Biosafety Manual

1.0 INTRODUCTION

The University of Notre Dame is committed to maintaining a safe working environment in all research and teaching laboratories where biological materials are used. As the foundation of that commitment, the University complies with all federal and state regulations and guidelines governing the use of biological materials in the laboratory. For specific information concerning these regulations and guidelines, see:

- National Institutes for Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules
- Biosafety in Microbiological and Biomedical Laboratories (BMBL)
- OSHA Bloodborne Pathogens Standard

2.0 PURPOSE

The purpose of this manual is to ensure a safe working environment for University biohazardous and recombinant DNA (rDNA) activities and for compliance with all applicable federal, state, local, and university regulations concerning the use of biological agents, biological toxins, select agents, and rDNA in the laboratory. The precautions and guidelines in this biosafety manual are compatible with current knowledge and regulations.

3.0 SCOPE

This Biosafety Manual is applicable to all personnel and affiliates associated with the University of Notre Dame who handle and/or may be exposed to biological hazards and/or recombinant materials in the course of research and teaching activities. Principal Investigators (PI) are responsible for the direct adoption of this manual in their teaching/research laboratories and incorporate the following elements:

- Approved IBC protocol.
- Safety protocols specific to the process or agent under consideration.
- All specialized practices essential for the particular process or agent.
- An emergency action plan covering spill response, needle sticks, and exposure of lab personnel.

All staff members and students are expected to read and adhere to all protocols and SOPs specified for their respective laboratories.

4.0 ACRONYMS AND DEFINITIONS

See Appendix A for common acronyms and definitions.

5.0 RESPONSIBILITIES

5.1 Institutional Biosafety Committee (IBC)

The IBC, whose members are appointed by the University President, is responsible for reviewing all research conducted at or sponsored by the University involving recombinant DNA (rDNA) subject to the National Institute of Health (NIH) Guidelines and biological research requiring biosafety containment. Their responsibilities include:

5.1.1 Independent risk assessment of research through IBC protocol review.
5.1.2 Verification of containment levels assigned by Principal Investigators (PIs).
5.1.3 Assessment of facilities, equipment, procedures, practices, training, and other elements of the research.
5.1.4 Reporting significant problems/violations to the NIH Office of Biotechnology Activities (NIH OBA).
5.1.5 Reporting guidelines and significant research-related incidents to NIH OBA.

5.2 Notre Dame Research (NDR)

NDR acts as the liaison between PIs, the IBC, granting agencies, and regulatory agencies. Their responsibilities include:

5.2.1 Serving as the Office of Record for IBC documentation.
5.2.2 Notifying PIs regarding the IBC's actions and results of their protocol review.
5.2.3 Keeping detailed records of meetings.
5.2.4 Providing necessary documentation for PIs to comply with University submission requirements.
5.2.5 Assisting PIs and researchers with export control and importing of biological agents and select agents.
5.2.6 Appointing IBC Chair and Institutional Biosafety Officer.
5.2.7 Filing an annual report with the NIH.
5.2.8 Administer the Occupational Health and Safety Program

5.3 Institutional Animal Care and Use Committee (IACUC)

The IACUC ensures the ethical and humane treatment of animals used in research, teaching, or testing in compliance with federal, state, and local regulations requirements. The IACUC holds several biosafety-related responsibilities, particularly when animal research involves biological materials or presents potential hazards. These responsibilities include:

5.3.1 Evaluating IACUC protocols that involve using biological materials to assess and mitigate potential risks to animals and personnel
5.3.2 Ensuring appropriate animal housing systems to minimize exposure to biohazardous agents.

5.3.3 Collaborating with PIs and IBC to develop and implement standard operating procedures and ensure alignment between animal care and research safety programs
5.3.4 Developing emergency plans for handling spills, personnel exposures, unintentional animal exposure, and equipment failure.
5.2.5 Assessing the potential biosafety risks associated with the genetic modifications in transgenic animals and their implications for animal welfare.

5.4 Biological Safety Officer (BSO) and Risk Management and Safety (RMS)
The BSO and RMS assist PIs in maintaining a safe working environment and provide industrial hygiene and safety support for laboratory operations. Their responsibilities include:

5.4.1 Serving on the University's IBC.
5.4.2 Reporting significant problems, violations, and incidents to the IBC.
5.4.3 Conducting laboratory inspections for compliance.
5.4.4 Assisting with hazard risk assessment for biological work activities.
5.4.5 Assisting in the transportation and disposal of biological or infectious waste.
5.4.6 Aiding in emergency response, cleanup, and decontamination of biological spills and accidents.
5.4.7 Determining shipping requirements for biological samples and assisting shipments of higher-risk samples.
5.4.8 Developing, reviewing, and updating the Biosafety Manual in accordance with regulatory requirements and institutional practices.
5.4.9 Coordinating with departments to ensure consistent implementation of safety policies and procedures across the University.

5.5 Principal Investigator (PI)
The PI is an individual who holds the ultimate responsibility for overseeing biological work conducted at the university and supervising the personnel engaged in such work. The PI's responsibilities include:

5.5.1 Complying with University policies, government regulations, and guidelines.
5.5.2 Ensuring lab personnel undergo online and hands-on required safety.
5.2.3 Maintain up-to-date logs of active personnel online and hands-on training.
5.5.4 Monitoring and approving the procurement, use, and disposal of biological agents.
5.5.5 Providing funding for vaccinations and testing.
5.5.6 Submitting rDNA registration and IBC protocols.
5.5.7 Implementing safety expectations and providing training.
5.5.8 Reporting problems, violations, and incidents to the BSO/IBC or Animal

Facility Director.

5.5.9 Developing IACUC and IBC protocols that adhere to biosafety guidelines, regulations, and institutional policies.

5.5.10 Amending approved IBC protocols to reflect current practices.

5.5.11 Providing and enforcing the use of appropriate Personal Protective Equipment (PPE).

5.6 Laboratory Personnel

Laboratory personnel responsibilities include:

5.6.1 Following established safety practices and procedures.

5.6.2 Completing required online and hands-on safety training.

5.6.3 Verifying equipment safety before use.

5.6.4 Reporting unsafe conditions to the PI.

5.6.5 Reporting spills, accidents, or injuries involving biological materials to the PI.

5.6.6 Informing the PI of health changes related to lab materials.

5.6.7 Reporting health status affecting susceptibility to infection to the University's healthcare provider.

5.6.8 Review IBC protocols on which they are approved personnel.

6.0 REGULATORY COMPLIANCE

6.1 Recombinant DNA (rDNA) Activities

6.1.1 The NIH defines recombinant and synthetic nucleic acids as molecules created by joining nucleic acid sequences that can replicate within a living cell (i.e., recombinant nucleic acids).

6.1.2 The NIH Guidelines for Research Involving Recombinant DNA Molecules oversee all rDNA activities and identify exempt activities.

6.1.3 DNA activities involving microorganisms and exempt rDNA microorganisms agents are not federally regulated.

6.1.4 The creation of transgenic rodents at levels above BSL-1 falls under the purview of the NIH recombinant guidelines.

6.1.5 The IBC and RMS have determined that the procedures and containment levels outlined in the CDC publication, “Biosafety in Microbiological and Biomedical Laboratories” (BMBL), shall govern such activities conducted at the University

6.2 Biological Agents and Toxins

6.2.1 Activities involving Biological Agents and Toxins are regulated by NIH and CDC guidelines, as well as OSHA (bloodborne pathogens), Department of Health & Human Services, and USDA regulations.

6.2.2 Best practices and guidelines for working with biological agents and toxins are provided by CDC's BMBL to ensure safe conduct of such work.

6.3 Tuberculosis

6.3.1 Research activities involving Mycobacterium tuberculosis must adhere to NIH or CDC guidelines. OSHA has published guidelines for activities that potentially expose individuals to tuberculosis.

6.4 Bloodborne Pathogen Standard (29 CFR 1910.1030)

6.4.1 The OSHA standard requires an Exposure Control Plan for exposure to blood or other potentially infectious materials (OPIM).
6.4.2 Notre Dame's Exposure Control Program outlines procedures for minimizing personnel exposure to human blood, blood products, or OPIM containing bloodborne pathogens.
6.4.3 Universal Precautions are implemented, treating all human/primate blood and other body fluids, tissues, and cells as if infectious for Bloodborne Pathogens (BBPs).
6.4.4 Lab activities must include precautions such as frequent hand washing, no mouth pipetting, no food or drink in the lab, proper disposal of biohazardous waste, and the use of Personal Protective Equipment (PPE).
6.4.5 Engineering controls, such as biosafety cabinets, ventilation systems, closed-top centrifuge rotors, etc., are the primary methods to control exposure

6.5 Hepatitis B Vaccine Program

6.5.1 The Hepatitis B vaccine is provided free of charge to all ND personnel deemed "at risk" due to occupational exposure.
6.5.2 While vaccination is encouraged by ND, it is not mandatory for employment.
6.5.3 Employees offered the vaccine must either accept it or sign the ND Declination Form.
6.5.4 ND personnel, including faculty, staff, post-doctoral fellows, graduate students, and undergraduates working for pay, can receive vaccination at the Wellness Center.
6.5.5 Non-paid students should visit the University Health Services (St. Liam's Hall) for vaccination.
6.5.6 For further details, consult the University Exposure Control Program.

7.0 PROTOCOL SUBMISSION AND REVIEW

7.1 Mandatory Submission Requirements

Prior to commencing any work involving the following, submission and approval of a completed Institutional Biosafety Committee (IBC) protocol form are mandatory:

7.1.1 Human and non-human primate blood, blood products, tissue, and related materials, or other potentially infectious materials (OPIMs).
7.1.2 Cell and organ cultures of human origin, encompassing established cell lines, human embryonic stem cells, and pluripotent stem cells and their derivatives.
7.1.3 Infectious agents rated BSL-2 or higher by the University's Biosafety Officer, including bacteria, viruses, prions, fungi, and protozoans.
7.1.4 Recombinant and/or synthetic nucleic acids, covering activities using viral vectors or plasmid vectors, creation of transgenic/knock-in/knock-out animals, maintaining a transgenic rodent colony at ABSL-2 containment or higher, purchasing or transfering transgenic rodents that require ABSL-2 containment or higher, and genetically modified plants (use and creation).
7.1.5 Biological toxins.
7.1.6 DHS Select Agents (Refer to the list of exclusions related to select agent activities).

7.2 Recombinant Materials Registration

Principal Investigators (PIs) are required to complete the recombinant DNA (rDNA) registration section of the IBC protocol as applicable. Protocols involving rDNA activities must adhere to the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules, as outlined in the latest edition and subsequent supplements published in the Federal Register. The IBC can approve non-exempt BSL-1, BSL-2, and exempt rDNA protocols not requiring federal agency review.

7.2.1 Areas to address when preparing rDNA protocols for submission include:
7.2.1.1 Synthetic and associated sequence(s).
7.2.1.2 rDNA insert.
7.2.1.3 Potential protein product.
7.2.1.4 Vector.
7.2.1.5 Carrier used for introducing rDNA into a host system for replication.
7.2.1.6 Types of Containment:

- **Biological Containment** - limiting infectivity of a vector or vehicle for specific hosts, and limiting dissemination and survivability in the environment.

- **Physical Containmen**t - specifically designed equipment and facilities to physically contain microbes, limiting access to the BSL-2 space.

7.2.2 Good Laboratory Practices design practices and procedures to physically contain microbes, mechanisms for inactivation, and disposal of microbes.
7.2.3 When applicable, standard operating procedures (SOPs) for lab activities involving biohazards and/or rDNA should be developed.

7.3 Review of IBC protocols and Registration Documents may involve:

7.3.1 Independent risk assessment associated with the research.
7.3.2 Verification of containment levels assigned by the PI.
7.3.3 Verification of training.
7.3.4 Assessment of facilities, equipment, procedures, practices, and all elements associated with the research.

Upon approval, the PI will receive a letter. Approved IBC Protocols are valid for 3 years from the approval date.

8.0 BIOLOGICAL RISK MANAGEMENT PROCESS

8.1 Biological Risk Assessment

The goal of the biological risk assessment is to address realistic, perceivable risks to protect personnel, the community, and the environment. The biological risk assessment process:

8.1.1 Is integral to the biological risk management process, focusing on biological agent and laboratory procedure hazards.
8.1.2 Implements a standardized approach for hazard identification.
8.1.3 Utilizes Agent Summary Statements in Section VIII of the BMBL, 6th Edition, designed to aid in risk assessments.
8.1.4 Targets agents linked to lab-acquired infections (LAIs) and those of heightened public concern.
8.1.5 Identifies known and suspected routes of transmission of LAIs, available infective dose, host range, and agent stability in the environment.
8.1.6 Is designed to be an ongoing process involving all personnel.

8.2 NIH Biological Agent Risk Groups

8.2.1 Classifies agents into four Risk Groups (RGs) based on pathogenicity and intervention availability (see Appendix A for definitions).
8.2.2 Section II of the NIH Guidelines (Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, April 2019 Edition)

specifies biosafety practices and containment principles.

8.3 Procedures for Conducting a Biological Risk Assessment

Step 1: Identify Biological Agent(s) Hazard(s)

8.3.1 Wild-Type Agents:

8.3.1.1 Characteristics include capability to infect, disease severity, preventative measures, effective treatments, routes of transmission, infectious dose, stability in the environment, host range, and indigenous or exotic status.

8.3.2 Genetically Modified Agents:

8.3.2.1 Considerations parallel those for wild-type agents.
8.8.2.2 Evaluate how genetic modifications affect pathogenicity, virulence, and susceptibility to treatments.

8.3.3 Cell Cultures:

8.3.3.1 Characteristics align with wild-type agents for known pathogens.
8.3.3.2 Assess potential for unanticipated pathogens, viral latency, and origin of cells, tissues, etc.

8.3.4 Other Hazardous Biological Substances:

8.3.4.1 Includes materials, apart from regulated waste, that may contain pathogenic biologicals
8.3.4.2 Environmental samples, such as water, soil, blood and bodily fluids, animals, and animal materials.

8.3.5 Viral Vectors (Including lentiviral vectors):

8.3.5.1 Pose potential safety concerns due to their ability to cause unintended infections, mutagenesis, and the risk of accidental exposure during handling.

Step 2: Identify Laboratory Procedure Hazards

8.3.6 The Principle laboratory procedure hazards are the following:
8.3.6.1 Agent Concentration
8.3.6.2 Suspension Volume
8.3.6.3 Use of sharps.
8.3.6.4 Facility control hazards

8.3.6.5 Exposure to zoonotic agents
8.3.6.6 Working with animals. Including Bites and Scratches.
8.3.6.7 Procedures/equipment generating aerosols consisting of, but not limited to:

- Pipetting
- Centrifugation
- Vortexing
- Sonicating
- Grinding
- Mixing
- Blending

Step 3: Determine Appropriate Biosafety Level

8.3.7 Select additional precautions based on the risk assessment.

Step 4: Review with University's Biosafety Officer & RMS

8.3.8 Before implementing controls, review the risk assessment and selected safeguards.

Step 5: Evaluate Proficiencies of Staff

8.3.9 Assess staff training, experience, proficiency in microbiological practices andequipment use, consistency with SOPs, ability to respond to adverse conditions, willingness to accept responsibility, history of bypassing safety controls, and attentiveness.

Step 6: Revisit and Verify

8.3.10 Regularly revisit the risk management process to verify strategies and determine if changes are necessary.

9.0 MEDICAL SURVEILLANCE AND VACCINATIONS

9.1 Medical surveillance

Medical surveillance may be necessary for employees handling biohazardous agents and animal caretakers responsible for animals exposed to disease-causing agents. Collaboratively, the Wellness Center and the Animal Facility Director will identify animal handlers potentially at risk of occupational exposure to infectious microorganisms during their tasks.

9.2 Medical Surveillance Questionnaire
Personnel with access to risk group 3 biological agents or animals inoculated with such agents are required to complete a medical surveillance questionnaire. These questionnaires must be renewed every three years or when there is change in health status (e.g., pregnancy, significant weight loss/gain), environmental risk conditions, or species used.

9.3 Vaccinations
Vaccinations are accessible for many laboratory-related disease-causing agents. Recommendations for vaccination usage will be determined on a case-by-case basis by the Wellness Center medical staff, in consultation with the Institutional Biosafety Committee (IBC) and Biosafety Officer (BSO). Please consult the Occupational Health Category A/B Process for further guidance."

10.0 PERSONAL PROTECTIVE EQUIPMENT (PPE)
The primary aim of personal protective equipment (PPE) is to safeguard employees from the risk of injury or fatality by creating a barrier against workplace hazards. While personal protective equipment is vital, it should not replace effective engineering or administrative controls or sound work practices. Instead, it should complement these controls to ensure the safety and well-being of lab personnel.

10.1 The PPE Hazard Assessment Tool
The PPE Hazard assessment Tool must be completed for laboratory work to ascertain any PPE necessities. Once completed, a copy of the PPE Hazard Assessment Tool should remain in the laboratory for personnel to review.

10.2 The Lab Personnel PPE Knowledge Certification
All laboratory personnel must sign a Lab Personnel PPE Knowledge Certification form. This form signifies that individuals comprehend and acknowledge the University's minimum Personal Protective Equipment (PPE) requirements.

10.3 The following PPE is mandatory while working with BSL-1/BSL-2 materials

10.3.1 Eye Protection: Safety glasses, goggles, or face shields meeting ANSI Z87.1 2015 standards. The ANSI designation should be imprinted on the equipment by the manufacturer.
10.3.2 Hand Protection: Gloves compatible with the biohazardous materials and/or chemicals being used.
10.3.3 Body Protection:
- Long pants or skirts (ankle-length)
- Lab coats (made of cotton or disposable)

10.3.4 Foot Protection: Closed-toe, closed-heel shoes, and booties as required.
10.3.5 Additional PPE may be necessary based on the PPE Hazard Assessment.
10.3.6 Gloves, lab coats, and any other PPE are not to be worn outside of the

lab in common areas, kitchenettes, personal offices, and group spaces. Additionally, lab coats are not to be worn in the hallways unless moving on non-carpeted flooring through hallways where food is neither stored nor consumed. If labs are spread across buildings such that travel requires moving on carpet or past food spaces, one lab coat per space per person should be provided. In instances where labs are BSL-2 rated, lab coats are never to be worn outside of lab

10.4 The following PPE is mandatory while working in a BSL-3 Laboratory

10.4.1 Eye Protection: Safety glasses, goggles, or face shields meeting ANSI Z87.1 1989 standards. The ANSI designation will be imprinted on the equipment by the manufacturer. Respirators with eye coverage are deemed as an adequate alternative.
10.4.2 Hand Protection: Gloves compatible with bio-hazardous chemicals. These gloves should be taped around the wrists for a complete seal, with double gloves as necessary.
10.4.3 Body Protection: Tyvek suit with attached booties or Tyvek suit with loose booties taped at the ankles.
10.4.4 Foot Protection: Closed-toe, closed-heel shoes, and booties attached to a Tyvek suit or loose booties taped to a Tyvek suit at the ankles.
10.4.5 Additional PPE may be required based on the PPE Hazard Assessment

10.5 Respirator Usage

When aerosols and sprays are likely to be generated and chemical substitution and effective engineering controls are not feasible, respirators may be necessary to protect against hazardous airborne particulates, aerosols, and sprays.

10.5.1 Compliance with the OSHA Respiratory Protection Standard at 29 CFR 1910.134 is mandatory for all personnel who are required or volunteer to wear a respirator. Please refer to the University's Respiratory Protection Plan.
10.5.2 Risk Management Services (RMS) shall be contacted before purchasing or using respiratory protection.
10.5.3 Users of respirators, including N95 masks, mandated for their research, must undergo fit testing for their masks prior to utilization.
10.5.4 When respirators are used voluntarily, the respirator user shall complete the "Information To Employees Who Wear Respirators For Voluntary Use" form in the Onbase system.
10.5.4 Dust masks may not necessitate participation in the University's Respiratory Protection Plan. However, when selecting a mask for the first time, contact RMS to confirm if participation is necessary.

11.0 TRAINING

11.1 Laboratory Safety Training
New laboratory personnel are required to undergo RMS's Laboratory Safety training, and, where applicable, Bloodborne Pathogen training. Additionally, all individuals working in or around biohazards must receive annual refresher training, which is accessible online.

11.2 Online Biosafety Training
All members of the Institutional Biosafety Committee (IBC), Principal Investigators (PIs), and laboratory personnel involved in activities with microorganisms or biotoxins are required to undergo biosafety training. This training must be completed initially and annually thereafter, regardless of the proposed activity level (BSL-1, BSL-2, or BSL-3). Training is conducted via eNDevour and fulfills the biosafety training criteria established by the University of Notre Dame.

11.3 Blood Borne Pathogens Training
Personnel who may reasonably come into contact with human/non-human primate blood, non-fixed tissue, or other potentially infectious materials (OPIM) as part of their job or research duties must undergo Annual Blood Borne Pathogens training.

11.4 BSL-3 Training
For BSL-3 training, individuals can access training sessions provided by the Lab Manager of the BSL-3 laboratory.

11.5 Hands On Training
The Principal Investigator (PI) is accountable for developing and executing training sessions on laboratory-specific biosafety Standard Operating Procedures (SOPs) for their team members and students. Hands-on training sessions should be documented with the trainee's signatures and dates, as well as those of the trainer. The training covers a range of topics, including:

- 11.4.1 Procedures and techniques
- 11.4.2 Laboratory safety protocols
- 11.4.3 Emergency action plans
- 11.4.4 Spill containment and disinfection/cleanup procedures
- 11.4.5 Guidelines for safely operating laboratory equipment such as chemical fume hoods, biosafety cabinets, microtomes, autoclaves, and centrifuges.
- 11.4.6 Refer to Appendix B for centrifuge safety and the Autoclave Safe Use and Validation Procedure for autoclave safety.

11.6 Training Needs Assessment Form
The Principal Investigator (PI) is tasked with filling out the University's Online Training Needs Assessment form, detailing the necessary eNDevour training for laboratory

personnel according to inherent risks. A copy of the Training Needs Assessment Forms should be accessible for review.

11.7 Training Assignments

The PI is responsible for assigning all required training to lab personnel outlined in the training needs assessment.

12.0 CONTAINMENT LEVELS

12.1 Biosafety Containment Types

12.1.1 **Primary (p):** Methods to safeguard the internal laboratory environment, including microbiological techniques and appropriate safety equipment.
12.1.2 **Secondary (s):** Methods to protect the external environment, including facility design and operational practices.
12.1.3 The IBC will assess the need for additional containment during protocol reviews.

12.2 Biosafety Level 1 (BSL-1) Containment Facility Requirements are as follows

12.2.1 Laboratory designed for easy cleaning.
12.2.2 Laboratory doors are to remain closed when work is in progress.
12.2.3 Non-porous work surfaces resistant to various chemicals.
12.2.3 Absence of carpets and rugs.
12.2.4 Accessible spaces for cleaning between benches, cabinets, and equipment.
12.2.5 Chairs and furniture made of non porous, easily cleanable materials.
12.2.6 Presence of sinks and eye wash stations within the lab.
12.2.7 Secure containment area with biohazard warning signage.
12.2.8 Adequate illumination without glare.
12.2.9 Lab windows fitted with screens.
12.2.10 Laboratory furniture capable of supporting anticipated loads.
12.2.11 Implementation of primary containment practices, including:

- Personal Protective Equipment (PPE) such as coats, gloves, and face/eye protection.
- Hair restraint to prevent contact with hazardous materials.
- Open bench-top work.
- Sharps program.
- Integrated Pest Management.

12.3 Biosafety Level 2 (BSL-2) Containment Facility Requirements are as follows

12.3.1 All BSL-1 containment facility requirements

12.3.2 Self-closing doors with locks as per institutional policies.
12.3.3 Biohazard warning signage supplied by RMS upon BSL-2 approval (refer to Section 13).
12.3.4 Properly maintained vacuum lines with liquid disinfectant traps and HEPA filters.
12.3.5 Installation and operation of biosafety cabinets (BSCs) to ensure effectiveness.
12.3.6 BSCs positioned away from airflow disruptions.
12.3.7 BSCs certified annually for correct performance.
12.3.8 Use of biological safety cabinets for aerosol-generating manipulations.
12.3.9 SOPs for handling agents.
12.3.10 Limited access to the lab.
12.3.11 Use of appropriate PPE and administrative controls for non-BSL-2 manipulations.
12.3.12 Availability of autoclave.
12.3.13 BSL-2 materials requiring open lab centrifugation, in large volumes or high concentrations, must use sealed rotors or safety cups. The loading and unloading of these rotors and cups should be conducted within a BSC or another suitable containment device.
12.3.14 Prohibition of unrelated animals and plants.
12.3.15 Routine decontamination of lab equipment.
12.3.16 Reporting incidents resulting in exposure to biological agents for immediate evaluation.

12.4 Biosafety Level 3 (BSL-3) Containment

12.4.1 All BSL1 & BSL2 containment facility requirements
12.4.2 Double-door entry with negative pressure anteroom.
12.4.3 Air movement from cleaner to contaminated areas.
12.4.4 Single pass air circulation without recirculation.
12.4.5 Aerosol-generating work conducted in biological safety cabinets.
12.4.6 Sealed penetrations in walls, ceilings, and floors.
12.4.7 Waterproof ceiling for easy cleaning.
12.4.8 Hands-on PPE donning and doffing training.

13.0 CONTAINMENT SIGNAGE AND MATERIAL LABELING

13.1 Signage

Biosafety signage is managed by RMS and requests for new or updated signs can be made by emailing [labsafety@nd.edu].

13.2 BSL-1 Signage Requirements

13.2.1 A biohazard warning sign (refer to Appendix E) featuring the universal

biohazard symbol and biosafety level must be displayed on the access door to the laboratory work area.

13.3 BSL-2 Signage Requirements

13.3.1 A biohazard warning sign (refer to Appendix E) must be posted on the access door to the laboratory work area, including the following information:

- Universal biohazard symbol and lab's biosafety level.
- Name and contact information of PI, supervisor, and lab manager.
- PPE requirements.
- General occupational health requirements.
- Immunization requirements, if any.
- Respiratory protection requirements.
- Medical surveillance requirements.
- Required laboratory entry and exit procedures.
- Information on biological agents.

13.4 BSL-3 Signage Requirements

13.4.1 A biohazard warning sign (refer to Appendix E) must be displayed on the access door to the laboratory work area, containing the following information:

- Universal biohazard symbol and lab's biosafety level.
- Name and contact information of PI, supervisor, and lab manager.
- PPE requirements.
- General occupational health requirements.
- Immunization requirements, if any.
- Respiratory protection requirements.
- Medical surveillance requirements.
- Required laboratory entry and exit procedures.
- Information on biological agents.

13.5 Material Labeling Requirements

13.5.1 All human tissue, body fluid, or potentially infectious materials must be stored in compliant containers (refer to Sections 19 and 21) and labeled with a biohazard symbol (refer to Appendix F).

13.5.2 Equipment such as refrigerators, freezers, and incubators used for storing or handling potentially infectious materials must be labeled with the biohazard symbol (refer to Appendix F).

13.5.3 Refrigerators, freezers, microwaves, blenders, and other common household kitchen appliances must also be labeled with a biohazard symbol and a "No food or drink" label.

13.5.4 Any food products utilized for research activities must be labeled "Not

for human consumption".

13.5.5 Any instruments or devices that are used, or may come into contact with, biological materials must be clearly marked with the biohazard symbol (see appendix F).

14.0 BIOSAFETY CABINETS

14.1 Types and Selection

Details regarding Biosafety Cabinet Types and Selection based on Risk Assessment are provided in Appendix C.

14.2 Biological safety cabinets (BSCs) offer threefold protection:

14.2.1 Safeguarding lab personnel from materials within the cabinet.
14.2.2 Shielding materials from contaminants.
14.2.3 Shielding the environment from materials within the cabinet.

14.3 Types of Biosafety Cabinets

There are three types of Biosafety Cabinets:

14.4.1 **Class I:** Provides protection for personnel and the environment only. Materials within the cabinet are not safeguarded and may be contaminated. The use of Class I BSCs is discouraged at the University of Notre Dame. Contact the BSO for inquiries.
14.4.2 **Class II:** Offers protection for personnel, products, and the environment.
14.4.3 **Class III:** Gas-tight and designed for handling high-risk (BSL-4) agents.

14.4 Biosafety Cabinet Usage and Maintenance

14.3.1 Biosafety cabinets are typically not designed for handling hazardous chemicals. While most BSCs filter out contaminated air using high-efficiency particulate air (HEPA) filters, it's important to note that HEPA filters are not effective in capturing chemical fumes.
14.3.2 A Chemical Fume Hood is not a suitable alternative to a BSC. The ventilation system of a fume hood is not designed to handle biological materials, and it may not have the necessary features, such as HEPA filters, to provide adequate protection.
14.3.3 The use of alcohol burners in cabinets can be hazardous and may void the manufacturer’s warranty. Safer alternatives such as micro-incinerators or disposable tissue culture supplies are recommended.
14.3.4 Cabinets must be installed by certified professionals. Notre Dame has a partnership with a certified company for installation, certification,

decontamination, and related services. Contact the BSO for support.

14.3.5 Certifications should be conducted annually or after cabinet relocation.

14.3.6 Installation or certification arrangements are managed through Maintenance. Costs associated with these services are the responsibility of the PI or Department.

15.0 SHARPS

15.1 Needle Recapping

The university strongly discourages the recapping of needles. If absolutely necessary to recap, use a device that aids in sharps recapping. These devices should adhere to the benchtop to prevent movement and hold the cap of the needle, allowing the user to uncap and recap with one hand. Contact RMS if assistance is needed in selecting or procuring a recapping device.

15.2 Sharps Waste

15.2.1 Sharps containers requirements include:

- Constructed of rigid, hard plastic,
- Labeled with the universal biohazard symbol,
- Not overfilled.

15.2.2 Mixed chemical and biohazardous sharps waste shall be placed into a sharps container that is labeled as Biohazardous sharps waste. Any mixed chemical and biohazardous waste shall be properly identified and labeled with a Biohazardous Waste Discard Tag.

15.2.3 Once the sharps container reaches the full line, ensure that the lid is securely closed and the locking tabs are engaged. Before disposal, label a Biohazardous Discard form container with the PI Name and Lab #. Decontaminate the exterior of the sharps container before RMS pickup.

15.2.4 RMS shall pick up all full sharps containers to ensure proper disposal.

16.0 EXPOSURES

For Major Incident, Injury, or Illness, Call 911 from a landline or NDPD from a cell phone: 574-631-5555

16.1 Exposure Procedure

In the event of exposure to a biohazard, rDNA, or needle stick incident, follow these

steps which are also outlined on the Incident, Injury or Illness Procedures flowchart posted in lab spaces (Appendix G) :

Step 1: Wash or Rinse exposed Skin or Eyes for 15 minutes

Step 2: Inform Supervisor (or designee) & Seek Medical Attention if Needed:

Undergraduate students collecting COLLEGE CREDITS
Go to University Health Services (St. Liam Hall) For after hours consultation call 574-631-7497.

Anyone working in a PAID capacity
Go to Notre Dame Wellness Center. For After hours consultation call 574-634-9355

UNPAID adults and minor researchers
Go to facility designated under individual's health insurance

Step 3: In all cases, supervisors or designee must complete an INCIDENT REPORT

17.0 EMERGENCY RESPONSE

17.1 Large Spills

If the spill is considered too large or too dangerous for laboratory personnel to safely clean up:

17.1.1 Secure the entire laboratory and
17.1.2 Call Notre Dame Police (NDPD) immediately for assistance. 911 from a campus phone or 574-631-5555 from a cell phone.

17.2 Bleach is the recommended disinfectant.

However, other disinfectants may be used provided they are effective against the particular agents, are the appropriate dilution, and sufficient contact time is utilized.

17.3 Spills Inside the Biosafety Cabinet

17.3.1 Wait at least five minutes to allow the BSC to contain aerosols.
17.3.2 Wear a laboratory coat, safety glasses and nitrile gloves during cleanup. (Latex gloves shall not be used when working with ethidium bromide).
17.3.3 Allow BSC to run during cleanup.
17.3.4 Apply disinfectant and allow a minimum of 20 minutes contact time.
17.3.5 Wipe up spillage with disposable disinfectant-soaked paper towels. Do

not place your head in the cabinet to clean the spill; keep your face behind the view screen.

17.3.6 Wipe the walls, work surfaces and any equipment in the cabinet with disinfectant-soaked paper towels.

17.3.7 Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures.

17.3.8 Place contaminated reusable items in biohazard bags or autoclavable pans with lids before autoclaving.

17.3.9 Expose non-autoclavable materials to disinfectant (20 minutes contact time) before removal from the BSC.

17.3.10 Remove protective clothing used during cleanup and place in a biohazard bag for removal.

17.3.11 Run BSC 10 minutes after cleanup (prior to resuming work or turning BSC off).

17.3.12 If the spill overflows the drain pan / catch basin under the work surface into the interior of the BSC, notify RMS. A more extensive decontamination of the BSC may be required.

17.4 For a spill inside the laboratory, but outside of the biosafety cabinet:

17.4.1 Evacuate Room - ensure all personnel are accounted for and doors are closed / locked. Post a notice on the door informing personnel of spill and not to enter; e.g., "Biohazardous Materials Spill! DO NOT ENTER!". Allow the spill's potential aerosols to settle for 30 minutes.

17.4.2 Assemble clean-up materials (disinfectant, paper towels, biohazard bags and forceps).

17.4.3 Don appropriate PPE, including lab coat, shoe covers, gloves and eye/face protection.

17.4.4 A respirator may be needed if aerosols are present. If you feel you need to use a respirator, STOP clean-up and consult RMS. If a respirator is not needed, continue to initiate clean-up.

17.4.5 Initiate cleanup with disinfectant as follows:

17.4.6 Place paper towels or other absorbent material over the spill area.

17.4.7 Carefully pour disinfectant around the edges of the spill and then onto the paper towels. Avoid splashing or generating aerosols.

17.4.8 Allow the disinfectant to remain in contact with the spill for at least 20 minutes.

17.4.9 Apply more paper towels to wipe up the spill.

17.4.10 Clean spill area with fresh towels soaked in disinfectant.

17.4.11 Dispose of all towels or absorbent materials using appropriate biohazardous waste disposal procedures. If any sharp objects are present, use forceps and discard in a sharps container.

17.4.12 Remove protective clothing and segregate for disposal or cleaning.

17.4.13 Wash hands with soap prior to leaving the area.

17.5 For a spill inside a centrifuge:

17.5.1 Clear area of all personnel.
17.5.2 Unplug the centrifuge.
17.5.3 Wait 30 minutes for aerosol to settle before attempting to clean up the spill.
17.5.4 If a spill is identified after the centrifuge lid is opened, carefully close the lid, evacuate the laboratory and close the laboratory door. Remain out of the laboratory for at least 30 minutes. Put notice on the door informing personnel of the spill and not to enter.
17.5.5 Wear a laboratory coat, safety glasses and gloves during cleanup. If there is splash potential, a face shield shall be worn.
17.5.6 A respirator may be needed if aerosols are present.
17.5.7 If you feel you need to use a respirator, STOP clean-up activities and consult RMS for appropriate response.
17.5.8 If a respirator is deemed not necessary, initiate clean up.
17.5.9 Remove rotors and buckets to the nearest BSC for cleanup.
17.5.10 Thoroughly disinfect the inside of the centrifuge.
17.5.11 Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures.

17.6 For a spill outside the laboratory:

17.6.1 To prevent a spill, transport labeled biohazardous material in an unbreakable, well-sealed primary container placed inside of a second unbreakable, lidded container (cooler, plastic pan or pail) labeled with the biohazard symbol.
17.6.2 Do not attempt to clean it up without appropriate PPE.
17.6.3 Secure the area, keeping all people well clear of the spill.
17.6.4 Call NDPD (Campus line: 911 or 574-631-5555) for assistance.
17.6.5 Standby during the spill response and cleanup activities to provide assistance as requested or as necessary.

18.0 STORING AND TRANSPORTING BIOHAZARDS ACROSS CAMPUS

18.1 Containment

Specimens of blood or other potentially infectious materials shall be placed in a primary container that prevents leakage (capped test tube, centrifuge tube, etc.) during collection, handling, and storage.

18.2 Transportation

If the specimens are transported through hallways or between buildings, the primary containers shall be placed in a closed secondary container (jar, cooler, sturdy box, etc.)

with absorbents which would contain the contents if the primary container were to leak or break during transit.

19.0 SHIPPING OF SAMPLES

19.1 Regulations

Specimens of blood or other potentially infectious materials shipping to or from the University of Notre Dame shall be shipped per DOT or IATA regulations.

19.2 Training

Personnel involved with shipping of biohazardous agents or potential BBPs shall have documented training prior to shipping.

19.1 Training for Biological Substance Category B Shipping and Dry Ice Shipping is available through Endeavour.

19.2 If your sample does not meet the criteria for Category B, or contain dangerous goods (e.g. specimens shipped in ethanol or formalin), contact RMS.

20.0 WASTE

20.1 Animal Carcass Waste

After proper euthanasia of laboratory animals (IACUC approved method), contaminated animal carcasses shall be placed in red bags and placed in an appropriate freezer until removal by RMS.

20.2 Infectious Waste That Can Be Autoclaved

20.2.1 Waste must be kept in an autoclavable bag containing the Universal Biohazard Symbol on the outside surface.

20.2.2 A closeable container lined with an autoclavable bag is required for the storage of autoclave waste prior to being autoclaved. A hands-free step activated red can is preferred.

20.2.3 During active operations, waste generated on the bench may be stored in an open container. However, it must be closed or emptied into a closable waste container when operations cease.

20.2.4 Contents of biohazardous waste shall not protrude or be overflowing from the waste container.

20.2.5 Biohazardous bags being prepared for autoclaving must be loosely tied and kept in secondary containment capable of containing the entire bag prior to being autoclaved. Bags may not be stored on the floor, bench, or any other location without secondary containment.

20.2.6 All infectious waste shall be stored in the location it was generated until

it can be autoclaved.

20.2.7 The top of the bag shall be secured with indicator tape or the bag shall have color indicator markings changing the color after sterilization has been attained.

20.2.8 Ensure the bag used for autoclaving can withstand the autoclave cycle without melting.

20.2.9 Once autoclaved, the sterilized waste shall be double bagged in a dark colored bag, sealed and labeled “Safe for Trash Disposal”.

20.2.10 See Autoclave Safe Use and Validation Procedure for guidelines on safe autoclave use.

20.2.11 Autoclaves shall be validated using a bio-indicator on a monthly basis. See Autoclave Safe Use and Validation Procedure for more information.

20.3 Chemically Treated Waste

20.3.1 Liquid biohazards can be rendered non-hazardous by treating with bleach or another appropriate disinfectant.

20.3.2 Contaminated pipettes/beakers can be treated with bleach, rinsed and then reused or disposed as nonhazardous waste.

20.3.4 All infectious waste shall be stored in a secure location until it can be disinfected by chemical treatment.

20.3.5 Waste treated with bleach should not be autoclaved.

20.4 Non-Autoclavable Waste

Includes biohazardous, medical, and infectious waste not able to be rendered non-hazardous through autoclaving or chemical treatment or contamination. Contact RMS for proper disposal assistance

21.0 WASTE LABELING REQUIREMENTS

21.1 Infectious Waste

All infectious waste shall be properly labeled with a biohazard symbol and the words “Biohazardous Waste”.

21.2 Biohazardous, Medical, and Animal Carcass Waste

All biohazardous waste shall be properly labeled with a biohazard symbol and the words “Biohazardous Waste”

21.3 Sharps Waste

Unless utilizing manufacturer-provided labeling, all Sharps containers must display a biohazard symbol along with the phrase "Biohazardous Sharps Waste." See Section 16 for more information.

22.0 RECORD KEEPING

22.1 IBC Protocols and Registration Documents

22.1.1 Protocols and Registration Documents are valid for 3 years from the date of approval.
22.1.2 Renewal protocols/registration documents shall be submitted no less than 30 days prior to expiration dates to the IBC.
22.1.3 Original or copies of approved protocols shall be included in the Lab Specific Biosafety Manual. The Lab Specific Biosafety Manual and protocols shall be kept in the laboratory.
22.1.4 IBC protocols and registration documents shall be reviewed annually by PI to ensure scope of work (including named personnel). If changes are identified, an amendment request shall be sent to the IBC.

22.2 Laboratory SOPs

22.2.1 SOPs shall be maintained for 3 years past the last time the procedure was conducted.
22.2.2 SOPs shall be reviewed at least biennially.

22.3 Training Records

22.3.1 PI shall maintain all lab specific safety training records for 5 years.
22.3.2 The records can be either electronic (digital) or hard copy (paper) format.
22.3.3 Lab specific training records shall include:

- Name and signature of trainee(s) and trainer
- Date training occurred
- Description of training or copy of SOP

23.0 REFERENCES

1. National Institute of Health publication, Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), January 2005 or as periodically updated
2. Centers for Disease Control (CDC) publication, Biosafety in Microbiological and Biomedical Laboratories, 6th Edition
3. Centers for Disease Control (CDC) publication, Biosafety in Microbiological and Biomedical Laboratories, 6th Edition
4. NIH publication, Laboratory Safety Monograph, A Supplement to NIH Guidelines for Recombinant DNA Research, January 1979
5. Laboratory Safety: Principles and Practices, 2nd Edition, ASM Press, Washington DC, 1995,
6. Risk Management and Safety, Chemical Hygiene Plan, February 2021
7. Occupational Safety and Health Act (OSHA), Part 1910, Subpart Z, Section1910.1030
8. Bloodborne Pathogens, December 1991
9. National Research Council, Biosafety in the Laboratory, National Academic Press, Washington DC (1989).September 1996 B-1
10. World Health Organization (WHO) publication, Laboratory Biosafety Manual, 4th Edition

24. REVISION TABLE

History	Effective Date
Confirmed and updated links as necessary	January 31, 2018
Removed Appendix for SOP template and linked to webpage.	January 31, 2018
Moved definitions within the document rather than appendix.	January 31, 2018
Added references to Autoclave Validation Procedure	February 2018
Updated formatting, typos, definitions of Vector, Host and rDNA insert.	April 2018
Removed section on tuberculosis.	April 2018
Added Appendix D – Biohazardous waste –storage and labeling for non-treated waste offered to RMS for disposal	April 2018
Updated formatting to latest document control format, typos, broken links, and grammar issues. Moved definitions section from procedure body to Appendix, replaced the image versions of appendices with editable text versions.	October 2019
Updated IBC protocols and Autoclave Safe Use and Validation Procedure links	March 2020
-Integrated the CDC's BMBL 6th Edition changes into the procedure. -Expanded Select Agent information and requirements.	February 2022

Major Restructuring and formatting changes. Minor additions and omissions in various sections to promote cross compatibility with other existing university programs.	May 2024

APPENDIX A – ACRONYMS AND DEFINITIONS

- **Access** – The freedom or ability to obtain or make use of or the ability to carry, use or manipulate select agents.
- **Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)** – A voluntary accrediting organization that enhances the quality of research, teaching, and testing by promoting humane, responsible animal care and use. It provides advice and independent assessments to participating institutions and accredits those that meet or exceed applicable standards.
- **Antiseptics** – Chemicals that destroy microorganisms on living tissue.
- **Biosafety Plan:** A written biosafety plan that is commensurate with the risk of the select agent or toxin, given its intended use. The plan must contain sufficient information and documentation to describe the biosafety and containment procedures for the select agent or toxin, including any animals (including arthropods) or plants intentionally or accidentally exposed to or infected with a select agent.
- **Blood-Borne Pathogens** – Pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).
- **Blood** – Refers to human-related blood, blood components, and blood products.
- **Baseline Serum** – A blood sample drawn from a human for archiving for future reference by a physician.
- **Biosafety in Microbiological and Biomedical Laboratories (BMBL)** – CDC publication outlining biosafety practices, biocontainment requirements, biosecurity measures, etc. to ensure the protection of the lab personnel, staff, students, and local community exposure to lab biological agents.
- **Biosafety Level 1** – Biosafety containment level where work involves well-characterized agents, which are not known to cause disease in immunocompetent adult humans, and which present minimal potential hazard to laboratory personnel and the environment.
- **Biosafety Level 2** – Biosafety containment level that builds upon BSL-1 and is suitable for work involving agents that pose moderate hazards to personnel and the environment.
- **Biosafety Level 3** – Biosafety containment level which is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.
- **Biological Substance Category B** – An infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs.
- **Class I Biosafety Cabinet** – An enclosure with an inward airflow through the front opening. Provides protection for the worker and the laboratory environment but not to product being utilized in the cabinet.

- **Class II Biosafety Cabinet** – An enclosure with an inward airflow through the front opening. Provides protection to the worker, the environment, and the product being utilized in the cabinet.
- **Containment** – Used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled and maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.
- **Disinfectants** – Chemicals that destroy microorganisms on inanimate objects.
- **Host** – Organism, such as the bacterium E.coli, in which the rDNA replicates.
- **Infectious Waste** – Any waste materials capable of producing a disease by an organism likely to be pathogenic to humans. Examples include the following: (1) Contaminated sharps or contaminated objects that could potentially become contaminated sharps; (2) Infectious biological cultures, infectious associated biologicals, and infectious agent stock; (3) Pathological waste; (4) Blood and blood products in liquid and semiliquid form; (5) Laboratory animal carcasses, body parts, blood and body fluids in liquid and semiliquid form; (6) Bedding of laboratory animals; and (6) Other waste that has been intermingled with infectious waste.
- **Laboratory Acquired Infection (LAIs)** – Infections acquired from an exposure to a biological agent within the laboratory.
- **Negative Airflow** – Directional airflow from areas exterior to a laboratory into the laboratory. Primary (p) Containment - methods to protect the internal laboratory environment.
- **On-going Suitability Assessment Program** – After the initial pre-access suitability assessment, a standardized procedure to determine if an individual displays behaviors that would increase the risk of a theft, loss, or release of a select agent or toxin.
- **Other Potentially Infectious Materials (OPIM)** – (1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and (3) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.
- **Personnel Suitability** – Evaluation of personnel with access to select agents or toxins to determine if the personnel display behaviors that would increase the risk of a theft, loss, or release of a select agent or toxin.
- **Pre-Access Suitability Assessment Program** – The evaluation of an individual using a standardized procedure to determine if the individual displays behaviors that would increase the risk of a theft, loss, or release of a select agent or toxin.
- **Recombinant DNA (rDNA)** – DNA prepared by breaking up and splicing together DNA from several different species of organisms.

- **rDNA Insert** – The foreign DNA being inserted into vector DNA so that the rRNA can replicate in a host.
- **Repository Materials** – Agents for which select agent inventory records will be maintained. These include: non-excluded select agent toxins, virulent select agents, nucleic acids that can produce infectious forms of select agent viruses, nucleic acids that encode for functional forms of select agent toxins (if they can be expressed in vivo or in vitro or are in a vector or recombinant host genome), and animal tissue containing virulent select agents.
- **Responsible Official (RO)** – The individual designated by the University of Notre Dame with the authority and responsibility to act on behalf of the University to ensure compliance with Section 9 of the select agent regulations.
- **Risk Groups** – National Institutes of Health (NIH) classification system for biological agents. Uses biological agent hazards characteristics and routes of transmission (focused on ability to cause disease in healthy human adults and spread in the community) to assign a value between 1 to 4.
- **Risk Group 1 Agents** – Biological agents not known to cause disease in healthy adults.
- **Risk Group 2 Agents** – Biological agents associated with human disease, infectious through auto- inoculation, ingestion, mucous membrane exposure, which is rarely serious and for which preventative or therapeutic interventions are often available.
- **Risk Group 3 Agents** – Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences for which preventative or therapeutic interventions may not be available.
- **Risk Group 4 Agents** – Indigenous or exotic agents likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available.
- **Sanitizers** – Chemicals that reduce the number of microbes to a safe level.
- **Secondary Containment** – Methods to protect the environment external to the laboratory.
- **Security Risk Assessment (SRA)** – An FBI procedure for obtaining approval under Section 73.10 for access to a select agent or toxin. An approved FBI security risk assessment is required for the Responsible Official, Alternate Responsible Officials, select agent authorized users, other select agent personnel and any other person who, in the normal scope of his or her duties, would have (or be permitted) unescorted access to a select agent or toxin. When FBI approval is granted individuals are considered, "SRA-approved." Access approval is valid for a maximum of 3 years.
- **Select Agent** – CDC and USDA defines as biological agents or toxins deemed a threat to the public, animal or plant health, or to animal or plant products.
- **Select Agent Authorized User** – A principal investigator who is authorized by EHS and the IBC to work with a select agent. The select agent authorized user may designate an SRA-approved co principal investigator for certain responsibilities as described in this chapter.
- **Select Agent Laboratory** – A room or suite of rooms, such as a laboratory or animal care area, which EHS has authorized for the storage or use of a select agent.

This area must be delineated in the Laboratory Safety Plan and meet the security standards described in the Security Plan. In most cases, security measures are also in place outside of the select agent laboratory, which creates a larger secure area.

- **Select Agent Tracking System** – A secure information system used to meet the Select Agent inventory, recordkeeping and tracking requirements.
- **Select Agent Worker** – A student, staff member, visiting scientist or faculty member (including the select agent authorized user) who has obtained a security risk assessment, has satisfied training requirements, and has met all other applicable training, occupational health and RMS requirements. Only select agent workers may ship, transport or access a select agent.
- **Sharps** – Any object that can penetrate the skin, e.g., needle, scalpel, knife, etc.
- **Sterilization** – Decontamination method that kills all microbes.
- **Tier 1 BSAT** – A subset of select agents or toxins designated in the select agent regulations as "Tier 1" because these agents and toxins present the greatest risk of deliberate misuse with the most significant potential for mass casualties or deleterious effects on the economy, critical infrastructure, or public confidence.
- **Vector** – DNA that facilitates replication of foreign DNA used to introduce rDNA in a host.

APPENDIX B – CENTRIFUGE SAFETY

The centrifuge uses centrifugal force to separate substances in liquid or solid media according to particle size and density differences. Hazards presented by all centrifuges, including microcentrifuges, if used and/or maintained improperly include:

- Physical hazards caused by mechanical stress, metal fatigue, and corrosion of the rotor over time.
- Exposure hazards: Aerosolization of biological, chemical, or radioactive materials.

STANDARD OPERATING PROCEDURE GUIDANCE

The following information may be integrated into a lab-specific standard operating procedure (SOP) for centrifuge use.

1. Planning for Use
   a. Complete lab-specific training for the centrifuge.
   b. Wear appropriate PPE: Including safety eyewear, gloves, lab coat, and appropriate street clothing (i.e., closed-toe shoes).
   c. Ensure gloves are compatible with hazard(s).
2. Inspecting Centrifuge (Pre-Use):
   a. Verify the rotor is compatible with the centrifuge and seated on the drive correctly.
   b. Ensure rotor and safety cups and buckets are free of cracks and deformities.
   c. Verify rotor O-ring is not cracked, missing, or worn.
   d. Ensure safety cups and buckets are attached properly and can move freely.
   e. Contact a qualified service technician if inspection identifies centrifuge components requiring repair or replacement
3. Preparing centrifuge tubes for loading:
   a. Inspect centrifuge tubes before use.
   b. Ensure tubes are rated for intended use (speed, temperature, and chemical resistance).
   c. Follow manufacturer's filling limits for tubes. Do not under- or overfill tubes.
   d. When centrifuging biohazardous materials, disinfect the outside of tubes prior to their removal from the biosafety cabinet and their loading into the rotor.
   e. When centrifuging hazardous materials, use tightly capped tubes, sealable safety cups, or sealable rotors that can be loaded and unloaded in a fume hood or biosafety cabinet (dependent on the hazard).
   f. Use in-line filters for high speed centrifuges and ultracentrifuges to prevent contamination of vacuum pump and pump oil. Use secondary containment for the vacuum pump.
4. Centrifuge Operation
   a. Balance Centrifuge
      i. Use a balance tube.
      ii. If a balance tube is not available, refer to Figure 1.

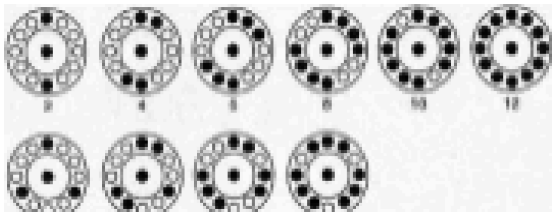


Figure 1. Balanced loading patterns for a 12-position micro centrifuge rotor.

b. Start the run
    i. Do not leave the centrifuge until full operating speed has been reached and appears to be running safely without incident.
    ii. Stop the centrifuge immediately if you notice any unusual noises or shaking and confirm the rotor is balanced.
    iii. To prevent rotor failure,
        1. Do not exceed maximum speed and maximum mass limits for the rotor.
        2. You must reduce rotor speed if sample density calculations. indicate maximum mass limits will be exceeded.
        3. Contact the manufacturer for guidance.
c. Sample Removal
    i. Stop run: Ensure centrifuge comes to complete stop before opening cover.
    ii. When centrifuging hazardous materials, wait at least 10 minutes after run to allow aerosols to settle before opening the centrifuge.
    iii. Check for leaks and spills in samples, rotor, safety cups, buckets, and centrifuge well.
    iv. In a fume hood or biosafety cabinet (depending on material) and wearing appropriate PPE, open sealable tubes, safety cups, rotors.

5. Centrifuge Maintenance
    a. Preventive Maintenance
        i. Establish a preventive maintenance schedule:
            1. Include regular cleaning of the centrifuge interior to prevent corrosion, damage, and avoid costly repairs.
            2. Reference centrifuge operator's manual or contact manufacturer for additional guidance.
        ii. Equipment repair and adjustments shall only be conducted by qualified service technicians.
    b. Maintain log book:
        i. For all high speed centrifuges and ultracentrifuges include run dates, durations, speeds, total rotor revolutions, and notes on rotor condition.
        ii. Retire rotors after the manufacturer's recommended life span except where an annual stress test demonstrates the absence of structural flaws. Note: Rotor life span may be reduced or warranty voided if autoclaved so contact the manufacturer for additional guidance.
6. Centrifuge Disposal
    a. If biohazardous materials were used,
        i. Clean and disinfect the centrifuge thoroughly.
        ii. Deface the biohazard sticker and attach a note on the centrifuge describing the decontamination process conducted.

b. If radioactive materials were used
   i. Appropriate radiation warning signs shall be placed on the centrifuge.
   ii. Prior to removal of the centrifuge, the Radiation Safety Officer (RSO) shall conduct a survey to determine if removable contamination above limits for release is detected.
      1. If contamination above these limits is detected, the unit shall, under the direction of the RSO, be cleaned and re-surveyed.
      2. If continued cleaning fails to bring the contamination below release limits, the centrifuge shall be disposed of as radioactive waste.

APPENDIX C – BIOSAFETY CABINET TYPES AND SELECTION BY RISK ASSESSMENT

Biosafety Cabinet Types		
BSC Class	**Airflow Pattern**	**Specific Uses**
Type I	Air flows in at the front and is exhausted through a HEPA filter.	• Material in BSC is not protected, provides protection only to personnel and the environment. • Can be used with non-volatile toxic chemicals and radionuclides and when exhausted outdoors may be used with volatile chemicals.
Type II A1	70% of air is recirculated in the cabinet and 30% is exhausted through a HEPA filter either to the room or through a canopy to outside.	• Do not use volatile chemicals. With 70% recirculation, levels of volatile chemicals can reach unsafe levels. • Only minute amounts of non-volatile toxic chemicals and radionuclides may be used.
Type II A2	Similar to Type II, A1, but has 100 lfm intake air velocity and plenums are under negative pressure to the room; exhaust air can be ducted to the outside through a canopy unit.	• Suitable for use with non-volatile toxic chemicals and radionuclides. • Can be used with minute amounts of volatile chemicals if ducted to the outside through an exhaust canopy.
Type II B1	30% of air is recirculated and 70% is exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter.	• Suitable for use with non-volatile toxic chemicals and radionuclides. • Can be used with minute amounts of volatile chemicals.
Type II B2	No air recirculation; total exhaust to the outside through a HEPA filter.	• Suitable for use with non-volatile toxic chemicals and radionuclides • Can be used with volatile chemicals in small amounts.

Selection of a Cabinet through Risk Assessment

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
BSL-1, -2, -3	YES	NO	YES	I

BSL-1, -2, -3	YES	YES	YES	II (A, B1, B2, B3)
BSL-4	YES	YES	YES	III B1, B2

Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, CDC/NIH, 2nd edition.

APPENDIX D – DECONTAMINATION AND SPILL RESPONSE

Decontamination is any process, which reduces biohazardous material (infectious agents, rDNA material, human material, biological toxins, etc) to an acceptable level below the level necessary to cause disease. Acceptable levels will depend on the biohazardous material in question, the type of work being conducted, and the method of decontamination.

In order to select the proper decontamination procedure one must consider many factors including; the biohazard's concentration and resistance to disinfectants, chemical compatibility with other materials present, surface being decontaminated, and hazards to humans and the environment associated with the disinfectant.

Note: All rDNA containing waste, including Biosafety Level 1 material, must be decontaminated prior to disposal or disposed as biohazard waste prior to being released from the laboratory.

The following two tables provide general information only. Phenolics and quats are available in many formulations with different properties. Follow the manufacturer's recommendations for use.

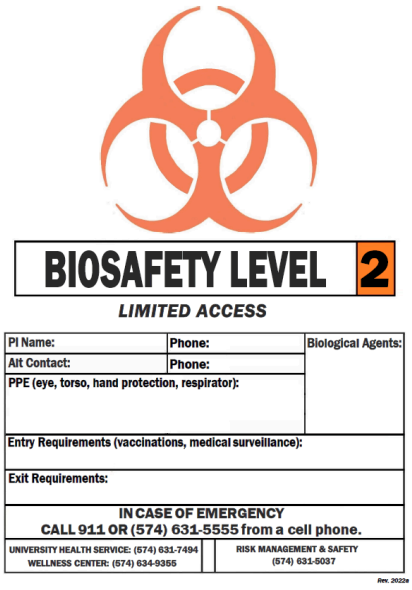
MICROBIAL RESISTANCE TO CHEMICAL DISINFECTANTS

	MICROORGANISM	EXAMPLES
MORE RESISTANT	Prions	BSE,vCJD Scrapie
	Bacterial Spores	Bacillus, Geobacillus, Clostridium sp.
	Protozoan Oocytes	Cryptosporidium
	Helminth Eggs	Ascaris, Enterobius
	Mycobacteria	M. tuberculosis
	Small non-enveloped viruses	Poliovirus, Parvoviruses, Papillomaviruses
	Protozoan Cysts	Giardia, Acathomoeba
	Fungal Spores	Aspergillus, Penicillium
	Gram-negative Bacteria	E. coli, Salmonella spp.
	Vegetative Fungi & Algae	Candida, Chlamydomonas
	Vegetative Helminths & Protozoa	Ascaris, Cryptospiridium, Giardia
	Large Non-enveloped Viruses	Adenovi ruses Rota viruses
	Gram-positive Bacteria	Staphylococcus, Streptococcus, Enterococcus
LESS RESISTANT	Enveloped viruses	HIV, Hepatitis B, Herpes Simplex Virus

Material	Tips For Use	Advantages	Disadvantages
Chlorine Compounds	-Dilute household bleach 1:9(v/v) solution of household bleach (10% bleach solution), make fresh monthly -Store diluted solutions in a sealed container and protected from light. -For spill cleanup, and to wipe down work surfaces -FINAL concentration of 10% bleach used for liquid infectious waste -Fisher Scientific Fisherbrand Bleach Solution Dispenser. It is a unique, Two-bottle design and fixed-ratio trigger sprayer automatically mixes concentrated bleach with tap water. Cat. No. 23-640- 127	-Relatively non-toxic -Low cost -Effective with detergents -Fast acting -Broad spectrum effectiveness	-Inactivated by organic material such as blood, -Do not use at less than 1:9 (v/v) dilution -Strong oxidizer; corrosive -Irritates mucous membranes, eyes, skin -No residual activity on surfaces -Can damage clothing -Incompatible with quats -Produces toxic chlorine gas if mixed with acids or ammonia compounds -Can't be used to disinfect radioactive iodine.
Alcohols	-Dilute to 70% in water, (loses effectiveness at concentrations above 90%) -Use to clean instruments and wipe down interior of Biological Safety Cabinets -Use as topical antiseptic on intact skin	-Non-corrosive -Effective with detergent	-Can have reduced effectiveness in organic material, does not penetrate organic material -Flammable -No residual activity and limited effective exposure time due to high rate of evaporation
Phenolics	-Dilute according to manufacturer's instructions -Commonly used to clean walls, floors, etc -Useful in areas where organic matter cannot always be removed, such as animal areas	-Good effectiveness in organic material -Effective with detergent -Has some residual Effectiveness	-Toxicity varies with specific compound, can be absorbed through skin -Some formulations may have unpleasant odor -Corrosive -Skin irritant -Not effective against spores
QUATS – Quaternary Ammonium Compounds	-Dilute according to manufacturer's instructions -Surfaces must be rinsed free of anionic soap or detergents before use	-Strong surface activity -Low toxicity -Non-corrosive	-Easily inactivated by organic materials, anionic detergents, and salts of metals in water (hard water)

(cationic detergents)	-Commonly used to clean walls, floors, etc.	-Effective over wide pH range	-Skin irritant

APPENDIX E – BIOSAFETY LEVEL CONTAINMENT SIGNS



APPENDIX F – INFECTIOUS / BIOHAZARD SYMBOL AND LEGEND

APPENDIX G- INCIDENT, INJURY OR ILLNESS PROCEDURES