

If human subjects are part of a study or research project WPI’s Institutional Review Board (IRB) Chair must be contacted. Under Federal mandate, this board was established to help investigators understand and comply with the ethical guidelines and regulatory requirements for research involving human subjects.

http://www.wpi.edu/Admin/OSP/IRB/

***Radiation, Health, and Safeguards Committee (RHSC)***

The WPI Radiation, Health, and Safeguards Committee (RHSC) is dedicated to providing a safe environment for the use of radioactive material at WPI. The RHSC is comprised of representatives appointed by the administration of WPI. CGSR membership is consistent with the federal provisions of 10CFR33.13.C.1 and the state provisions of 105CMR120.127.B.3.a.

http://www.wpi.edu/Admin/Safety/RSO/rhsc.html

***Licensing Requirements:* City of Worcester Recombinant DNA Ordinance**

WPI additionally complies with local regulations pertaining to work with recombinant DNA technology. The following information is posted on the City of Worcester website, under Department of Inspectional Services, http://www.ci.worcester.ma.us/

In accordance with Revised Ordinances of the City of Worcester 1986, Chapter 17, Section 6: “No institution may employ rDNA technology in the City of Worcester without a special permit issued by Public Health”.

**Massachusetts Department of Public Health:** Minimum requirements for the management of medical or biological waste (State Sanitary code Chapter VIII), see Chapter 11 of this manual.

**Chapter 3**

**Biosafety Regulations and Guidelines**

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.

1. Centers for Disease Controls and Prevention (CDC) and the National Institutes of Health (NIH): Biosafety in Microbiological and Biomedical Laboratories (BMBL) <http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm>

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.

2. National Institutes of Health (NIH): Guidelines for Research Involving Recombinant DNA

 Molecules (NIH Guidelines)

<http://oba.od.nih.gov/rdna/nih_guidelines_oba.html>

 This document provides guidelines for constructing and handling recombinant DNA molecules (rDNA), and organisms containing rDNA. Although these guidelines are not subject to regulatory enforcement, institutions that receive any NIH funding for rDNA 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 rDNA research using the NIH Guidelines as a minimum standard.

3. Occupational Safety and Health Administration (OSHA): Bloodborne Pathogens

<http://www.osha.gov>

This regulation covers occupational exposure to human blood and other potentially infectious material, including human tissue and cells. OSHA specifies a combination of engineering controls, work practices, and training to reduce the risk of infection. Personnel potentially exposed to human blood and other potentially infectious material must be offered immunization against Hepatitis B and receive training. Personnel who work with HIV or Hepatitis B in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens

1. Select Agents: 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.

<http://www.selectagents.gov/>

This regulation requires that laboratories comply with the BMBL (see above) and the OSHA Laboratory Standard. Each transfer of a Select Agent must be accompanied by a specific CDC/APHIS form that requires the signature of the Worcester Polytechnic Institute Responsible Official and serves to document the chain of custody. Several written safety- and security-related documents are required and must be developed and maintained by affected laboratories. Background checks are required for persons seeking approval for access to select agents.

See Chapter 12 of this manual, “CDC/USDA Select Agents”, for additional information.

**Chapter 4**

**Biosafety Principles**

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 area from exposure to biological agents. This 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.

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 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 Worcester Polytechnic Institute biosafety program has been developed to provide general policies and procedures when working with biological agents. Each individual laboratory must supplement this manual with laboratory specific policies, procedures and training that will minimize the specific risks present in their experiments or laboratories.

Safety Equipment

Safety equipment includes engineering controls designed to minimize exposure to biological agents (such as Biosafety Cabinets, aerosol-containing centrifuge rotors or cups, aerosol capturing filters, etc). Biological safety cabinets (BSCs) are the most important safety equipment for protection of personnel and the laboratory environment, and most BSCs also provide product protection. Safety equipment is only effective when workers are trained on the proper use of such equipment, and the equipment is regularly inspected and maintained.

Personal Protective Equipment

Personal protective equipment includes safety eyewear, lab coats, and gloves, and is used to supplement the containment provided by laboratory practices and safety equipment. Personal protective equipment is considered the least desirable containment method since its failure results in direct exposure of personnel to the biological agent.

Facility Design

Facility design and security features provide 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 specific infectious organisms. Descriptions of the biosafety levels, as well as assigned biosafety levels for specific organisms, are contained in the CDC/NIH document; *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*. The recommended biosafety level for an organism represents conditions under which the agent can normally be handled safely; however, specific circumstances may dictate that the recommended conditions be raised or lowered. As outlined in the BMBL, the four biosafety levels are summarized below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biosafety Level** | **Agents** | **Practices** | **Safety Equip.** | **Facilities** |
| 1 | Not known to cause disease in healthy adults. | Standard Microbiological Practices | None required | Open bench top, sink required |
| 2 | Associated with human disease, *hazard:* auto-inoculation, ingestion, mucous membrane exposure | BSL-1 practice plus:1. Limited access
2. Biohazard warning signs
3. Sharps precautions
4. Biosafety manual
 | *Primary barriers:* Class I or II BSCs or other containment used for manipulations of agents that cause splashes or aerosols of infectious materials; *PPE:* lab coats; gloves; eye/face protection as needed | BSL-1 plus:Autoclave available |
| 3 | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences | BSL-2 practice plus:1. Controlled access
2. Decontamination of all waste
3. Decontamination of lab clothing before laundering
4. Baseline serum
 | *Primary barriers:* Class I or II BSCs or other physical containment devices used for all manipulations of agents; *PPE:* protective lab clothing; gloves; respiratory protection as needed | BSL-2 plus:1. Physical separation from access corridors
2. Self-closing, double door access
3. Exhausted air not recirculated
4. Negative airflow into laboratory
 |
| 4 | Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agent with unknown risk of transmission | BSL-3 practices plus:1. Clothing change before entering
2. Shower on exit
3. All material decontaminated on exit from facility
 | *Primary barriers:* All procedures conducted in Class III BSCs or Class I or Class II BSCs in combination with full-body, air-supplied, positive pressure personnel suit | BSL-3 plus:1. Separate building or isolated zone
2. Dedicated supply/exhaust, vacuum, and decon systems
3. Other requirements outlined in BMBL
 |

Consult the BMBL for a more complete description of the four biosafety levels, as well as recommended biosafety levels for specific organisms.

In addition to the four biosafety levels described above, there are also four biosafety levels for work with infectious agents in vertebrate animals. For a complete description of the animal biosafety levels, consult the BMBL.

***Routes of Transmission of Biohazards into the Human Body***

Skin and Mucous Membrane Contact

Together, with direct contact to biohazards, 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 that may land on the skin. Splashes or aerosols in contact with the eyes is also a recognized route of exposure.

Ingestion

Mouth pipetting presents the highest risk for ingestion of infectious material. Other modes of accidental ingestion of infectious lab materials are: splashing of material directly into the mouth or nose, indirect exposure through touching the mouth with contaminated hands, as well as eating or drinking in the laboratory. Application of cosmetics (i.e. lipstick or chapstick), changing contact lenses, applying eye drops can also present a risk of ingestion and are prohibited in the lab.

Percutaneous Inoculation

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 sharps (broken glassware, surgical tools, scratches from animal cages, etc.), and animal bites. Spills or splashes onto broken skin can also result in percutaneous inoculation.

Inhalation

Many procedures have the potential for generation of respirable aerosols, including: sonication, centrifugation, “blowing out” of pipettes, heating inoculating loops, and changing litter in animal cages.

**Chapter 5**

**Laboratory Biosafety Practices**

Basic Laboratory Practices

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

|  |  |  |
| --- | --- | --- |
| Biosafety Practice1. Do not mouth pipette
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols
3. 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 for removal as biowaste
4. Use lab coats, gloves, safety eye wear, and other personal protective equipment
5. Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents
6. Decontaminate work surfaces before and after use, and immediately after spills
7. Do not eat, drink, store foods, or smoke in the laboratory
 |  | Routes of Exposure BlockedInhalation, ingestion, skin and mucous membrane contactInhalation, skin and mucous membrane contactPercutaneous, inhalationSkin and mucous membrane contactSkin and mucous membrane contactSkin and mucous membrane contactIngestion, skin and mucous membrane contact |

**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 sources of hazard information for biological agents.

Microbial Agents

1. The CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) has descriptions of biosafety levels and recommended biosafety practices for specific biological agents.

1. The Canadian Laboratory Centre for Disease Control (LCDC) maintains Material Safety Data Sheets for microbial agents [www.phac-aspc.gc.ca/msds-ftss/index.html](http://www.phac-aspc.gc.ca/msds-ftss/index.html)

Toxins

Isolated biological toxins are chemical hazards, although many such toxins produce adverse effects at doses significantly below that of “traditional” laboratory chemicals. Laboratory use of isolated toxins falls under the site Chemical Hygiene Plan, and Material Safety Data Sheets (MSDSs) must be maintained and available. Biological toxins that are on the Select Agent list require specific security, control and oversight. Contact the IBC chair for more information and prior approval. See information in Chapter 12

1. MSDSs for the specific toxin should be received from the vendor upon receipt of the toxin.

2. Toxicology textbooks such as Casarett’s and Doull’s Toxicology are also good sources of hazard information for toxins.

This manual, in combination with the referenced CDC/NIH publications *Biosafety in Microbiological and Biomedical Laboratories* and *Guidelines for Research Involving Recombinant DNA Molecules*, provides general standard operating procedures (SOPs) for working with biological agents.

However, since these SOPs cover relatively general topics, individual laboratories are required to develop laboratory specific SOPs that cover the biosafety concerns and laboratory procedures for that particular laboratory. For example, safe manipulation of specific organisms with exact exposure control methods, and specific decontamination and waste handling requirements should be included in the SOP for that particular experiment. There is no standard format required for SOPs and laboratories are encouraged to use any format that effectively conveys the biosafety information (including use of pictures and illustrations). The laboratory specific SOPs do not need to duplicate the more general SOPs contained in this manual or the CDC/NIH documents, but should supplement these other documents.

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 Chapter 12) and other higher risk microorganisms and toxins must be stored in a locked container, and the PI must maintain an inventory with sufficient detail to enable identification of missing materials.

Prevention of Aerosols and Droplets

Handling of liquids or dry powders is likely to generate aerosols or droplets. Procedures such as centrifuging, mixing, and pipetting that involve high energy tend to produce respirable aerosols that stay airborne for extended periods and are small enough to be inhaled. Low energy procedures including opening containers and streaking plates produce droplets that settle quickly on surfaces, skin, and mucous membranes.

Biological Safety Cabinets

Procedures involving infectious material should be performed inside a biological safety cabinet (BSC) whenever possible. A properly operating and properly used BSC (see Chapter 8) will contain any aerosols and droplets generated during handling of infectious agents.

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 tight fitting lid with a sealable gasket.

Centrifugation

The potential for contamination and infection is high if liquid and aerosol is released during centrifugation. Sealed centrifuge rotors, 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 microincinerator will effectively control spatter resulting from sterilization of inoculating loops. Single-use, presterilized disposable inoculating loops (and needles) are an alternative to flaming or incineration.

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 soon as a spill is known to occur, or at the end of the laboratory procedure as part of the final cleanup, or at least daily during use. Used sheets should be placed in biowaste collection boxes.

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 the least desirable exposure control method since its failure results in direct exposure to the agent. PPE is most effective when used to supplement primary 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 and is generally recommended over polyester-cotton blends. Wearing lab coats is considered to be standard microbiological practice for BSL1 and BSL2 laboratories. For BSL 3 laboratories, CDC/NIH guidelines recommend solid-front or wrap-around gowns or suits, rather than front-buttoning lab coats. 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 (or even all chemicals!).

Thin nitrile gloves are an alternative to latex examination gloves that provide similar dexterity and increased chemical resistance. Nitrile gloves provide protection against microbiological hazards, but without the latex allergy hazard. These should be used instead of latex gloves. Standard latex examination type gloves provide protection against microbiological hazards, including human blood and body fluids but do not generally provide adequate protection against liquid chemicals; additionally, many people develop latex allergies as a result of wearing latex gloves.

Contamination control requires that gloves be removed immediately after a known spill or splash, at the end of a procedure, before moving onto another experiment or area of the lab, prior to exiting a BSC and certainly before touching noncontaminated 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. They also prevent infection that can result from the splashing of infectious or pathogenic organisms in the eye. Normal prescription eyeglasses are *not* safety glasses and *do not* provide adequate eye protection in the lab. Further guidance on the use of protective eye and face wear for chemical hazards can be found in the Chemical Hygiene Plan. 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 EH&S if respiratory protective equipment is required or if there are questions about the respiratory protection program.

**Storage and Labeling of Biological Agents**

Biological agents 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 (see below) as physical space on the container permits. At a minimum, secondary (or outside) containers must include the universal biohazard symbol (identity of contents is also desirable). Freezers, refrigerators, incubators, centrifuges and other storage areas must also be labeled with the biohazard symbol; exceptions to this policy will be considered on an individual basis by the IBC. Waste, and contaminated equipment or other objects to be decontaminated must also be labeled with the Biohazard Symbol.

Universal Biohazard Symbol

The OSHA Bloodborne Pathogen Standard specifically requires that containers of human blood, or other potentially infectious material (OPIM), contaminated waste, refrigerators, freezers, incubators and containers used to store or transport blood or OPIM, be labeled with the universal biohazard symbol (fluorescent orange or orange-red). See the site Bloodborne Pathogens Plan for additional information on handling and labeling of blood and OPIM.

Biohazard Labels and Signs

Signs must be posted at or on the access doors indicating that biological agents are used within the room. 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). The following areas require posting:

1. Entrances to laboratories and animal rooms that use agents classified as BSL2 or BSL3.

2. Cages or animal rooms used for housing animals infected with ABSL2 or ABSL3 agents.

**Chapter 6**

**Laboratory Training**

Laboratory Training

Training is required for all laboratory workers (faculty, staff, students, and visiting scientists). The exact training required for a particular person will depend on the hazards to which he or she is exposed.

Chemical Hygiene training covering the following topics: chemical hygiene, hazardous waste, chemical spill response, laboratory ventilation.

Biosafety training is also required for laboratory workers who work with biological agents. It is the responsibility of the Principal Investigator (PI) or Laboratory Supervisor to ensure that all personnel receive training that is appropriate for their job duties and exposure potential.

General Biosafety Training

Biosafety training that covers regulatory requirements, WPI’s Biosafety Program, general biosafety work procedures, and biohazardous waste (aka “biowaste”) disposal is required. Laboratory workers and project students potentially exposed to biological agents are required to receive this training. However, individual departments can conduct equivalent training on their own if they desire (all training must be documented by submitting a copy of the training materials and participant rosters to the EOS office). Workers and project students must receive training prior to beginning laboratory work with biological agents and then annually afterwards.

Bloodborne Pathogens Safety Training

Laboratory workers and project students who are exposed to human blood and body fluids, unfixed human tissue, or human cells/human cell products are within the scope of the OSHA Bloodborne Pathogens Standard. The Centers for Disease Control and Prevention (CDC) and OSHA specifically identify all cell lines of human origin as potentially infected with bloodborne pathogens, and that these materials be handled using a minimum of BSL2 containment and procedures. Consequently, all personnel and project students who work with human cell lines are required to be in the Bloodborne Pathogens Program and receive annual training.

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 safety 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. Additionally, workers must have prior experience in handling human pathogens before working with HIV or HBV. Personnel lacking experience with human pathogens must be trained in a laboratory before working with HIV or HBV. Initial training must not include the use of infectious agents, rather training and work activities should be progressive as proper techniques are demonstrated. Workers are permitted to handle infectious agents only after demonstrating proficiency to the satisfaction of the Laboratory Supervisor.

Packaging and Shipping of Infectious Agents Training

Personnel who package and ship infectious agents such as microorganisms, blood samples, rDNA and clinical samples for pathological testing, are required by federal and international regulations to receive training every two years.

Laboratory Specific Training

Individual 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 present 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, and exposure response/evaluation procedures. This laboratory specific training should supplement general biosafety training, the use of this manual and any other guidelines. Training records must be maintained by each laboratory.

The names and signatures of the instructor(s) and laboratory personnel, signature of the PI (if not the instructor), topic of training, and date that training was conducted, shall be recorded on a documentation form and maintained by the laboratory. Ongoing training is required as new hazards and procedures are introduced into the laboratory.

**Chapter 7**

**Decontamination**

Decontamination

Decontamination of cultures and objects contaminated by biological 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, as well as the release of infectious organisms to the outside environment (primarily through person to person transmission). Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of cultured organisms. The PI is responsible for maintaining decontamination and disinfection supplies.

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. Laboratory workers should remember that there are hazards associated with all of these chemical disinfectants. Inhalation and skin contact should be minimized, and eye contact avoided. Appropriate gloves and safety eyewear should always be worn when handling these chemicals. Pertinent information for some of the common chemical disinfectants is summarized in table format at the end of this chapter, but PIs must determine the appropriate disinfectant to use on the particular biohazard present

Autoclaving

Autoclaving uses saturated steam under pressure (approximately 15 psi) to achieve a temperature in the autoclave of at least 121o C (250o F). Autoclaving can be used to destroy vegetative bacteria, bacterial spores, and viruses. When decontaminating biohazardous waste, it is recommended that the temperature in ALL portions of the waste reach a minimum of 121o 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 of autoclaving:

1. Temperature - autoclave uses steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121o C. Although the autoclave chamber may reach 121o C, this does not necessarily mean that the interior of the load will reach this temperature.
2. Time - a minimum autoclave cycle time of twenty minutes at a chamber temperature of 121o C (time does not begin as soon as the autoclave cycle is initiated) is commonly recommended for sterilization of clean items. 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.
3. 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.

See Chapter 11 regarding WPI’s specifically approved biological waste handling practices.

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 nor closed containers. The highest dry heat equivalent temperature that these materials will reach in an autoclave is approximately121o C. The highest temperature that material will reach in a dry-heat oven will be the actual temperature inside the oven. A temperature of 160-180o C for 3-4 hours is recommended for decontamination of dry waste or empty containers using a dry heat oven.

| **Disinfectant** | **Summary of Chemical Disinfectants****Use Parameters** | **Effective Againsta** | **Important Characteristics** | **Potential Application** |
| --- | --- | --- | --- | --- |
| Vege-tative cells | Lipo-philic viruses | Tubercle bacilli | Hydro-phillic 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; make up at least weekly | 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** | *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 |
| **Formaldehydeb (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 |

From: Laboratory Safety: Principles and Practices, second edition, Diane O. Fleming, John H. Richardson, Jerry J. Tulis, and Donald Vesley, eds., American Society for Microbiology, Washington, D. C.

a: + = very positive response, ± = less positive response. A blank denotes a negative response or not applicable.

b: due to its irritating characteristics and status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation.

\* Presence of other solutions & chemicals, detritus, dirt, oils, etc. all reduce the effectiveness of chlorine bleach as a disinfectant, therefore, at the laboratory bench a fresh 10% dilution (or higher concentration) of commercial grade chlorine bleach (such as UltraClorox) is an appropriate disinfecting solution. This higher concentration will allow enough free chlorine to meet this minimal standard for effective decontamination.

**Chapter 8**

**Laboratory Ventilation for Biosafety**

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 inward 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 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.

Horizontal Laminar Flow Clean Bench

With horizontal laminar flow clean benches, High Efficiency Particulate Air (HEPA) filtered air (which is virtually sterile) flows horizontally across the workspace directly toward the user. These clean benches provide product protection and originally designed to provide particulate-free environments for manufacturing semiconductor components. 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 provide product protection against microbial contamination, but they do not provide personal 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 only acceptable to make sterile transfers of materials free of organisms (solution or media prep), or with cells known to be of low risk to laboratory workers, visitors and immunocompromised individuals.

Biological Safety Cabinets (BSCs)

There are three classes of biological safety cabinets (BSCs), class I, II, and III (see schematic below). 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.

Classes and Types of Biosafety Cabinets

Class I Class II Class III

Type A Type B

Type B1 Type B2 Type B3

Class I BSCs

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 BSL 1, 2, or 3 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. 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 BSL 1, 2, or 3 agents. Recirculated 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. Some Type A cabinets contain potentially contaminated air plenums that are under positive pressure behind the walls of the cabinet. Any breach of the positively pressured plenum or ducting would result in loss of containment and possible release of material.

Class II Type B BSCs

Type B biological safety 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 B3 BSCs***

Type B3 cabinets are a modified Type A BSC that has no plenums under positive pressure, and

which is exhausted directly to the outside. Type B3 cabinets are similar to Type A cabinets in that approximately 70% of air is recirculated as HEPA filtered downflow air. Type B3 cabinets are suitable for BSL1, 2, or 3 agends and minute quantities of volatile toxic chemicals or tracer amounts of volatile radionuclides.

***Type B1 BSCs***

Type B1 cabinets are designed for safe handling of small quantities of carcinogens and volatile radionuclides required for microbiological work. 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 BSL 1, 2, or 3 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 BSL 1, 2, or 3 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies.

Class III BSCs

Class III BSCs are of a glove-box design (gas-tight absolute containment) providing 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***. 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 BSL 4 agents.

**Certification of BSCs**

Generally, 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. The PI is responsible for assuring that the annual certification is completed.

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. 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

**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 supplement a safe worker. The following are general recommendations for BSC use.

1. Ready Work Area

a. Turn off UV lamp (if equipped); turn on fluorescent light.

b. Check air grilles for obstructions and remove them; turn on fan (blower).

c. Allow air to purge workspace for 3 minutes.

2. Pre-disinfect

a. Spray or swab all interior surfaces with an appropriate disinfectant (Chapter 7).

b. Allow the surfaces to air dry.

3. Assemble Materials

a. Only introduce materials that are required to perform the procedure.

b. Position materials so that clean and contaminated items do not touch, with contaminated items downstream (ventilation-wise) of clean items.

c. Ensure the view screen is properly located and secured.

4. Pre-Purge Cabinet

a. Allow the BSC fan to run for at least three minutes with no activity inside (leave fan on!).

5. Personal Protective Equipment

a. Don protective clothing, gloves, mask, etc., as appropriate

6. Perform Procedures

a. Minimize movement of arms during procedure; move arms straight in or out of the BSC

 when entering or exiting the hood face.

b. Work from a clean area to more contaminated work areas (see figure below).

c. Remove gloves into waste container.

d. Avoid blocking air flow to front and back grills with excessive supplies/materials.

7. Cleanup and disinfection

a. Place potentially contaminated materials in a biohazard bag or other appropriate container.

b. Wipe surfaces of all items in the BSC with an appropriate disinfectant (Chapter 7).

c. Remove all items from the BSC and decontaminate (or otherwise collect or disinfect) waste and other contaminated materials as appropriate.

d. Disinfect all interior surfaces of the BSC.

8. Personal Hygiene

a. Remove protective clothing, mask, etc., and dispose of as appropriate.

b. Wash hands.

9. Post-Purge Cabinet

a. Allow air purge period (minimum of three minutes) with no activity inside (leave fan on!).

10. Shutdown cabinet

a. Turn off blower and fluorescent lamp.

b. Turn on UV lamp (if equipped).

c. Lower sash

**A typical layout for working “clean to dirty” (left to right)**

 **Horizontal Flow “Clean Bench”**

A. front opening

B. supply grille

C. supply HEPA filter

D. supply plenum

E. blower

F. grille

**Class I BSC**

A. front opening

B. sash

C. exhaust HEPA

D. exhaust plenum

**Class II Type A BSC**

A. front opening

B. sash

C. exhaust HEPA filter

D. rear plenum

E. supply HEPA filter

F. blower

**Class II Type B3 BSC**

(Tabletop model)

A. front opening

B. sash

C. exhaust HEPA filter

D. supply HEPA filter

E. positive pressure

 plenum

F. negative pressure plenum

 **Class II Type B1 BSC**

A. front opening

B. sash

C. exhaust HEPA filter

D. supply HEPA filter

E. negative pressure exhaust plenum

F. blower

G. additional HEPA filter for air supply

 **Class II Type B2 BSC**

 A. front opening

 B. sash

 C. exhaust HEPA filter

 D. supply HEPA filter

 E. negative pressure exhaust plenum

 F. supply blower

 G. filter scree

**Chapter 9**

**Human Tissue and Cell Culture**

**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 (BSL2) practices and procedures or higher. Persons who are exposed to these materials in the laboratory are considered to have potential exposure to bloodborne pathogens such as human immunodeficiency virus (HIV) , hepatitis B virus (HBV), and Hepatitis C virus (HCV) and must be included in WPI’s Bloodborne Pathogens Safety Program. These persons must be offered the hepatitis B vaccination (they do not have to accept) and receive annual Bloodborne Pathogens Safety Training. The Institutional Biosafety Committee will make the final determination on exemption of cell lines based on evidence provided to the IBC on a case by case basis.

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 and disinfection procedures including chemicals (formalin, alcohol), boiling, dry heat, and irradiation, and these agents can be present in fixed tissue from infected persons. Consult with the BSO to develop specific procedures to decontaminate prion-containing waste. 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. It is prudent to consider nerve tissue (even fixed tissue) potentially infectious. BSL2 containment and practices or higher are recommended for all activities utilizing known or potentially infectious tissues and fluids from naturally infected humans and from experimentally infected animals.

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 BSL2 or higher containment and procedures:

1. All cultured cells derived from human sources, including immortalized and “well established” cell lines.

2. All cultured cells derived from primate lymphoid or tumor tissue.

3. All cultured cells exposed to or transformed by a primate oncogenic virus.

4. All clinical materials, such as samples of human tissue obtained from surgery, biopsy, or autopsy.

5. All human and non-human primate tissue.

6. All uncharacterized cultured cells new to the laboratory until proven to be free of infectious agents.

7. All virus-containing primate cultured cells.

8. All mycoplasma containing cultured cells.

**Chapter 10**

**Biohazardous Spill Response**

Preplanning for Biohazardous Spill Cleanup

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 biohazard exposure when cleaning up a spill and will increase the likelihood that the spill is handled appropriately. Laboratory supervisors and PI’s should prepare their laboratory for typical spill scenarios expected in the laboratory, including appropriate disinfection and decontamination materials (see Chapter 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 (Chapter 7)
* Forceps or other devices to pick up contaminated material (especially sharps)
* Sharps disposal container
* Autoclavable biohazard bags

The chemical spill kits in the laboratories 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.

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, chemical, or radiological) the **safety of personnel is the most important consideration**. Cleanup is to begin only after it is determined that the personnel who will clean up 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. If in doubt, initiate the site emergency response procedures.

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

 disinfectant-soaked paper-towels, changing them frequently

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.

Biohazardous Spills In the Laboratory, Outside the Biological Safety Cabinet

1. If a BSL1 agent (or less than 100 ml of a BSL2 agent) is spilled, proceed to step 4.

2. If the spill is greater than 100 ml of a BSL2 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. Initiate site emergency response procedures.

4. Remove 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. Put on 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.

Biohazardous Spills Inside a Centrifuge

1. Clear the area of all personnel and allow aerosol to settle 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-soaked paper towels, 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.

Biohazardous Spills Outside the Laboratory During Transport

1. At Gateway, do not remove biological from the laboratory section of the building. Immediately clear the area of all personnel and secure the area.

2. Cleanup should be initiated as soon as possible to prevent spread of aerosols. Attempt cleanup only if appropriate cleanup materials and protective clothing are available.

3. Initiate emergency response procedures.

*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.

For example, biowaste removal from a class lab should be in a sealed secondary container with proper warning labels, or samples carried from one lab to the next should be done in a leak-tight bin, with absorbant material (paper towels) lining the floor of the container. Always consider the best way to move samples in advance.

**Chapter 11**

**Biohazardous Waste**

The following state and local agencies regulate or provide guidelines covering the management of biological waste streams. A summary of these regulations and guidelines is provided below.

105 CMR 480.000: MINIMUM REQUIREMENTS FOR THE MANAGEMENT OF MEDICAL OR BIOLOGICAL WASTE (STATE SANITARY CODE CHAPTER VIII). The purpose of 105 CMR 480.000 is to set forth minimum requirements for the storage, treatment, disposal and transportation of medical or biological waste.

City Of Worcester, Department of Health and Human Services, Division of Inspectional Services. This agency is tasked with the inspection of laboratory facilities engaged in rDNA research. Upon satisfactory inspection, submission of IBC minutes and a fee, an annual rDNA license will be approved.

**Biohazardous Waste**

Biohazardous waste includes waste materials derived from cultures and stocks of infectious agents, human pathological wastes, contaminated animal carcasses and body parts, all sharps, and human blood and blood products. 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, EPA and State regulations require that biohazardous waste be properly labeled, stored, and disposed.

Waste Minimization

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

* Small sample size
* Avoiding generating mixed biohazardous waste that includes radioactive and/or hazardous waste
* Minimize the amount of biohazardous waste that requires commercial disposal

**Labeling and Storage of Biohazardous Waste**

Labeling

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.

**Handling and Disposal of Biohazardous Waste**

Sharps

Sharps include all syringes, lancets, scalpels and other similar medical instruments (whether contaminated or not), as well as contaminated Pasteur and serological 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, seal the container and place in the designated area for a waste pickup.

In order to dispose of autoclaved infectious waste in the normal trash, solid waste regulations require that all infectious waste must be treated (i.e. disinfected or autoclaved) to make it non-infectious in order to be labeled as non-infectious.

* Sharps must be ground up or otherwise have their physical hazard eliminated
* State requirements for treatment, labeling and disposal must be followed.

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).

**It is the policy of WPI to collect all sharps and dispose of them via an offsite biowaste vendor contracted by the EOS . No onsite treatment of sharps waste is approved by the IBC. See Chapter 14 for information on handling sharps.**

Uncontaminated Laboratory Glassware and Broken Glass

Collect 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” and can be disposed of as ordinary trash in those boxes.

Solid Biohazardous Waste aka “Solid Biowaste”

* Solid biowaste includes microbial agents, tissue culture, and contaminated material (such as petri dishes, pipettes, gloves, towels, and liquids that have been solidified to a gel inside of the original container, etc.). On site decontamination of large quantities by autoclaving of solid biohazardous waste is not an IBC approved practice at WPI. All solid biowaste is to be collected in red, biohazard bag lined cardboard boxes that are also labeled with the universal biohazard markings and DOT shipping information. When the box is full, tie off the red bag, seal and tape the box, and place it in the designated area of the lab for waste pick up. (See Chapter 14)

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, supernatents, spent media, etc.). Collect liquid waste in closeable, rigid plastic, leak proof containers labeled with the universal biohazard symbol.

All other liquid biowaste must be either treated with a disinfectant or solidified prior to collection as solid biowaste. Small quantities of liquid biowaste can be disinfected on site, and the decontaminated waste poured down the laboratory drains. Do this by adding enough Clorox Ultra Bleach (hospital grade, provided by EOS in GP2224 & SL322) to create a 10% bleach solution with the liquid waste, let it sit for a recommended 24 hours, or overnight at a minimum, and dispose of it via the lab sink while flushing with plenty of water. Always wear a lab coat, gloves and safety glasses to avoid a potential splash. Be sure that the disinfectants used are compatible with chemicals present in liquid biowaste before decontamination.

Alternately, a small container (i.e. 6-well plate or small t-flask) of liquid waste can be solidified into a gel using a small quantity of solidifier powder sprinkled into the liquid. When the liquid turns into a gel, dispose of the entire container in the biohazard solid waste box. Do not pour liquids directly into the biohazard box. For incidental amounts of liquid such as that associated with 96 well plates, sprinkle a few tablespoons of the solidifier powder into the red bag prior to adding any waste. This will serve to absorb small quantities of liquids that my drip from wastes added to the box. (See Chapter 14)

Animal Carcasses, Body Parts, and Tissue

Non-infectious carcasses are to be placed in an opaque plastic bag and the bag taped shut with 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 or BSO**. 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. Carcasses will be stored in a labeled freezer prior to packaging in red bag lined biohazard boxes and shipped offsite via the Biowaste vendor. Decontamination of infectious carcasses onsite by steam autoclaving is not an approved practice per the WPI IBC

Mixed Waste

All mixed waste (biohazardous waste in a hazardous chemical or radioactive matrix) must be managed in a method approved by EOS.

**Autoclave Quality Assurance Program**

Autoclaving is not currently an alternative approved by the WPI IBC , it is an industry-accepted procedure for decontamination of certain biohazardous waste. MDPH regulations impose strict conditions under which on-site autoclaving of waste may be performed. Solid biohazardous wastes at WPI are currently shipped offsite via a biowaste vendor, and an MDPH compliant on-site autoclave program is not required. Any change in this policy requires approval of the Biosafety Officer and IBC, as well as compliance with MDPH criteria

The following information is included for reference only. Biological cultures and stocks, contaminated solid waste, liquid waste, and small animal carcass waste can be sterilized through autoclaving under MDPH laws if certain procedures are followed, specific records kept (load logs of quantity/nature of material/real-time printouts of temperature and pressure, periodic spore challenge testing, yearly autoclave certification (third party testing), oversight by the IBC with yearly reviews of all biowaste decontamination records and equipment at the site.

After complete and verified 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.

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

1. Infectious waste must be treated in an autoclave for a minimum of **30 minutes at 121o C (250o 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 o 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 “breathe”) 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.

2. All autoclaved waste must include a steam sterilization indicator (the use of biohazard bags with a “built-in” indicator is recommended).

3. Steam autoclaves used to treat infectious waste must operate at a minimum temperature of 121o C. The operating temperature of the autoclave must be verified for each run by maintaining a ecord of the temperature either as a chart or paper tape recording or a manual recording in a logbook.

4. 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, measure and record the maximum temperature achieved during the autoclave cycle through the use of a maximum registering (or “holding”) thermometer. See the testing procedure below.

5. Maintain records of B. stearothermophilus testing and maximum autoclave temperature recordings for a minimum of three years (see MDPH Autoclave Log in Chapter 14).

Quarterly Spore Testing Procedure

1. Place ampoule of B. stearothermophilus spores and holding thermometer in the center of an autoclave load.

2. Process the load under normal operating procedures.

3. The highest temperature indicated on the holding thermometer is entered on the Autoclave QC Log. If this temperature is less than 121 C, 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 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 end of chapter) and retain for at least three years.

**Chapter 12**

**CDC/USDA Select Agents**

**Introduction**

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. This chapter will refer to all these agents simply as “select agents”. The current list of select agents is provided at the end of this chapter. 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. The information in this Chapter is a summary of the select agent regulations; it is not a complete description of the regulatory requirements associated with select agents. **Investigators must review and understand the select agent regulations and their responsibilities prior to acquiring or working with, any select agent (see above links to regulatory information).**

**Responsible Official**

Select agent regulations require that a Responsible Official (RO) be designated for each institution that possesses and uses select agents. Currently, Worcester Polytechnic Institute is **not** registered to possess select agents. **The RO has institutional responsibility for the biosafety, security, and regulatory compliance of select agents, and as such, must be contacted prior to obtaining any select agents.**

**Authorization to Possess and Use Select Agents**

Prior to obtaining any select agent, a Biological Project Registration Form (see Chapter 2) covering the proposed work must be submitted to, and approved by the Institutional Biosafety Committee. In addition, PIs who want to acquire, possess, or use any biological agent or toxin listed as a select agent **must be registered and approved with the appropriate agency (CDC or USDA) prior to obtaining the agent(s) or toxins(s).** Both the institution and the individual laboratory must be approved by the appropriate agency. Investigators who want to possess and use select agents must contact the RO or the EH&S Office for assistance with the registration application process. Approval by the CDC or USDA can take several weeks to a several months (plan a minimum of three to six months), and PIs should plan research projects accordingly.

The select agent regulations contain very strict requirements regarding biosafety, training, emergency response, security and accountability, as well as other requirements. Investigators wanting to acquire select agents should review the CDC or USDA select agent regulations (whichever is appropriate) thoroughly before initiating the registration application process.

**Exemptions and Exclusions**

Diagnostic labs that do not maintain select agents are largely excluded from the CDC and USDA select agent regulations; however, there are notification and possession time limits and other requirements that do apply. Additionally, the CDC and USDA can grant exclusions for temporary public health emergency situations, and other special circumstances. Consequently, any laboratory that conducts diagnostic or verification testing for any select agent must identify themselves as soon as possible. **Identification of any select agent in a specimen or isolate must be reported to the CDC as soon as possible.**

Several specific select agent microbial strains and toxin forms have been determined to not present a severe threat to public health and safety, and are therefore excluded from the CDC/USDA select agent regulations. The list of excluded biological agents and toxins is dynamic and the most current list is available on the CDC and USDA select agent web sites. Toxins are excluded based on threshold quantities. Laboratories maintaining exempt quantities of select agent toxins must keep an accurate inventory of toxin amounts to verify that total quantities are below the threshold.

**Safety Plan**

Each select agent laboratory must develop and implement a written safety plan that addresses the biological and chemical safety issues associated with the specific select agents maintained by the laboratory before the agents arrive. In particular, the plan must address the hazards associated with the select agents, methods to be used to prevent exposures, including use of laboratory ventilation (biological safety cabinets and lab hoods) and personal protective equipment, disinfection and decontamination methods, waste handling and disposal procedures, and the proper response to spills, personal contamination, and other incident response.

**Security Requirements**

**All persons who will have access to any select agent must be approved by the Department of Justice.** Approval requires that each individual successfully pass a background security check (conducted by the FBI in accordance with the USA PATRIOT Act) and submit fingerprints to the FBI. Anyone who has not been approved for access to select agents must be denied access unless they are escorted by an approved person. Everyone who enters a laboratory where select agents are accessible must have security approval or be accompanied by an approved person. This includes visiting scientists, maintenance workers, custodians, and vendors.

Each select agent laboratory must have a written security plan that addresses the following topics:

* Physical security
* Cyber security
* Inventory of select agents
* Select agent transfers
* Training
* Reporting of unauthorized persons and missing materials
* Provisions for cleaning, maintenance, and repairs

**Any theft or loss of select agents must be immediately reported** to the CDC or USDA as appropriate.

**Emergency Response**

**Each laboratory that possesses or uses select agents must develop a written emergency plan** before the agent arrives that is laboratory specific, but which is coordinated with the department, building, and location emergency plans. The plan must address the hazards of the select agents, planning and coordination with emergency responders, building evacuation, site security and control, decontamination and emergency medical treatment, and other emergency response issues.

**Training**

All persons approved for access to select agents must receive documented training covering biosafety of select agents and their safe handling, use, and disposal; security requirements and procedures; inventory and accounting procedures; and emergency response procedures. **Training is required before beginning work with select agents and annually thereafter.**

**Transfers**

Select agents can only be transferred between entities that are currently approved by the CDC or USDA to possess and use select agents. All transfers of select agents (including intrafacility transfers) require prior approval of the CDC or USDA. Both the sender and recipient must complete a common transfer form, and the recipient submits the completed form to the CDC or USDA. The form requires the signature of the RO from both the sender and recipient facilities. When the select agent is consumed or destroyed, the recipient must submit documentation to the CDC or USDA notifying them of this fact. The form is only available hard-copy through the RO.

**Inventory and Disposal of Select Agents**

**A accurate record of all select agents, from receipt to destruction or disposal, must be maintained by the RO.** The inventory must include specific information on individual containers and vials, as well as a record of each use, and ultimate disposal. The select agent inventory must be verified at least monthly to account for all quantities and containers of select agents. Any discrepancies between the inventory record and the actual inventory must be reported as soon as possible.

Records

The select agent regulations require that several records be maintained, including the following: detailed inventory of each select agent and associated containers; access to select agents; access to the area where select agents are used or stored; safety, security, and emergency response plans; training records; transfer documents and safety and security incident reports. Each individual laboratory is responsible for maintaining these records. Laboratories must maintain records of training conducted by laboratory personnel or any other applicable training. The recordkeeping requirements are complex, and therefore the CDC or USDA regulations should be reviewed for a complete description of the recordkeeping requirements.

**List of Select Agents**

**Check for recent updates through the Centers for Disease Control**

**CDC Non-Overlap Select Agents and Toxins USDA High Consequence Livestock Pathogens**

Crimean-Congo haemorrhagic fever virus **and Toxins (Non-Overlap Agents and Toxins)**

*Coccidioides posadasii* Akabane virus

Ebola viruses African swine fever virus

Cercopithecine herpes virus 1 (Herpes B virus) African horse sickness virus

Lassa fever virus Avian influenza virus (highly pathogenic)

Marburg virus Blue tongue virus (Exotic)

Monkeypox virus Bovine spongiform encephalopathy agent

*Rickettsia prowazekii* Camel pox virus

*Rickettsia rickettsii* Classical swine fever virus

South American haemorrhagic fever viruses: *Cowdria ruminantium* (Heartwater)

 Junin Foot and mouth disease virus

Machupo Goat pox virus

Sabia Lumpy skin disease virus

Flexal Japanese encephalitis virus

Guanarito Malignant catarrhal fever virus (Exotic)

Tick-borne encephalitis complex (flavi) viruses: Menangle virus

 Central European tick-borne encephalitis *Mycoplasma capricolum*/M.F38/*M. mycoides capri*

 Far Eastern tick-borne encephalitis *Mycoplasma mycoides mycoides*

 Russian spring and summer encephalitis Newcastle disease virus (VVND)

 Kyasanur forest disease Peste Des Petits Ruminants virus

 Omsk hemorrhagic fever Rinderpest virus

Variola major virus (Smallpox virus) Sheep pox virus

Variola minor virus (Alastrim) Swine vesicular disease virus

*Yersinia pestis* Vesicular stomatitis virus (Exotic)

Abrin

Conotoxins **Listed Plant Pathogens**

Diacetoxyscirpenol *Liberobacter africanus*

Ricin *Liberobacter asiaticus*

Saxitoxin *Peronosclerospora phillippinensis*

Shiga-like ribosome inactivating proteins *Phakopsora pachyrhizi*

Tetrodotoxin Plum Pox Potyvirus

 *Ralstonia solanacearum* race 3, biovar 2

**High Consequence Livestock Pathogens** *Schlerophthora rayssiae* var *zeae*

**and Toxins/Select Agents (Overlap Agents)** *Synchytrium endobioticum*

*Bacillus anthracis Xanthomonas oryzae*

*Brucella abortus Xylella fastidiosa* (citrus variegated chlorosis strain)

*Brucella melitensis*

*Brucella suis*

*Burkholderia mallei* (formerly *Pseudomonas mallei*)

*Brukholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)

Botulinum neurotoxin producing species of *Clostridium*

*Coccidioides immitis*

*Coxiella burnetii*

Eastern equine encephalitis virus

**Chapter 13**

**Packaging and Shipping Infectious Agents & Biological Waste**

**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. Only trained persons can ship dry ice and/or biological material. Contact the Biosafety Officer or EOS for information on who is authorized.

**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.

Medical Waste Tracking form - A paper or electronic form approved by the Department that provides confirmation to a generator of receipt of medical or biological waste by an off-site treatment facility.

Training Requirements

Those involved in the packaging and shipping of diagnostic specimens or infectious substances including biowaste must undergo training every two (2) years or when activities change. It is the responsibility of the department to assure training is completed.

**Chapter 14**

**Appendices**

Biological Waste - Sharps

ALL razor blades

ALL syringes

ALL needles

ALL sharp metals

Serological pipettes

Pasteur pipettes

1. No Liquids
2. Do not overfill
3. Capped when not adding sharps

For more information, contact the Environmental and Occupational Safety Office at x 5216

Biological Waste Box Contents

**Solids Only in the Box:**

**Pipette tips**

**Paper towels**

**Solidified liquids**

**Petri dishes**

**Plastic ware**

**Non-sharps**

**Gloves**

**Not Allowed in the Box:**

**Liquids, Chemicals,**

**Radioactive Material,**

**Trash**

**Sharps**

For more information, contact the Environmental and Occupational Safety Office at x 5216

**PROPER DISPOSAL OF PIPETTE TIPS**

* **Collect ALL pipette tips at the point of generation in 8 ½ x 11 Biohazard bags**
* **When ¾ full, place Biohazard bags in the Biological Waste boxes for offsite disposal**
* **Do not place any pipette tips in the trash**

**For more information, contact the Environmental and Occupational Safety Office at x 5216**

Disposal of Solidified Liquids

**Add Solidifier Powder to liquid in disposable container**

**Allow to completely gel**

**Dispose of entire container in Biohazard Box**

For more information,

contact the Environmental

and Occupational

Safety Office at x 5216

Disposal of Bleached Liquids

**Add Bleach to Liquid to create 10% Solution**

**Allow to set 24 hrs or Overnight**

**Dispose of contents via sink, flush with water**

 **WEAR PPE!**

For more information,

contact the Environmental

and Occupational

Safety Office at x 5216

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**BIOWASTE Standard Operating Procedure Form**

To be submitted annually by Principal Investigator to the IBC once per year, and whenever there are changes in volumes, locations, nature of experiment or organisms used or changes of personnel.

1. Date:
2. Principal Investigator Name:
3. Laboratory Location(s), include building name and specific room number(s):
4. Names & E-mail addresses of Faculty, Staff & Graduate Students working in the laboratory:

a.

b.

c.

d.

e.

f.

1. List of Biological Agents Present In the Laboratory (please fill in the table electronically, the spaces will expand automatically):

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Biological Agent Name | Acquisition Origin | Experimental use (brief description) | Storage Location of active biological  | Estimated Volume of Liquid biowaste per week (in gallons) | Location(s) of Liquid waste disposal | Estimated Volume of Solid biowaste per week (in pounds) | Location of Solid biowaste collection container(s) |
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