



# MARINE BIOLOGICAL LABORATORY

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## BIOSAFETY MANUAL

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PAUL SPEER      CHIEF OPERATING OFFICER

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DATE

4-6-2022

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ANNE SYLVESTER      DIRECTOR OF RESEARCH

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DATE

4-6-2022

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ERIC H. JENSEN      BIOSAFETY OFFICER

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DATE

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## 1 INTRODUCTION

The Marine Biological Laboratory (MBL) is committed to providing a healthy and safe learning, teaching, research, and work environment. The Biosafety Manual is part of the MBL's biological safety program designed to minimize exposure and mitigate the risk associated with handling potentially infectious biological materials.

This manual serves as a resource document for working safely in research and teaching laboratories at MBL in order to:

- (a) Inform and educate laboratory personnel and students on biosafety practices and procedures.
- (b) Protect the MBL community (staff, students, and visitors) and the general public from exposure to potentially infectious agents and recombinant DNA.
- (c) Prevent environmental contamination.
- (d) Provide an environment for high quality learning, teaching, and research while maintaining a safe workplace.
- (e) Comply with all pertinent federal, state, and local regulations and guidelines.

The Biosafety Manual provides institutional-wide safety guidelines for working with biological hazards. Biosafety practices and procedures in all research and teaching laboratories at the MBL should comply with those outlined in this manual.

The success of any biosafety program mainly depends on the collaborative efforts among the Responsible Researchers (Course Directors, Resident Faculty, Whitman Scientists, Visiting Scientists, and other Principal Investigators), Authorized Individuals (laboratory personnel, postdoctoral researchers, research scientists, and graduate students, Biosafety Officer (BSO) and the Institutional Biosafety Committee (IBC). The Responsible Researcher have the ultimate responsibility for ensuring that the policies and procedures outlined in this manual are implemented in their respective research or teaching laboratories.

### 1.1 Purpose

The purpose of the Biosafety Manual is to establish policies and procedures designed to protect laboratory personnel, the general public, and the environment from biohazardous materials. The policies and procedures are meant to ensure compliance with regulatory requirements without unduly impeding academic research, innovation, and scientific discovery at the MBL.

### 1.2 Scope

The Biosafety Manual applies to all research and teaching activities involving biological agents. All faculty, staff, students, and visiting scientists working on research projects or with education courses are included in the scope of this manual.

Individual laboratories are strongly encouraged to use the information provided in this manual to develop their own laboratory-specific standard operating procedures (SOPs) that should meet or exceed the requirements outlined in this manual. A current copy of the Biosafety Manual and laboratory-specific SOPs should be made readily available to all laboratory personnel at all times.

### 1.3 Biosafety Manual Review

The Biosafety Manual shall be reviewed and updated, as necessary, to reflect new or revised regulations, or modified tasks and procedures. Submit any suggestions and comments regarding this manual to the Biosafety Officer at 508-289-7424 or [safety@mbi.edu](mailto:safety@mbi.edu).

## 2 ROLES AND RESPONSIBILITIES

The specific responsibilities for implementation of the biosafety program at MBL are outlined below:

### 2.1 Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) is an MBL Standing Committee responsible for reviewing research activities using recombinant DNA (rDNA), infectious agents, Select Agents, non-native species, and projects with blood products. In addition the IBC is involved with overseeing the safe conduct of research, and assessing biocontainment levels. The Committee provides oversight of the biosafety program to ensure research is conducted in compliance with federal, state and local regulations and MBL policies. The "**NIH Guidelines for Use of Recombinant or Synthetic Nucleic Acid Molecules**" require that all procedures involving the use of rDNA undergo review by the IBC.

<https://osp.od.nih.gov/biotechnology/nih-guidelines/>

For more information related to MBL's Institutional Biosafety Committee (IBC), please refer to the IBC Policy & Procedures Manual. The manual can be found at:

<https://www.mbl.edu/research/health-safety/biological-safety/biosafety-committee>

### 2.2 Department Chairs & Center/Division Directors

Each Center Director, Division Director or other unit leader is responsible for providing adequate support to researchers in their administrative unit. They should ensure that adequate facilities are available to control biohazards, and to enable laboratory personnel to comply with applicable regulations and MBL policies.

### 2.3 Biological Safety Officer

The Environmental Health & Safety Manager serves as the designated Biosafety Officer (BSO) in accordance with NIH Guidelines. The BSO is responsible for providing guidance on the safe handling of biological agents and overall management of the biosafety program. The BSO is a member of the IBC.

The BSO's responsibilities include:

- Managing the biosafety program and implementation of IBC policies and procedures at the MBL.
- Ensuring records of infectious agents, recombinant DNA activities and Select Agents are kept current.

- Assisting research and teaching laboratories in conforming to applicable regulatory guidelines and IBC policies by providing training, facility inspection, and communication of program requirements.
- Collaborating with Responsible Researchers, faculty, and staff to determine biosafety training needs.
- Developing and conducting laboratory biosafety training to meet identified needs.
- Conducting periodic inspection of research and teaching laboratories using biological materials and Select Agents Toxins.
- Providing technical advice to Responsible Researchers and IBC on research safety procedures.
- Developing emergency response plans for handling biological spills and personnel contamination and for investigating accidents involving rDNA research.
- Reporting to the IBC and the MBL senior management any significant challenges, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the BSO becomes aware.

## **2.4 Responsible Researchers**

The Responsible Researchers are responsible for the health and safety of all personnel and students in their laboratories, and for full compliance with all applicable federal, state, and local regulations. The specific responsibilities of the Responsible Researchers include:

- Ensuring that specific laboratory hazards are effectively communicated to laboratory personnel.
- Ensuring that laboratory personnel or students have received appropriate training and are competent to perform procedures used in the laboratory.
- Developing laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory.
- Ensuring that engineering controls are available, operational, and are used properly to minimize exposure to biohazardous agents.
- Ensuring that required personal protective equipment (PPE) is available and used by laboratory personnel.
- Ensuring that laboratory personnel are provided immunizations and medical surveillance prior to, and in the event of, exposure to biohazardous agents as appropriate (based on current CDC or IBC recommendations).
- Notifying the BSO of any spills and incidents involving biological agents that may result in exposure to laboratory personnel, the general public, or release to the environment.
- Ensuring that biological agents are disposed of according to applicable federal, state and local regulations, and MBL policies as outlined in this manual.
- Ensuring that biohazardous materials for transportation and shipping are packaged and handled in compliance with applicable regulations.
- Conducting periodic laboratory safety inspections in collaboration with the BSO and/or Laboratory Safety Representative.

## 2.5 Laboratory Staff and Students

Laboratory personnel and students are responsible for their own health and safety and that of their colleagues. Specific responsibilities include:

- Following the biosafety practices and procedures outlined in the MBL Biosafety Manual and recommended by the Responsible Researcher and the BSO.
- Conducting all laboratory work in a safe manner as described in the Biosafety Manual and specific standard operating procedures (SOPs) established for their laboratory.
- Informing the Responsible Researchers, Laboratory Supervisor or the BSO of any potentially hazardous situations or conditions (e.g. biological spills).
- Reporting any exposures to infectious materials including animal bites and sharps injuries, to the Responsible Researchers or Laboratory Supervisor and the BSO.
- Attending biosafety training and other laboratory safety training, as appropriate.
- Report all injuries and illnesses sustained while conducting research to the Responsible Researchers or Laboratory Supervisor and the BSO and seek prompt medical treatment.
- Using engineering controls (e.g., biosafety cabinets) to prevent exposure to biological agents and contamination of personnel and facilities.
- Wearing appropriate personal protective equipment (PPE).

## 3 BIOSAFETY LEVELS AT MBL

Biological safety (biosafety) is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure or injuries that result from working with biological materials. Biosafety defines the containment requirements under which biohazardous materials can be safely handled. The objective of containment is to confine biohazards and to minimize the potential exposure of the laboratory personnel, individuals outside of the laboratory, and the environment to biohazardous agents.

### 3.1 Biosafety Levels for Infectious Agents

The CDC's *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) classifies agents into four distinct Biosafety Levels (BSL-1, BSL-2, BSL-3, and BSL-4). Biosafety levels consist of combinations of:

- Laboratory practices and techniques.
- Safety equipment.
- Laboratory facilities.

Each combination of the above factors is specifically appropriate for:

- The operations performed.
- The known or suspected routes of transmission of the infectious agents.
- The laboratory function or activity.

MBL only allows work with BSL-1 and BSL-2 biohazard levels. All BSL-2 work must be approved through the IBC application process.

### **Biosafety Level 1 (BSL-1)**

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

### **Biosafety Level 2 (BSL-2)**

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.
- Access to the laboratory is restricted when work is being conducted.
- All procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets (BSCs) or other physical containment equipment.

### **Summary of Biosafety Levels for Infectious Agents.**

<b>Biosafety Level</b>	<b>Agent Characteristics</b>
BSL-1	<ul style="list-style-type: none"> <li>• Not known to consistently cause diseases in healthy adults.</li> </ul>
BSL-2	<ul style="list-style-type: none"> <li>• Agents associated with human disease.</li> <li>• Routes of transmission include percutaneous injury, ingestion, and mucous membrane exposure.</li> </ul>
BSL-3	<ul style="list-style-type: none"> <li>• Indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route of exposure.</li> </ul>
BSL-4	<ul style="list-style-type: none"> <li>• Dangerous or exotic agents that pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or effective treatments.</li> <li>• Agents with a close or identical antigenic relationship to an agent requiring BSL-4 containment until data are available to re-designate the level.</li> <li>• Related agents with unknown risk of transmission.</li> </ul>

### 3.2 Laboratory Specific Operating Procedures (SOPs)

A BSL-2 laboratory must adopt a laboratory-specific biosafety operating procedure that is tailored to the specific hazards and procedures associated with the laboratory. The required BSL-2 procedures must be described in the laboratory SOP with any additional practices that are to be implemented. This SOP should be submitted together with a research protocol to the IBC for review. A copy of the laboratory-specific SOP should be readily available and readily accessible in the laboratory (hard copy in a binder or in electronic form).

## 4 BIOLOGICAL RISK ASSESSMENT

Biological risk assessment is the process of evaluating biological research projects for potential hazards and characterizing the hazards into classes with proper containment and work practices to ensure research can be conducted safely and effectively. Biological risk assessment is an ongoing process during the biological research. Research involving recombinant DNA and synthetic nucleic acids will be reviewed by the MBL's IBC. Research involving animals will be reviewed by the MBL's Animal Care and Use Committee (IACUC).

### 4.1 Risk Assessment

The BSO, MBL Veterinarian, faculty and staff will participate in risk assessments. Information obtained from the risk assessment will be used as a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility design to limit personal exposure to biohazards.

The primary factors to consider in risk assessment and selection of safety precautions fall into two broad categories: (a) agent hazards and (b) procedure hazards.

During a risk assessment, the following factors should be evaluated:

- (a) The biological and physical hazard characteristics of the agent.
- (b) The sources likely to harbor the agent.
- (c) Host susceptibility.
- (d) The procedures that may disseminate the agent.
- (e) The best method to effectively inactivate the agent.

The competence of the laboratory personnel to control hazards should be considered. This will depend on the training and technical proficiency, adhering to safe work practices by laboratory staff and the effectiveness of the containment equipment and facility safeguards.

The following five steps for conducting risk assessment are recommended:

- (a) Identify the hazards of the agents and perform an initial assessment of risk.
- (b) Identify the hazards of the experimental procedures.
- (c) Make a determination of the appropriate biosafety level and select additional safety precautions based on the risk assessment.
- (d) Evaluate the competences of the laboratory staff regarding biosafety practices and the effectiveness of the safety equipment.

- (e) Review the risk assessment with the PI/CDs, subject matter expert, BSO and the IBC, as appropriate.

#### 4.2 Classification of Infectious Agents Based on Risk Groups

Biohazardous materials are classified according to risk levels. Following a risk assessment, biohazardous agents are placed into appropriate containment levels. Some biological materials that may not be normally considered as biohazardous may be regulated. There are four risk groups (RG) which are defined in Appendix B of the NIH Guidelines:

<https://osp.od.nih.gov/biotechnology/nih-guidelines/>

Microorganisms that are in RG1 require basic laboratory facilities and standard microbiological practices. The biological agents that are likely to be encountered in MBL research and teaching laboratories are RG1 or RG2 pathogens, designated as low and moderate hazard, respectively.

The progression from invasion to infection following contact with an infectious agent depends upon the route of transmission, infectious dose, pathogenicity of the agent, and resistance of the person exposed (innate or acquired immunity). Not all contacts result in infection. It is prudent, to assume virulence and handle biological agents at the appropriate biosafety level.

##### Classification of Infectious Microorganisms by Risk Group.

Risk Group	CDC/NIH/WHO	Examples
<b>Risk Group 1</b>	Agents not associated with disease in healthy adult humans. No or minimal individual and community risk.	Adeno-associated virus (AAV) <i>Escherichia coli</i> K-12 host vector systems
<b>Risk Group 2</b>	Agents associated with human disease that is rarely serious and for which preventive/therapeutic interventions are often available. Moderate individual risk but low community risk.	<i>Salmonella enterica</i> <i>Escherichia coli</i> <i>Staphylococcus aureus</i> Toxoplasma Hepatitis B virus
<b>Risk Group 3</b>	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. High individual risk but low community risk.	<i>Mycobacterium tuberculosis</i> <i>Bacillus anthracis</i> West Nile virus Yellow fever HIV
<b>Risk Group 4</b>	Agents likely to cause serious or lethal disease in humans and animals for which preventive or therapeutic interventions are not usually available. High individual risk and high community risk.	Ebola virus Lassa virus Herpes viruses

RG3 and RG4 agents require more sophisticated engineering controls (e.g., facilities and equipment) than standard laboratories, and special handling and decontamination procedures. Laboratory research on RG3 and/or RG4 agents is not permissible at the MBL. Containment facilities and work practices for these agents have not been established.

### 4.3 Routes of Exposure

An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overwhelm the body's defense system. Below are the routes of possible infection:

#### Ingestion

- Eating or drinking in the laboratory.
- Mouth pipetting.
- Transfer of microorganisms to mouth by contaminated fingers or articles.

#### Injection

- Accidental inoculation with a hypodermic needle or other sharp instrument.
- Abraded skin through cuts, scratches, skin rashes, etc.
- Animal bites or scratches.

#### Absorption

- Splashes of infectious material into the eye.
- Transfer of microorganisms to eyes by contaminated fingers.
- Through contact with skin.

#### Inhalation

- Inhalation of airborne microorganisms.

### 4.4 Exposure Sources

Exposure sources in the research laboratory are hazards that could result in infection of laboratory personnel and students or members of the public through work involving biological agents.

Some of the most common hazards that should be considered include the following:

#### 4.4.1 Pathological Specimens

Any specimens from human and animal sources may contain infectious agents. Animals may harbor endogenous pathogens that are virulent for humans. When handling these animals or their tissues/body fluids, laboratory personnel should follow Universal Precautions.

The laboratory personnel are required to undergo Bloodborne Pathogen (BBP) training, in compliance with OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030). The training will be provided by the BSO.

#### 4.4.2 Laboratory Aerosols

Various laboratory procedures can generate biological aerosols that can be potentially hazardous to personnel and surrounding environment. Standard microbiological practices provide aerosol minimization techniques, but some special precautions can help enhance the level of prevention.

##### **Standard Precautions:**

- When working with infectious or potentially infectious materials, aerosol-generating procedures must be performed in a Biosafety Cabinet (BSC).
- Use respiratory protection whenever required (as approved by BSO).
- When a spill occurs, leave the area for 30 minutes to allow droplets to settle and aerosols to be removed by HVAC before cleaning up a spill.

##### **Specific Precautions:**

- **Pipetting:** Dispense liquids as close to the reservoir as possible. Rinse with the appropriate disinfectant before disposing pipettes or pipette tips. Take caution when removing pipette or pipette tips.
- **Proper Use of Syringes:** Avoid air bubbles. Do not recap needles of syringes unless using a safety device that permits singlehanded recapping.
- **Inoculation loops:** Use pre-sterilized, disposable loops when possible. When heat-sterilizing reusable loops, allow loop to cool before introducing it to any potentially infectious materials.
- **Decanting Liquids:** Pour carefully and as close to the vessel receiving as possible.
- **Animal inoculation:** Remove syringe needles from animals slowly, with a smooth and steady motion.
- **Vortexing:** Ensure all substances being vortexed are in a durable container with a tight-fitting lid.
- **Blending:** Use a laboratory grade blender with a tight-fitting lid. Operate the blender in a biosafety cabinet if infectious material is used. Allow aerosols to settle before opening the lid.
- **Sonication:** Operate the sonicator in a BSC or aerosol containment if infectious material is being used.
- **Centrifuging:** Use aerosol-proof rotors or safety buckets with caps that seal with O-rings. Before use, inspect O-rings and safety caps for cracks, chips, and erosion. Use tubes with threaded caps. Avoid overfilling the tube and getting caps/closures wet. Wipe tubes down with disinfectant after filling. Load and unload rotors and buckets inside the BSC. Balance buckets, tubes and rotors before centrifuging. Wait a few minutes after centrifuge completes to allow aerosols to settle. Disinfect the centrifuge after use.
- **Vacuum flask and aspiration set-up:** Vacuum lines and vacuum pumps must be protected by using a hydrophobic filter installed between the collection flask and vacuum source. A HEPA filter may be required to protect against infectious aerosols.

#### 4.5 Health Status

Certain medical conditions increase the risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions include diabetes or other metabolic disorders, pregnancy, certain autoimmune diseases, immunodeficiency or immunosuppression, animal-related allergies, chronic skin conditions or respiratory disorders, and steroid therapy. Individuals with these conditions should consult with their personal physician.

Women who plan to become pregnant or who are pregnant are encouraged to inform their Responsible Researchers or Laboratory Supervisors, the MBL's EH&S Office, and/or the MBL Human Resources Department. Such individuals are encouraged to discuss occupational exposure concerns relating to fetal infection with their personal physician.

#### 4.6 Medical Surveillance

Medical surveillance is a pro-active approach to preventing occupational illnesses. It is used as a tool to monitor individuals for occupational diseases acquired at the workplace.

The aim of the medical surveillance program is to:

- Provide active immunization when indicated by a health professional.
- Provide early detection of laboratory-acquired infections.
- Exclude highly susceptible individuals (e.g. pregnant women or immunocompromised individuals) from highly biohazardous laboratory work. This determination can be made during a risk assessment or medical consult with the occupational health professional or primary care physician.

The Responsible Researchers, IBC and BSO have shared responsibilities in identifying laboratory personnel who should be included in the medical surveillance program. The risk assessment process for IBC protocol registration provides the best opportunity to address any issues which might require medical surveillance.

## 5 BIOSAFETY TRAINING

The Responsible Researcher ensures all personnel working with biohazardous materials under their supervision are trained appropriately for the work they will be involved in.

At a minimum, personnel in a BSL-2 laboratory must complete biosafety training provided by the MBL BSO. Level BSL-1 training is covered through the annual general safety training provided by the EH&S Manager. The Responsible Researcher is encouraged to contact the BSO regarding any questions on the appropriate biological safety training for their laboratory.

#### Summary of Required Biosafety Training

BIOSAFETY LEVEL	BIOLOGICAL MATERIALS USED	TRAINING REQUIRED
BSL-1	Risk Group 1 Agents	General Safety Training

BSL-2	Risk Group 2 Agents	Biosafety Level 2
BSL-2	Human Derived Materials	Biosafety Level 2 OSHA Bloodborne Pathogens
BSL-2	None (Custodial, POM and Security)	Biosafety Awareness/Hazard Communication OSHA Bloodborne Pathogens
BSL-1 or BSL-2	Materials to be Autoclaved	Autoclave Training
BSL-1 or BSL-2	Shipping Hazardous Materials	Shipping Biological or Infectious Substances

## 6 BIOHAZARD CONTAINMENT

The following procedures are recommended for the management of small spills (<100mls) or large spill (>100mls) of infectious material, blood, tissue, or other potentially infectious materials in the laboratory.

### 6.1 Management of Small Spills (Spills <100 mL)

- Put on personal protective clothing (laboratory coat, gloves, face and eye protection) and assemble clean-up materials (disinfectant, autoclave bag, forceps, and paper towels).
- If the spill has occurred inside a BSC, keep the cabinet turned on.
- Cover the spill with paper towels and carefully pour disinfectant (10% bleach solution) to completely moisten the paper towels/contaminated area.
- Allow the disinfectant stand for 30 minutes.
- Remove any broken glass with forceps and dispose in a sharps container.
- Soak up the disinfectant and spill area with additional paper towels.
- Discard all disinfected materials into the trash. Any exposed reusable items (laboratory coats or forceps) must be autoclaved.
- Remove disposable gloves (and masks) and place into a biological waste container for autoclaving. Reusable PPE (safety glasses, etc.) should be cleaned with the proper disinfectant.
- Wash hands and exposed skin areas with soap and water.

### 6.2 Management of Large Spills (Spills >100 mL)

- If the spill occurs inside a BSC, close the sash and leave the BSC running.
- Restrict people from entering the area. Add sign "Warning Spill, Do Not Enter"
- Before leaving room, remove any contaminated clothing and place into a biohazard bag for decontamination later.
- Wash hands and exposed skin thoroughly with soap and water.
- Call MBL Campus Security at x7911 to report the size, location, and composition of the biological spill. Remain in the vicinity in a safe area to direct the spill team to the spill area upon their arrival.

- Inform the Biological Safety Officer at 508-289-7424 or [safety@mbi.edu](mailto:safety@mbi.edu) and your supervisor about the spill.

## 7 LABORATORY PRACTICES AND PROCEDURES

With the use of proper practices and procedures in the laboratory or work area, exposure to biohazards can be minimized. The following practices are important for the prevention of laboratory infection and disease, and for the reduction of the potential for contamination of experimental material.

### 7.1 Personal Hygiene

- Wash hands frequently after handling infectious materials.
- Always wash hands for at least 20 seconds before leaving the laboratory or work area.
- Do not touch your face with your hands.
- Use good microbiological techniques to protect your research, colleagues and yourself.
- Do not eat, drink, or store food, beverages or medications in the laboratory.
- Do not apply cosmetics, eye drops or lip balm in the laboratory.
- Do not handle contact lenses in the laboratory.

### 7.2 Laboratory Procedures for Handling Infectious Agents

- A laboratory specific biosafety manual must be developed outlining activities and Standard Operating Procedures (SOPs).
- Keep laboratory doors closed to restrict access to only authorized laboratory personnel.
- When RG2 (or higher) pathogens are used in long-term studies, post a biohazard sign at the laboratory entrance identifying the agents in use and dates for which used. In addition, all laboratories must have a door sign that states the names and contact numbers for emergency laboratory personnel.
- All laboratories must contain a sink for hand washing and access to an eyewash station.
- All individuals should wear a laboratory coat and protective gloves when handling potentially hazardous materials, including human blood and body fluids.
- Remove and leave all PPE within the laboratory before exiting. If transport of research materials through public spaces is required, then wash hands, wear a clean glove to carry materials, and use an ungloved hand for door handles, elevator buttons, etc.
- Perform all aerosol-generating procedures such as pipetting, shaking, grinding, sonicating, mixing, and blending in a BSC, whenever feasible.
- Centrifuge materials containing infectious agents in rigid, shatter-resistant tubes with caps. Use a centrifuge with sealed heads or screw capped safety cups. After centrifuging, open the tubes within a BSC.
- Avoid using needles, syringes, razor blades, and other sharps whenever possible.
- Cover benchtops and work surfaces with absorbent materials to absorb spills.

- Decontaminate work surfaces at the end of procedures and immediately after a spill. Limit bench-top items to those in immediate use; cluttered areas are more likely than well-maintained spaces to be the sites of accidents and are harder to clean and disinfect.
- After use, place reusable sharps such as surgical instruments, into a puncture-resistant container with disinfectant solution. Label the container with the biohazard symbol.
- Minimize splashing and aerosol generation. When pipetting, expel liquids against the sidewall of a tube rather than against the tube bottom.
- Use secondary containers (e.g., trays, specimen transport bags) for the prolonged storage or transport of infectious materials.
- Use only mechanical pipetting devices (no mouth pipetting) and cotton-plugged pipettes.

### 7.3 Hazard Communication

A biohazard label is required for all areas or equipment in which biohazardous materials (Risk Groups 2 or 3 agents) are handled or stored, or where BSL-2 procedures are required.

- A biohazard warning sign incorporating the Universal Biohazard Symbol shall be posted on the access door to the laboratory work area.
- All human tissue, body fluid, or other potentially infectious materials shall be stored in a container labeled with a biohazard symbol.
- Refrigerators, freezers, incubators, or other pieces of equipment where potentially infectious materials are stored or handled shall be labeled with the biohazard symbol.
- Refrigerators, freezers, microwaves and blenders shall be labeled with a biohazard symbol and “NO FOOD OR DRINK”.
- Any food products (e.g., dry milk, fruit juices, etc.) used for research activities shall be labeled “NOT FOR HUMAN CONSUMPTION”.

Contact the BSO at 508-289-7424 or [safety@mbi.edu](mailto:safety@mbi.edu) if you need biohazard labels and instructions on where to post them.

#### Example biohazard labels and signs for posting in BLS-2 Areas.



### 7.4 Personal Protective Equipment

Personal protective equipment (PPE) is used to protect personnel from contact with infectious agents. Responsible Researchers and Laboratory Supervisors are responsible for conducting laboratory PPE assessments, providing PPE, and training

personnel in the proper use of PPE. PPE must not be taken home or worn outside the laboratory.

Appropriate laboratory PPE should be determined following a thorough risk assessment of the work being performed. For assistance in selecting PPE for work with biological materials, contact the BSO at 508-289-7424 or [safety@mbi.edu](mailto:safety@mbi.edu).

The recommended laboratory PPE includes, but is not limited to, the following:

### **Laboratory Coats**

- Coats are used to prevent contamination of the skin and street clothes. MBL offers a laboratory coat program to current resident staff. Contact EH&S office.

### **Gloves**

- Gloves must be worn when working with infectious and potentially infectious materials.
- Gloves are recommended for all handling of biological materials to avoid cross contamination of experiments.
- If personnel develop or have latex allergies, then a suitable alternative should be available for use.
- Double-gloving provides further protection when applied appropriately.
- Gloves must be removed before leaving the laboratory. A One Glove Rule approach is acceptable with glove hand holding materials while the ungloved hand is available for opening doors,

### **Face Protection**

- Safety glasses are available to all MBL staff through MBL EH&S office. Visiting Scientists and Education Courses are to provide their own safety glasses which may be purchased through the MBL Stockroom.
- Safety glasses are recommended for all laboratory procedures.
- Splash goggles, safety glasses with solid side shields, or face shields are required when there is a potential risk for exposure from splashes, sprays or splatters from infectious or other hazardous materials.

### **Respiratory Protection**

- If respiratory protection is deemed necessary, contact the MBL EH&S office for risk assessment, training and fit testing information. Please reference MBL's Respiratory Protection Plan
- Personnel who are required to wear respiratory protection must be evaluated by a physician and trained in respirator selection and usage.

## **8 BIOLOGICAL SAFETY CABINETS**

Biological safety cabinets (BSC) are the primary means of containment for working safely with infectious materials. Biosafety cabinets operate by controlling airborne contaminants during work by use of laminar airflow and High Efficiency Particulate Air (HEPA) filtration.

They are designed to provide three types of protection when used together with standard microbiological practices: (a) Personal protection from material inside the cabinet; (b) Protection for the material inside of the cabinet; and (c) Protection for the environment from the material inside of the cabinet.

### 8.1 High Efficiency Particulate Air (HEPA) Filters

Control of airborne particulate materials is achieved with HEPA filters that efficiently remove microscopic contaminants from the air. The HEPA filter removes particles equal to and greater than 0.3 microns ( $\mu\text{m}$ ) this includes all bacteria, spores, and viruses with an efficiency of 99.99%.

Air flow is balanced so that some air is taken from the room and, together with sterile cabinet air, sucked into a horizontal grill at the front of the work surface. A BSC shall generally not be used for work with hazardous chemicals. The BSCs at MBL exhaust the contaminated air through HEPA filters back into the laboratory.

### 8.2 Classes of Biosafety Cabinets

There are three types of BSCs, designated as Class I, II, and III, based on specific airflow patterns within the BSC and on the locations of HEPA filters within the unit.

**Class I Biosafety Cabinets** are negative pressure cabinets with room air being drawn in from the front of the cabinet across the work area and HEPA filtered as it is exhausted back to the surrounding room. This type of cabinet provides no protection for the research materials but does protect the researcher(s) and the surrounding laboratory environment from materials worked with in the cabinet.

**Class II Biosafety Cabinets** are the most widely used and versatile cabinets at MBL. Airflow is drawn into the front grille of the cabinet. Exhaust air and the air supplied to the work surface pass through HEPA filters providing protection of the research materials, research personnel, and the environment. A fraction of the air (70%) is recirculated through a supply HEPA filter back into the cabinet workspace. The remaining 30% of the air in the BSC passes through the exhaust filter into the room or to the outside.

**Class III Biosafety Cabinets** are completely gas-tight enclosures with non-opening view windows. They are ducted to the building's exhaust system. Operations in the cabinet are conducted through attached rubber gloves. The cabinet is maintained under negative air pressure. Supply air is drawn into the cabinet through HEPA filters. Air from the cabinet is 100% exhausted and passes through two HEPA filters. A separate supply HEPA filter allows clean air to flow onto the work surface. They are designed for use with high-risk (BSL-4) agents.

### 8.3 Horizontal and Vertical Laminar-Flow Hoods (Clean-Air Benches)

Horizontal and vertical Laminar Flow Hoods (Clean-Air Benches) are not equivalent to BSCs. These hoods discharge HEPA-filtered air across the work surface and towards the user, thus only providing protection for the product. Laminar Flow hoods are not to be used when working with potentially infectious materials.

## Biological Safety Cabinet Characteristics

BSC CLASS, TYPE	Face Velocity (fpm)	Airflow pattern	Nonvolatile toxic chemicals /radionuclides	Volatile toxic chemicals or radionuclides
<b>Class I</b>	75	Enters at the front of cabinet. Exhausted through HEPA filter to the outside or room.	YES	YES (when exhausted outside)
<b>Class II, A1</b>	75	70% recirculated to the cabinet work area through HEPA filter. 30% balance can be exhausted through HEPA filter back into the room or to the outside through a canopy unit.	YES (small amounts)	NO
<b>Class II, A2</b>	100	Same as II, A1, but higher face velocity. Plenums are under negative pressure to room Exhausted air can be canopy-ducted to the outside through a HEPA filter.	YES	YES (small amounts)
<b>Class II, B1</b>	100	30% recirculated 70% exhausted through dedicated HEPA-filtered duct to the outside.	YES	YES (small amounts)
<b>Class II, B2</b>	100	No recirculation Total exhaust to the outside through hard-duct and a HEPA filter.	YES	YES (small amounts)
<b>Class III</b>	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filter in series.	YES	YES (small amounts)

### 8.4 Proper Use of Biosafety Cabinet

#### Before beginning work:

- Monitor alarms, pressure gauges, or flow indicators for any changes.
- If the UV light is on, turn off the UV light.
- Switch the cabinet on and let it run for about 3 to 5 minutes.

- Wipe work surface with an appropriate disinfectant (e.g. 70% ethanol).
- Plan your work and place everything needed for the procedure inside the BSC.
- Wipe items with disinfectant before placing inside the BSC.

**During work:**

- Avoid airflow disruption that could affect the level of protection provided by the BSC.
- Keep the BSC free of clutter.
- Do not place objects over the front air intake grille.
- Do not block the rear air intake grille.
- Limit traffic around the area when the BSC is in use.
- Ensure laboratory door is closed and avoid opening or closing the door if it is located near the BSC.
- Move arms slowly when removing or introducing items. Avoid sweeping arm motions.
- When working with infectious materials, change gloves when moving hands in and out of the cabinet.
- Keep all materials at least 4 inches inside the front sash.
- Keep clean materials at least one foot away from any aerosol generating activities to minimize the potential for cross contamination.
- Place any equipment that creates air turbulence in the back one third of the BSC and stop other work while the equipment is running.
- Do not operate a Bunsen burner inside the BSC to avoid disrupting air flow patterns within the BSC.
- Segregate contaminated and clean items. Work from “*clean to dirty*.”
- Clean up all spills in the cabinet immediately. Allow the BSC to run for about 3-5 minutes before resuming work.

**After completion of work:**

- Wash hands for 20s after completion of work.
- Using a new pair of gloves, wipe down all items with an appropriate disinfectant before removing from the hood.
- After removing all materials from hood, wipe all interior surfaces of the BSC with an appropriate disinfectant.
- Laboratory personnel should remove gloves and laboratory coats, and then wash hands for 20s as the final step in safe microbiological practices.
- Periodically decontaminate and clean under work grilles.

**8.5 Certification of Biosafety Cabinets**

The MBL requires that all BSCs installed on campus be tested and certified annually by technicians accredited by the National Science Foundation (NSF). BSCs must be tested and certified annually or if:

- A new cabinet is being installed.
- A cabinet has been moved.
- A cabinet requires maintenance and repairs.

All certification, maintenance, and repairs of BSCs are to be conducted by an NSF-approved vendor contracted by the MBL. If encountering an issue with a BSC, please contact the EH&S Manager at 508-289-7424 or [safety@mbi.edu](mailto:safety@mbi.edu).

## 8.6 Purchase, Installation, and Maintenance of Biosafety Cabinets

1. Before purchasing a BSC, the BSO must be consulted for an evaluation of its suitability for the intended research and the available space. Arrangements for installation or disposal of BSCs shall be requested through the MBL Plant Operations & Maintenance (POM) Department and the BSO.
2. Installation of BSCs shall be done by certified professionals. The BSO will coordinate with vendor to provide installation, certification, and decontamination of BSCs. Contact the BSO at 508-289-7424 or [safety@mbi.edu](mailto:safety@mbi.edu) for assistance.
3. Certifications shall be done annually or whenever a BSC has been moved, or requires maintenance and repairs. The BSO will schedule the annual certification of all BSCs at the MBL.
4. Payment for services including certification, repairs, and decontamination of the BSC is the responsibility of the owner (Principal Investigator or Department/Center/Division).

## 8.7 Decontamination of Biosafety Cabinet

BSCs must be professionally decontaminated in the following situations:

- Before being moved to another location.
- Before removal for disposal or long-term storage.
- Before maintenance, service and repairs can be made to the unit.

Decontamination must be performed by a certified professional accredited by NSF International. MBL uses a certified external vendor for decontamination. Vendor may use paraformaldehyde gas as the method of decontaminating BSC filters, which is an NSF/ANSI Standard 49 approved decontamination method. Paraformaldehyde is carcinogenic.

The BSO shall schedule for the decontamination with outside vendor. The cost of the decontamination shall be incurred by the owner of the BSC (Responsible Researcher, Department, Center, or Division).

To prepare a BSC for maintenance, certification or repair:

- The BSC must be completely cleared of any equipment, pipettes, waste, liquids and tubing.
- The surfaces of the BSC must be decontaminated with an appropriate disinfectant (e.g. 10% bleach solution followed by 70% ethanol solution).
- Once a specific time has been set for the maintenance to be performed, a laboratory member must be available to meet with the certification technician.
- The exterior of the BSC must be decontaminated with a suitable disinfectant.
- In the case of removal or disposal, all biohazard stickers must be removed from the exterior surfaces.

## 9 APPROVAL FOR BIOHAZARD WORK AT MBL

All research at the MBL involving biohazards (or utilizing Non-Native Species) must be documented and submitted to the Institutional Biosafety Committee (IBC) for review and approval prior to the initiation of work. Approved protocols are valid for three years. For the purposes of the IBC, biohazards are defined as potentially infectious agents, organisms or materials, biological toxins, organisms containing recombinant DNA, or other genetically altered organisms and agents. Please note: if you plan on using or collecting biological agents, samples, etc. from live vertebrate animal sources or identifiable human sources, or your research will involve radiation/radioactive isotopes, you will must seek approval from the Institutional Animal Care and Use Committee (IACUC), Institutional Review Board (IRB), or Radiation Safety Committee (RSC).

The MBL's IBC shall meet at least once every six months (or as often as necessary) to discharge its institutional obligations. To register or amend research, download the application form "Institutional Biosafety Committee Biosafety Application" at:

<https://www.mbl.edu/research/health-safety/biological-safety/biosafety-committee>

### 9.1 Recombinant DNA

The National Institutes of Health (NIH) has developed guidelines on the use and containment of recombinant DNA materials in the research laboratory. Strict adherence to the NIH Guidelines for Research Involving Recombinant of Synthetic Nucleic Acid Molecules is critical to maintain effective regulatory oversight of rDNA at the MBL. The NIH Guidelines require individuals conducting such research to file a registration form with the IBC which must approve the protocols related to rDNA molecules.

The NIH Guidelines are designed to outline the practices for constructing and handling rDNA, both natural and synthetic, as well as cells, organisms, and viruses that contain these molecules. Recombinant DNA refers to either: (a) molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecule that can replicate in a living cell, or (b) molecules that result from the replication of those described in (a) above.

The NIH Guidelines specify different levels of approval and registration requirements (Sections III-A through Section III-F) that must be met prior to or upon initiation of work. Certain uses of rDNA (Section III-F) are exempt from any approval or registration requirements.

#### Summary of Experiments Falling Under NIH Guidelines

All recombinant DNA research proposals require the Responsible Researcher to make an initial determination of the required level of physical and biological containment. The NIH has developed six categories (**III-A** to **III-F**) addressing different types of rDNA research.

**1. Section III-A: Experiments Requiring IBC Approval, Recombinant DNA Advisory Committee (RAC) Review, and NIH Director Approval Before Project Initiation.**

If the proposed research falls within **Section III-A** of the NIH Guidelines, the experiment is considered a "Major Action". This includes experiments involving human gene transfer experiments. The experiment cannot be started without submission of relevant information to the Office of Recombinant DNA Activities at NIH. The proposal has to be published in the Federal Register for 15 days, be reviewed by the NIH Recombinant DNA Advisory Committee (RAC), and specific approval by the NIH must be obtained. The containment level for such an experiment will be recommended by the RAC and set by the NIH at the time of approval. The proposal requires IBC approval before initiation.

**2. Section III-B: Experiments Requiring IBC and NIH/OBA (Office of Biotechnology Activities) Approval Before Project Initiation.**

Research cannot be initiated without submission of relevant information on the proposed experiment to NIH/ Office of Biotechnology Activities (OBA). Experiments include the cloning of toxic molecules. Containment conditions for such experiments will be determined by NIH/OBA in consultation with ad hoc experts. IBC approval is required before initiation. Please refer to the guidelines for specific details.

**3. Section III-C: Experiments Requiring IBC and IRB (Institutional Review Board) Approval and NIH/OBA registration before Project Initiation.**

Experiments with human subjects are covered, which require IBC and IRB approval and NIH/OBA registration before initiation.

**4. Section (III-D) requires IBC Approval Before Project Initiation.**

Experiments with whole animals or plants, and projects involving DNA from Risk Group 2, 3 or 4 agents. Prior to initiation, the Responsible Researcher must submit an IBC application to the IBC for review and approval before commencement of work.

**5. Section (III-E) requires IBC notice simultaneous with initiation.**

Section III-E experiments require the Responsible Researcher to submit an IBC application to the IBC at the time the experiment is initiated. The IBC reviews and approves all such proposals, IBC review and approval prior to initiation of the experiment is not required (but recommended).

**6. Section (III-F) EXEMPT (MBL IBC review is still required).**

Section III-F experiments are exempt from the NIH Guidelines. However, the PI must still submit an IBC application to be reviewed by the IBC to verify the exempt status of the registration. Approval is not required before initiation of experiment, but highly recommended. Responsible Researcher must submit an IBC application to the IBC for review.

## 9.2 Research Involving Animals

The use of vertebrates in research is subject to federal law (Animal Welfare Act) and state regulations. The MBL complies with all federal and State and local regulations regarding animal use and care. All animal protocols using vertebrates or cephalopods must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). To register or amend research involving animals, download the appropriate application form at:

<https://www.mbl.edu/research/research-administration/compliance-and-ethics/iacuc>

## 9.3 Select Agent Toxins

Some select biological toxins are highly regulated and subject to registration with the Centers for Disease Control and Prevention (CDC) or the United States Department of Agriculture (USDA), due to their potential to pose a severe threat to public health and safety. All users of select agents must complete an IBC application and must have appropriate training before commencement of work. Below are the exempt quantities for the select toxins. See MBL's Select Agent Toxin Policy:

<https://www.mbl.edu/research/health-safety/biological-safety>

### Exempt Quantities of Select Agent Toxins

HHS Toxins [§73.3(d)(7)]	Amount
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

## 10 DECONTAMINATION OF EQUIPMENT AND WORK AREAS

Any equipment that is going to be either moved for an area, reused or disposed must be decontaminated. This includes both single use and multiple use equipment.

Decontamination is defined as chemical or physical treatment that destroys microorganisms that produce disease to an acceptable level. This can be achieved through disinfection or sterilization. **Disinfection** is the physical or chemical treatment that destroys most microbial agents, except spores. Although viable microbes may still be present, they are below the level necessary to cause disease. **Sterilization** is the process by which all forms of microbial life, including spores, viruses, and fungi, are destroyed.

Selecting the appropriate method for inactivating a biohazardous agent is challenging. The choice depends mainly on the equipment available, the biohazardous agent, and the presence of interfering substances (e.g., high organic content) that may protect the

organism from decontamination. For example, decontamination of a surface such as a laboratory bench may be achieved with a disinfectant, while decontamination of biohazardous waste is accomplished via sterilization in an autoclave.

There are four main categories of physical and chemical methods of decontamination are described below:

### 10.1 Heat (Autoclave)

Heat can be applied in dry or wet form in order to kill microbial agents. Compared to dry heat, wet heat exhibits better heat transfer to and into the cell resulting in overall shorter exposure time and lower temperature. Steam sterilization (autoclaving) uses pressurized steam at 121 to 132 degrees Celsius (°C) (250-270 degrees Fahrenheit) for 30 to 40 minutes. The heat kills all microbial forms, including heat-resistant spores. A similar effect can be achieved using dry heat in an oven when the temperature is raised to 160-170 °C (320 to 338 °F) for 2 to 4 hours. Autoclaves are routinely used at MBL. Training is provided by the Safety Office and the Autoclave Standard Operating Procedures can be found at:

<https://www.mbl.edu/research/health-safety/biological-safety>

### 10.2 Liquid Chemicals Used as Disinfectants

Several disinfectants are commonly used in the research laboratory settings, especially to wipe down surfaces or equipment to remove infectious agents. The appropriate liquid disinfectant should be chosen after carefully evaluating biohazardous agent and the type of material to be decontaminated.

The factors to be considered while selecting a disinfectant include:

- Nature of surface being disinfected: The more porous and rough the surface is, the longer contact time a disinfectant will need to be effective.
- Number of microorganisms present: Higher concentrations of microorganisms require a longer contact time or higher concentration of disinfectant.
- Resistance of microorganisms: Microbial agents can be classified according to increasing resistance to disinfectants and heat.
- Presence of organic material: The proteins in organic materials (e.g. blood, bodily fluids, and tissue) can prevent or decrease the activity of certain disinfectants.
- Duration of exposure and temperature: Increased exposure time increases the effectiveness of disinfectants. Low temperatures may reduce the activity of the disinfectant, and longer exposure times may be required.

Liquid disinfectants are useful for surface decontamination. At an appropriate concentration, they may be used for decontamination of liquid biohazardous waste prior to disposal into the sanitary sewer. EPA approved disinfectants along with contact time for deactivating biohazard can be found at:


<https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants>

For disinfectants at MBL, the following are recommended for use:

**(a) Alcohols (Ethanol and Isopropanol)**

Ethanol or isopropyl alcohol (>70%) is very effective as a general use disinfectant against vegetative forms of bacteria and fungi, and enveloped viruses. Some organisms (e.g., *Mycobacterium tuberculosis*) are not inactivated by 70% ethanol.

**Increasing resistance to chemical disinfectants.**

Degree of Resistance	Microbe	Examples
<b>LEAST RESISTANT</b>  <b>MOST RESISTANT</b>	Lipid or Medium-Size Viruses	Herpes simplex virus, HIV Cytomegalovirus, Respiratory syncytial virus, Hepatitis B virus.
	Vegetable bacteria	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Salmonella choleraesuis</i>
	Fungi	<i>Trichophyton</i> sp., <i>Cryptococcus</i> sp., <i>Candida</i> sp.
	Non-lipid or Small Viruses	Poliovirus, Coxsackievirus Rhinovirus
	Mycobacteria	<i>Mycobacterium tuberculosis</i> <i>Mycobacterium bovis</i>
	Bacterial spores	<i>Bacillus subtilis</i> <i>Clostridium sporogenes</i>

Since alcohols evaporate rapidly there is limited exposure time, so sufficient liquid must be used to ensure extended contact time. And with ethanol being highly flammable, be mindful of use near sources of heat or ignition.

**(b) Halogens (Bleach)**

Chlorine-containing solutions are the least expensive disinfectants. Sodium hypochlorite (bleach) is the most common base for chlorine disinfectants. Household bleach (5% available chlorine) can be diluted 1:10 with water to yield an effective disinfectant solution. A freshly prepared dilute solution of bleach is highly effective in decontaminating large biological spills.

Excess organic matter inactivates chlorine-containing disinfectants. These compounds are strong oxidizers and very corrosive, even in dilute solutions. Always use appropriate personal protective equipment when using these compounds. It is recommended that the decontamination step of a surface with a hypochlorite agent should be followed by a wipe-down using 70% alcohol or water to remove the corrosive residue of the bleach.

**(c) Hydrogen Peroxide**

Solutions of Hydrogen Peroxide at concentrations as low as 0.25% can provide effective disinfectant. At low concentrations, Hydrogen peroxide can be safely handled for both individuals and around animal enclosures throughout MBL.

Hydrogen peroxide can be applied by spray or use of a damp towelette which is more appropriate near animal life.

For Hydrogen Peroxide to serve as an effective disinfectant, a longer contact time is required. In use of a low (0.25-0.50%) solution, the disinfected surface should be allowed to stay wet 5-10 minutes before wiping dry.

#### (d) Quaternary Ammonium Compounds

Less commonly used at MBL are solutions containing quaternary ammonium. These solutions are cationic detergents that have strong surface activity and are generally nontoxic (ideal for general use). They are active against Gram-positive bacteria and lipid-containing viruses, but less active against Gram-negative bacteria. They are ineffective against non-lipid-containing viruses. They are rendered inactivate by organic materials, anionic detergents or metal salts found in water.

## 11 BIOLOGICAL WASTE DISPOSAL

Proper handling and disposal of biohazardous waste is critical to prevent infection of personnel (laboratory workers, students, custodial staff, laboratory visitors, etc.) and release to the environment. The Commonwealth of Massachusetts regulations (105 CMR 480.000) and OSHA require that biohazardous waste be properly labeled, stored, and disposed of.

There are three categories of biohazardous waste: biohazardous solid waste, biohazardous sharps waste, and biohazardous liquid waste. At a minimum, all biohazardous waste must be labeled with the Universal Biohazard Symbol.

### 11.1 Biohazardous Solid Waste

Biohazardous solid waste includes microbial agents, tissue culture, and contaminated material (e.g., petri dishes, pipettes, contaminated glass, plastic plates, paper towels, gloves, etc.). Non-sharp biohazardous solid waste must be decontaminated before disposal using an autoclave.

Solid waste to be autoclaved should be collected in a clear autoclave bag without the Universal Biohazard symbol or markings. The autoclave bag should be used to line a rigid, leak-proof waste container for BSL-1 or BSL-2 laboratories marked with the Universal Biohazard symbol.

#### Biohazard container, Sharps and Glass Box used in MBL laboratories



## 11.2 Biohazardous Sharps Waste

A sharp is any instrument that is capable of causing punctures, cuts, or scrapes to the body. Sharps includes but is not limited to needles, syringes, scalpels, razor blades, slides, coverslips, Pasteur pipettes, capillary tubes, sharp or broken glass, lancets, suture needles, and microtome blades. Use of sharps should be restricted to trained personnel and cases in which no alternative is available.

### Sharps Precautions

- Avoid the use of needles and other sharps whenever possible. Use plastic ware instead of glassware if available.
- Minimize any contact with sharps by disposing of or storing them immediately after use.
- Needles must never be recapped, removed from the syringe, sheared, bent or broken. Dispose of the entire syringe-needle combination in a sharps container.
- Use a mechanical device to remove scalpel blades. Never use your fingers.
- Be careful during cleanup; some sharp items may be hidden in the waste materials.
- If a needle stick occurs, allow the wound to bleed for a few minutes, gently wash the area with water and soap, and then get medical attention immediately.

#### 11.2.1 Disposal of Laboratory Sharps

Sharps are considered as regulated medical waste under federal and state regulations. They must not be disposed of as regular trash. All sharps must be disposed of into an approved, puncture resistant, sharps container.

- Sharps used with genetically modified and biological materials must be collected in a rigid, leak-proof, puncture resistant, and shatter-proof red biohazard sharps containers for disposal. The sharps container should be marked with the Universal Biohazard symbol and the word "BIOHAZARD".
- Do not overfill the sharps container.
- Never force materials into a sharps container.
- Never reach into the sharps container to retrieve an item.
- Do not remove the lid from the sharps container.
- When the sharps container is  $\frac{3}{4}$  full, close lid and contact the Environmental Health & Safety Manager at 508-289-7424 or by email [safety@mbl.edu](mailto:safety@mbl.edu) to request a pickup of the sharps containers, or delivery of waste disposal supplies.

Pasteur pipettes present a special challenge. If pasteur pipettes are contaminated with BSL-2 contaminants, the pipettes must be disposed of in the sharps container that is identified as a Biohazard. If pasteur pipettes are contaminated with BSL-1 or any non-BSL related contaminants, dispose of the pipette in the broken glass boxes.

Disposal of uncontaminated laboratory glassware and broken glass should be placed into the standard broken “GLASS” cardboard boxes. However biologically contaminated glassware, place directly into a sharps containers for a small container (100ml or less). A larger contaminated container (>100ml) must be autoclaved prior to being placed into the broken glass box.

### 11.3 Biohazardous Liquid Waste

Biohazardous liquid waste is material that is contaminated with biological agents, including human blood and body fluids, liquid culture media, viral supernatant, and media from infected cells. Collect biohazardous liquid waste in closeable, rigid, plastic leak-proof containers labeled with the Universal Biohazard symbol. The waste must be decontaminated by autoclaving (see Section 10.1) or chemical disinfection prior to disposal into the sink.

If chemical disinfection is chosen, full-strength household chlorine bleach may be added to the waste container (e.g., an aspiration flask). Such that the final solution concentration of bleach will be 10% (add 1 part bleach to 9 parts liquid waste). Contact time for bleach with biohazardous liquid must be at least **30 minutes** prior to disposal. This decontaminated waste is acceptable to put down the laboratory sink drain. When disposing down drain allow tap water to run as the solution may be slightly corrosive.

In the case that bleach is not an effective disinfectant for the biological agent in use, a disinfectant approved by the U.S. Environmental Protection Agency (EPA) must be used. Ensure the proper contact time prior to disposal.

### 11.4 Mixed Biological and Chemical Waste Disposal

For non-sharps, disinfect the infectious material with a chemical disinfectant and dispose of as chemical waste. Select chemical disinfectants carefully because some disinfectants can react with chemicals. Once the biological component is inactivated, the waste can be managed as Hazardous Chemical Waste. Refer to the MBL’s Chemical Hygiene Plan for guidance or contact the EH&S Manager at 508-289-7424 or [safety@mbl.edu](mailto:safety@mbl.edu) with questions on appropriate disinfectant.

For sharps, mixed chemical and biohazardous waste will be placed into a sharps container that is labeled as “CHEMICAL SHARPS WASTE”. Any mixed chemical and biohazardous waste must be properly identified and labeled with a Hazardous Waste Label.

### 11.5 Mixed Biological Waste and Radioactive Waste

- All mixed radioactive waste and biohazardous waste must be properly segregated prior to disposal.
- Decontaminate infectious material with an appropriate disinfectant. Disinfectant used must be compatible with radiation waste storage and packaging requirements (pH, etc.). Autoclaving of radioactive waste is not permitted.
- Deface all “biohazard” and “regulated medical waste” symbols and markings from decontaminated material before disposing of as radioactive waste.

- The labeled mixed radioactive waste is picked up by the Radiation Safety Officer (RSO) and transported to the Radioactive Waste Storage Facility for packaging and disposal. Contact the RSO 508-289-7424 or email [safety@mbi.edu](mailto:safety@mbi.edu) for assistance.

### **11.6 Animal Carcasses and Bedding**

Animal waste MUST NOT be discarded in the regular trash. Animal carcasses shall be placed in ziplock plastic bags labeled with date, user name and room number. If the material is infectious, place the ziplock bag inside a red biohazard bag labeled with the biohazard symbol. The waste then needs to be taken to the biohazard freezers in Loeb room G11 or Rowe room 107 for storage. The pathological waste is stored frozen until it is pick-up by a licensed Regulated Medical Waste contractor for disposal. Contact the Safety Office 508-289-7424 or email [safety@mbi.edu](mailto:safety@mbi.edu) for assistance.

Animal bedding should NOT be autoclaved. Bring all used bedding (stored in tied plastic garbage bags) to Loeb G11 and place in specified biowaste disposal container. This bedding is then disposed of as biowaste through MBL's outside vendor.

## **12 TRANSPORT AND SHIPPING OF BIOLOGICAL MATERIALS**

All biological materials shall be transported in a manner that maintains the integrity of the material during normal transport conditions, and minimizes the chance of any accidental release and exposure to the MBL Community, general public, and the environment. The transportation must comply with U.S. Department of Transportation (US DOT) regulations for ground transportation, and the International Air Transport Association (IATA) regulations requirements for transportation via air. Transportation of regulated materials must be covered by transport and use permits, as designated by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS)

<https://www.aphis.usda.gov/aphis/home/>

### **12.1 Transporting Biological Materials on MBL Campus**

Biological materials can be safely transported between MBL buildings when the materials are properly packaged and clearly labeled to minimize the potential for accidental spills especially in public areas and roadways.

The following procedures for preparing and transporting biological materials between MBL Campus buildings should be followed:

- Biohazardous materials (e.g., microbes, biological toxins, human and non-human primate materials, and recombinant or synthetic nucleic acid molecules) must be packaged in a rigid primary (specimen) container that is leak-proof and secured with a tight-fitting cap.

- Place the primary container(s) in a secondary transport container that is also sealed and labeled with a biohazard symbol. The secondary container must be strong enough to remain closed in case the container is dropped.
- Place sufficient absorbent material (i.e. paper towels) in the secondary container to absorb all free liquids in the event that primary containers rupture or break during transport.
- Package primary containers in the secondary container in a manner that will reduce shock, rupture, or breakage.
- Carry a pair of clean disposable examination gloves with you when transporting biohazardous materials.
- Avoid transporting biohazardous materials through eating areas, break rooms, or public walkways with heavy human traffic.
- Label all secondary containers with a brief description of the contents and an emergency contact name and phone number (e.g., Responsible Researcher or Laboratory Supervisor).
- Containers used for transporting blood specimens (regardless of source) or specimens known or suspected to contain a pathogen should be labeled with the universal biohazard symbol.

### **12.2 Transportation of Biological Materials off MBL Campus**

Transportation of biological materials off MBL Campus property is regulated by federal (U.S. DOT) and international (IATA) regulations, and will require specific packaging, labeling and documentation. Only trained personnel can prepare and transport biohazardous material. Contact the BSO at 508-289-7424 or [safety@mbledu](mailto:safety@mbledu) for additional information. Transportation of some regulated materials is defined by and regulated by USDA-APHIS.

### **12.3 Shipping of Biological Materials to an Off MBL Campus Destination**

Shipping of biohazardous materials requires training. This includes shipment of diagnostic specimens (from humans or animals), cultures of infectious substances (infectious to humans and/or animals), genetically modified organisms and any biological materials shipped on dry ice.

Training is mandatory for shippers (the person sending out the package) and handlers (the people who transport the package). Persons packaging biological specimens or hazardous materials/dangerous goods for shipment must receive function-specific training.

The BSO will provide training and consultation for MBL personnel who plan to ship biological materials. The training is required every two years or when there is change in the regulations.

## **13 IMPORT AND EXPORT OF INFECTIOUS AGENTS**

Various federal and international regulations exist which require permits for the import, export and interstate transportation of biologicals materials.

### 13.1 Import of Infectious Materials

The CDC's Import Permit Program (IPP) regulates the importation of infectious biological agents, infectious substances, and vectors of human disease into the United States. Items that most often require a permit from CDC are:

1. **Etiologic agents:** Any infectious (etiologic) agent known or suspected to cause disease in humans.
2. **Biological Materials:** Unsterilized specimens of human and animal tissues (such as blood, body discharges, fluids, excretions or similar material) containing an infectious or etiologic agent.
3. **Hosts and Vectors:**
  - *Animals:* Any animal known or suspected of being infected with an organism capable of causing disease in humans may require an import permit.
  - *Insects:* Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to man.
  - *Arthropods:* Any living insect or other arthropod that is known or suspected of containing an etiologic agent (human pathogen).
  - *Snails:* Snail species capable of transmitting a human pathogen.
  - *Bats:* All live bats require an import permit from the CDC and the U.S. Department of Interior, Fish and Wildlife Services.

Import permits are issued only to the importer, who must be located in the United States. A CDC Import Permit can be obtained on their website:

<https://www.cdc.gov/cpr/ipp/applications/>

### 13.2 Import of Soils and Animals

The USDA Animal and Plant Health Inspection Service (APHIS) require permits for the import of animal and plant materials due to the potential risk of exotic disease introduction into the U.S. or transmission of diseases across state lines or due to regulated biotechnology in agricultural plants or animals. Examples of items requiring permits are:

- Infectious agents of livestock and biological materials containing animal material.
- Tissue culture materials and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origins.
- Plant pathogens, weeds and seeds.
- Plant seeds of genetically modified crop species.

### 13.3 Export Controls Related to Biologicals and Toxins

The export of various biological materials or agents of human, plant, and animal diseases may require a license from the U.S. Department of Commerce for export from the United States to areas abroad. All select agents, and numerous biological agents and toxins, are controlled for export. They require authorization (via export license) by the federal government before they can be shipped worldwide.

### 13.4 Material Transport Agreements

Material Transfer Agreements (MTAs) address the exchange of research materials between individuals at separate organizations and address ownership, intellectual property, publications, and liability related to the research materials.

## 14 REGULATIONS AND GUIDELINES

1. CDC and the National Institute of Health (NIH) Guidelines on: ***Biosafety in Microbiological and Biomedical Laboratories*** (BMBL)  
<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF>
2. National Institutes of Health (NIH Guidelines): **Guidelines for Research Involving Recombinant DNA Molecules**  
<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>
3. OSHA **Bloodborne Pathogens** Standard  
<https://www.osha.gov/SLTC/bloodbornepathogens/standards.html>
4. U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), and Veterinary Services (VS), **Import of Animal or Plants**  
[www.aphis.usda.gov/animal-health/organisms-vectors](http://www.aphis.usda.gov/animal-health/organisms-vectors)
5. Commonwealth of Massachusetts Regulations (105 CMR 480.000), **Management of Biological Waste**  
<http://www.mass.gov/eohhs/docs/dph/regs/105cmr480.pdf>