**Biosafety Manual**

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**Note:**

This is a general template. The principal investigator or other responsible party is responsible for including laboratory and protocol-specific procedures for addressing hazards in their laboratory.

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# Introduction

Provide a general introduction that gives background on your institution and individuals in charge and that describes the purpose of the biosafety manual.

# Important Contact Information

Insert important contact information for key individuals and departments.

#  General Responsibilities

ADD/DELETE ADDITIONAL SECTIONS AS NECESSARY

## Principal Investigator (PI)

* Ensures that laboratory and support personnel receive appropriate training for the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures
* Ensures biosafety procedures are incorporated into standard operating procedures (SOPs) for the laboratory
* Ensures personal protective and safety equipment is provided and used
* Ensures compliance by laboratory personnel with the relevant regulations, guidelines, and policies
* Reviews the Biosafety Manual periodically with lab personnel and provides lab-specific updates as necessary
* Ensures all applicable training is completed by individuals in the laboratory

##  Laboratory Personnel

* Participate in appropriate training and instruction
* Comply with biosafety procedures
* Report all accidents, major spills, or exposure incidents to immediate supervisor
* Review the Biosafety Manual periodically

# General Safety Practices & Equipment

## Personal Protective Equipment

Personal protective equipment (PPE) is used in any laboratory environment to protect the user from potentially harmful biological and chemical exposures. Proper PPE needs to be determined and approved prior to starting any type of work and will vary depending on the person conducting the research and the type of research conducted. All PPE must be selected with the goal of providing protection from a hazard.

The selection of alternate choices of PPE should be considered if the user is at risk of physiological discomfort (e.g., contact dermatitis from latex gloves or asthma from wearing certain face masks). Proper training on the wearing and function of PPE is required PRIOR to using PPE.

All PPE shall be removed prior to leaving the work areas and placed in designated areas for disinfection or disposal. ***At no time will personnel be permitted to take home any PPE, including lab coats, for laundering or cleaning.***

## Safety Engineered and Needleless Sharps

Over the last few years, manufacturers have developed “engineered sharps”; these are commonly used items (e.g., scalpels, syringes, needles) that have various mechanical devices to vastly decrease the occurrence of injuries due to sharps.

## Biological Safety Cabinets

Biological safety cabinets (BSCs) are designed to provide three types of protection:

* Personnel protection from the material inside the cabinet.
* Protection of the material inside of the cabinet.
* Protection for the environment from the material inside of the cabinet.

### Comparison of BSCs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Type** | **Face Velocity (lfpm)** | **Airflow Pattern** | **Radionuclides/ Toxic Chemicals** | **Biosafety Level(s)** | **Product Protection** |
| Class I\*Open front | 75 | In at front; rear and top through high efficiency particulate air (HEPA) filter | No | 2,3 | No |
| Class II Type A1 | 75 | 70% recirculated through HEPA; exhaust through HEPA | No | 2,3 | Yes |
| Type A2 | 100 | Same class II A1, but plena under negative pressure to room and exhaust air is ducted | Yes | 2,3 | Yes |
| Type B1 | 100 | 30% recirculated through HEPA; exhaust via HEPA and hard ducted | Yes (Low levels/volatility) | 2,3 | Yes |
| Type B2 | 100 | No recirculation; total exhaust via HEPA and hard ducted | Yes | 2,3 | Yes |
| Class III | NA | Supply air inlets and exhaust through 2 HEPA filters | Yes | 3,4 | Yes |

\* Glove panels may be added and will increase face velocity to 150 lfpm; gloves may be added with an inlet air pressure that will allow work with chemicals/radionuclide.

Biosafety Cabinets should be certified at the following times:

* When newly installed
* After filter replacement
* After the cabinet has been moved
* Annually

Expand on types present at your institution and maintenance schedules.

## Signs and Hazard Communication

The biological hazard warning symbol should be used throughout the institution to notify workers about the presence of infectious agents. It is the responsibility of the responsible party to ensure that all necessary postings are installed and properly maintained. The warning symbol should be removed when the hazardous agent is no longer in use or present. The biohazard symbol should be orange or red in color with a contrasting background.

In general, the door to any laboratory containing an infectious agent should have a biohazard symbol posted. Additionally, postings should also be affixed to items such as BSCs, freezers, transport containers, or other work and storage areas or equipment where potentially infectious agents are used or stored.

## Use of Open Flames in Biosafety Cabinets/Tissue Culture Hoods

Describe your institution’s policy on open flame use. It is NOT recommended that open flames be used in BSCs/tissue culture hoods due to the presence of paper HEPA filters which are flammable, disruption of airflow, and inactivation of manufacturer’s warranty.

## Chemical Fume Hoods

Chemical fume hoods are not to be confused with BSCs. They offer no sample protection, nor do they offer personnel or environmental protection against biological agents. Fume hoods should only be used for chemical and radioactive work. The hoods need to be verified yearly (by facilities maintenance or other assigned party).

Air intake from the fume hood is not filtered and is 100% exhausted. The fume hood is hard ducted to the outside.

It is important to keep the sash open within its proper operating position.

* Verify that the chemical hood is exhausting.
* Work with the sash lowered to the 100-110 ft/min level. The sash must be below chin level.
* Work at least 6 inches inside the hood.
* Do not block the face of the hood (e.g., with shielding or large pieces of equipment).
* Do not block the rear exhaust slot. Place bulky items to the rear and sides on a supporting mesh elevated at least two inches from the work surfaces to allow passage of air to the rear slot.
* Remember, the fume hood work surface is not a storage area.

## Exposures

Describe your exposure procedures here.

## Spill Response

Describe spill procedures at your institution (e.g., inside containment, outside containment, inside a centrifuge, outside the laboratory)

# Infectious Agents

## Universal Precautions

The concept of Standard Precautions is to treat all human/primate blood and other body fluids, tissues, and cells (including established cell lines) as if they were known to be infectious. Standard precautions are frequent hand washing, no mouth pipetting, no food or drink in the lab, and proper disposal of medical waste as well as engineering controls and PPE.

## Risk Groups

There are four risk groups for biological agents: Risk Group (RG) 1, 2, 3, and 4. RG1 is considered to present the lowest hazard.

* RG1: Agents that are not associated with disease in healthy adult humans.
* RG2: Agents associated with human disease, which is rarely serious and for which preventive or therapeutic interventions are often available.
* RG 3: Agents associated with serious or lethal human diseases for which preventive or therapeutic interventions may be available (high individual risk but low community risk). RG 3 agents are generally transmitted via aerosols.
* RG4: Agents likely to cause serious or lethal human diseases for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

## Biosafety Levels

There are four biosafety levels as designated in the U.S. Centers for Disease Control and Prevention (CDC)/National Institutes of Health (NIH) Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th edition. The levels were established by taking into account the risk group and characteristics of the infectious agent to be used, the activities to be performed coupled with the facilities, prescribed laboratory procedures, and safety equipment to be utilized:

* Biosafety Level 1 (BSL-1): The practices and equipment utilized in a BSL-1 facility are appropriate for work with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. Examples of these types of microorganisms include *E. coli* K12 derivatives, *Saccharomyces cerevisiae*, and others.
* Biosafety Level 2 (BSL-2): The equipment, practices, and facilities used in BSL-2 laboratories are established for a broad range of indigenous moderate-risk group 2 agents. Examples include *Salmonella* sp. and *Toxoplasma* sp. The primary hazards to workers associated with these agents are accidental auto-inoculation, ingestion, and skin and mucous membrane exposure. Procedures with the ability to produce aerosols must be conducted in primary containment devices such as a biological safety cabinet.
* Biosafety Level 3 (BSl-3): BSL-3 facilities are established for the use of indigenous or exotic agents that possess the potential for infection by aerosol, and the results of such infection may have serious or lethal consequences. Typical examples of agents designated as requiring Biosafety Level 3 facilities include *Mycobacterium tuberculosis* and *Coxiella burnetii*.
* Biosafety Level 4 (BSL-4): BLS-4 facilities are designed for work with dangerous and exotic agents that pose a high risks to individuals to contract a life-threatening disease. In these facilities, all manipulations are considered to be of high risk, and the procedures and safety equipment are designed to prevent exposure.

Four biosafety levels have also been outlined for activities involving infectious agents and animals. These are referred to as Animal Biosafety Levels (ABSL) 1, 2, 3, and 4. These are also outlined in the BMBL. Add another section to describe ABSLs if necessary.

## Facility Requirements

Each BSL has its own requirements as laid out in the BMBL 6th edition. Each level builds upon the requirements from the previous.



Source: [2201\_EHS\_Biosafety\_Manual\_v5-final\_web\_comp\_3.pdf (stanford.edu)](https://ehs.stanford.edu/wp-content/uploads/2201_EHS_Biosafety_Manual_v5-final_web_comp_3.pdf)

## Select Agent and Toxins

Describe select agent and toxin use at your institution if applicable.

## Dual Use Research of Concern (DURC)

Describe DURC at your institution if applicable.

# Training

## New Employee Orientation

All new employees will be required to complete new employee orientation training, which includes:

List topics.

## Chemical Safety Training (if applicable)

## Radiation Safety Training (if applicable)

## Emergency Procedures

## Bloodborne Pathogen Training (if applicable)

## List of Annual Trainings

## Add additional training topics/courses here

# Waste & Decontamination

## Waste

### Biohazardous Solid Waste

Describe procedures for solid biohazardous waste disposal.

### Biohazardous Liquid Waste

Describe procedures for liquid biohazardous waste disposal.

### Sharps Waste

Describe procedures for sharps waste disposal.

### Chemical Waste (if applicable)

Describe procedures for chemical waste disposal.

### Radioactive Waste (if applicable)

Describe procedures for radioactive waste disposal.

### Mixed Waste (if applicable)

Describe procedures for mixed waste disposal.

### Animal Carcasses (if applicable)

Describe procedures for animal carcass disposal.

## General Decontamination

### Ethylene Oxide

### Autoclaves

### Other Specific Decontamination Solutions/Procedures (e.g., vaporized hydrogen peroxide)

### Bleach solutions

### Agent Specific Decontamination Procedures

# Lab Deactivation & Equipment Removal/Disposal/Surplus

## Agent Deactivation/Disposal

## Lab Close Out Process/Checklist

## Disposal of Used Laboratory Equipment

# Medical Surveillance

## Medical Surveillance

## Occupational Health Information/Clinic Location

## Laboratory Animal Occupational Health Program (if applicable)

## Field Work Considerations (if applicable)

## Vaccinations

# Recombinant DNA & Synthetic Nucleic Acid

Add information on institution specifics surrounding rDNA work. Add and delete sections as needed.

## NIH Guidelines

## Exempt rDNA/sNA

## Non-Exempt rDNA/sNA

## Viral Vectors and Transgenes

## Human Gene Transfer

## Transgenic Plants

## Genome Editing and Gene Drives

# Safety Committees and Oversight Panels

Add information on institutional safety committees/oversight panels.

# Toxins

Add information on toxins used at your institution and procedures that surround their use.

# Transportation

Add information on the shipment of biological materials, hand transport, personal car transport, permits, and packaging to be used.

## Transportation of Infectious Agents on Campus

## Shipping Infectious Agents Domestically

## Importing Infectious Agents

## Exporting Infectious Agents

## Special Cases

# References

The following publications were used as informational resources in the preparation of this document.

1. Biosafety in Microbiological and Biomedical Laboratories, 6th edition. June 2020. <https://www.cdc.gov/labs/BMBL.html>
2. Stanford University Biosafety Manual. 2018. <https://ehs.stanford.edu/wp-content/uploads/2201_EHS_Biosafety_Manual_v5-final_web_comp_3.pdf>
3. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. 2019. <https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf>
4. World Health Organization: Laboratory Biosafety Manual. 4th edition, 2020.
5. Public Health Agency of Canada: Material Safety Data Sheets <https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html>

# Appendix 1: Biohazardous Agent Inventory Form

This agent inventory form may be used to help specifically adapt the manual to each individual lab.

**Principal Investigator:**

**Lab Building/Room(s)***:*

**Biohazardous Agents Used (list):**

|  |  |
| --- | --- |
| ***Please include all microorganisms, human-derived materials, potential carcinogens, and toxins.*** | ***Room #*** |
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**Principal Investigator’s Certification**

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating practices.

Signature \_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# Appendix 2: Signature and Acknowledgement of Risk Form

This training documentation form may be used to help specifically adapt the manual to each individual lab.

We, the undersigned, understand that the listed agents we are working with are potentially hazardous. We have read and understand this manual and agree to follow the stated policies and procedures presented, along with those specifically developed by the PI for this laboratory.

| **Name** | **Signature** | **Date** |
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