Biosafety Manual

1. 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. PURPOSE

This procedure is to ensure a safe working environment for University biohazardous and recombinant DNA (rDNA) activities and for compliance with all applicable federal, state, and local 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. SCOPE

This Biosafety Manual applies to all University of Notre Dame lab personnel handling and/or potentially exposed to biological hazards in research and teaching laboratories. This manual shall be adopted directly by Principal Investigators (PI) for use in their teaching / research laboratories, unless the PI generates their own biosafety manual. Lab specific SOPs and approved IBC protocols are required to accompany this or PI generated biosafety manuals.

4. ACRONYMS AND DEFINITIONS

See Appendix A for common acronyms and definitions.

5. RESPONSIBILITIES

5.1 The Institutional Biosafety Committee (IBC) (Members appointed by the University President)

5.1.1 Reviews all research conducted at or sponsored by the University involving rDNA subject to the NIH Guidelines and biological research requiring biosafety containment. This review shall include:

- Independent assessment of the risks inherent to the research.

- Verification of containment levels assigned by the PIs.
- Assesses facilities, equipment, procedures, practices, training and all other elements of the research.

5.1.2 Notifies PIs of the committee's actions.

5.1.3 Keeps records of meetings, in a manner, which provides sufficient detail to serve as a record of major points of discussion, committee's rationale for particular decisions, and proof the IBC has fulfilled its review and oversight responsibilities.

5.1.4 Reports any significant problems or violations to the National Institutes of Health Office of Biotechnology Activities (NIH OBA).

5.1.5 Reports guidelines and any significant research-related accident or illness to NIH OBA.

5.1.6 Files an annual report with the NIH.

5.2 Notre Dame Research (NDR)

5.2.1 Provides the necessary liaison between PIs, the IBC, granting agencies, and regulatory agencies.

5.2.2 Serves as the Office of Record for documentation involving the IBC.

5.2.3 Provides all necessary documentation for PIs to comply with University submission requirements.

5.2.4 Assists PIs and researchers regarding export control and importing of biological agents and select agents.

5.2.5 Appoints IBC Chair.

5.2.6 Appoints Institutional Biosafety Officer.

5.3 Risk Management and Safety Department (RMS)

5.3.1 Provides industrial hygiene and safety support for all laboratory operations.

5.3.2 Transports and disposes of all biological or infectious waste in compliance with all applicable federal, state, and local ordinances.

5.3.3 Assists, as necessary, in the emergency response, cleanup, and decontamination of biological spills and accidents.

5.3.4 Provides assistance in determining shipping requirements for all biological samples and oversees shipments if the sample is deemed to be of greater risk to health and safety than Biological Substance Category B.

5.4 Institutional Animal Care and Use Committee (IACUC)

5.4.1 Provides appropriate assistance to ensure animal care meets or exceeds federal, state, and local requirements and specifications.

5.4.2 Ensures animal housing systems are designed and utilized in a manner, which minimizes the potential exposure of other animals or personnel to potentially biohazardous agents.

5.4.3 In cooperation with the PI and the IBC, develops and implements specific standard operating procedures, in adherence to the ABSL

classification of the agent being used addressing animal care, research, and accident / equipment failure procedures.

5.4.4 Ensures all animal care personnel are adequately trained and aware of the potential risk associated with each agent.

5.4.5 Develops emergency plans for handling accidental spills, personnel exposures, unintentional animal exposure, equipment failure, etc..

5.4.6 Reports any significant problems or violations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) regulations and any significant research- related accident or illness to AAALAC.

5.5 Biological Safety Officer (BSO)

5.5.1 Assists PIs with establishing and maintaining a safe working environment in research and teaching laboratories.

5.5.2 Serves as a member of the University's Institutional Biosafety Committee.

5.5.3 Reports to the IBC any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses unless already reported by the PI.

5.5.4 Conducts laboratory inspections to ensure compliance with standards and containment conditions established by the IBC.

5.5.5 Assists PI with hazard risk assessment for biological work activities.

5.5.6 Provides technical advice to PIs and the IBC.

5.6 Principal Investigator (PI)

5.6.1 Complies with all University policies, applicable government regulations, and guidelines associated with their research and lab space(s).

5.6.2 Completes laboratory safety training successfully, as required.

5.6.3 Monitors and approves the procurement, use, and disposal of biological agents used in the laboratory.

5.6.4 Informs all employees and students working under their supervision how safety and health are high priorities.

5.6.5 Ensures all employees and students working under their supervision are trained on the safety and health policies, rules, regulations, procedures, and responsibilities identified in the unit safety plan.

- Ensures additional training and updates are provided to all employees and students working under their supervision when equipment, procedures, or policies change.
- Training shall be documented with a signature sheet and retained so it is available upon request.

5.6.6 Provides funding for vaccinations, testing and/or baseline serum samples as needed.

5.6.7 Submits rDNA registration for research laboratory activities with the IBC protocol as appropriate.

5.6.8 Submits IBC protocols of research laboratory activities containing work with biological hazards. The IBC protocol shall be submitted prior to research and shall include:
- Identity of the organisms and/or biological materials used in the laboratory, along with all recognized risks (including source, species, quantity, storage, and any testing conducted on the biological materials).
- A completed laboratory risk assessment describing the research activities specific to biohazards, the risks associated with laboratory procedures, and the implementation of appropriate controls and/or practices to minimize those risks.

5.6.9 Sets expectations and provides lab specific training on safety equipment, devices, personal protective equipment, and apparel regarding provision, maintenance and use by individuals present in the laboratory, including personnel from other laboratories. These expectations include:
- Individuals working under the PI or supervisor complete training and operate under the relevant expectations and requirements when present or using equipment in other laboratories.
- In the case of laboratories occupied by multiple PIs, each PI or supervisor has these responsibilities for their own personnel.

5.6.10 Refers to NIH's Guidelines for Research Involving Recombinant DNA Molecules or CDC's Biosafety in Microbiological and Biomedical Laboratories for specific containment requirements.

5.6.11 Ensures secondary methods, e.g., facility design features or special practices are incorporated into the protocol when primary or standard practices are not sufficient for containment.

5.6.12 Ensures practices to minimize risks are written into the laboratory's standard operating procedures.

5.6.13 Ensures all laboratory personnel have been informed about the risks connected with work in the laboratory.

5.6.14 Ensures compliance with IBC approved emergency plans for spills and personal exposures in the laboratory.

5.6.15 Reports any significant problems and violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the BSO/IBC (where applicable) and/or Animal Facility Director (where applicable).

5.6.16 Consults with IBC or IACUC, if reporting to CDC, NIH or AAALAC is required, so IBC or IACUC can conduct applicable notifications.

5.7 Laboratory Worker

5.7.1 Follows established laboratory safety practices and standard operating procedures.

5.7.2 Completes Biosafety, Biocontainment, BBP, and any other applicable training in accordance with compliance requirements.

5.7.3 Verifies the performance and safety of all equipment prior to its use. This includes personal protective equipment (PPE), biosafety cabinets, centrifuges, fume hoods, etc..

5.7.4 Communicates to the PI any unsafe practices or conditions in the laboratory.

5.7.5 Reports any spills, accidents, or injuries involving biological materials to the PI.

5.7.6 Informs the PI of any health changes potentially caused by biological and chemical exposure or that affect susceptibility to any lab materials.

5.7.7 Informs the University's healthcare provider of a health status affecting one's susceptibility to infection and one's ability to receive available immunizations or prophylactic interventions. Particularly, those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (organ transplant, medical immunosuppressive agents, etc.).

6. REGULATORY COMPLIANCE

6.1 Recombinant DNA (rDNA) Activities

6.1.1 The NIH Guidelines for Research Involving Recombinant DNA Molecules governs all rDNA activities and identifies exempt activities.

6.1.2 DNA activities involving microorganism and exempt rDNA microorganisms.

6.1.3 Activities involving these agents are not federally regulated.

6.1.4 Creation of transgenic rodents at level above BSL-1.

6.1.5 The IBC and RMS has determined 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 These agents are governed by NIH and CDC guidelines, as well as OSHA (bloodborne pathogens), Department of Health & Human Services and USDA regulations.

- CDC's BMBL provides best practices and guidelines to ensure work with biological agents and toxins can be safely conducted.

6.3 Tuberculosis

6.3.1 NIH or CDC guidelines shall be followed for all research activities involving Mycobacterium tuberculosis.

6.3.2 OSHA has published guidelines for activities that potentially expose people to tuberculosis.

6.4 Bloodborne Pathogen Standard (29 CFR 1910.1030)

6.4.1 OSHA standard that applies to the exposure to blood or other potentially infectious materials (OPIM).
- An Exposure Control Plan is required.
- The Notre Dame Exposure Control Plan describes how to eliminate or minimize exposure of all Notre Dame personnel to human blood, blood products, or OPIM that might contain bloodborne pathogens.

6.4.2 Universal Precautions
- The concept of Universal Precautions is to treat all human/primate blood and other body fluids, tissues and cells as if they were known to be infectious for BBPs
- Precautions to include in lab activities:
  - Frequent hand washing
  - No mouth pipetting
  - No food or drink in the lab
  - Proper disposal of biohazardous waste

6.4.3 Personal Protective Equipment (PPE)

6.4.4 Engineering controls include items such as biosafety cabinets, ventilation systems, closed top centrifuge rotors, etc. These are the primary methods to control exposure.

6.4.5 Hepatitis B Vaccine Program
- The vaccine is offered free of charge to all ND personnel considered "at risk" due to occupational exposure
- While ND encourages employees to be vaccinated, accepting vaccination is not a condition of employment.
- Employees that are offered the vaccine are required to either accept the vaccine or sign the ND Declination Form.

6.4.6 ND personnel (including faculty, staff, post-doctoral fellows, graduate students, and undergraduates working for pay) shall go to the Wellness Center for vaccination. Students as part of a class shall go to the University Health Services (St. Liam's Hall). Refer to the University Exposure Control Plan for more information.

7. PROTOCOL SUBMISSION AND REVIEW

7.1 Submission and approval by the IBC of a completed IBC protocol form is required before initiation of any work conducted with:

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, including established cell lines, human embryonic stem cells, and pluripotent stem cells and their derivatives

7.1.3 Infectious agents, including bacteria, viruses, prions, fungi, and protozoans

7.1.4 Recombinant and/or synthetic nucleic acids, including activities using viral vectors or plasmid vectors, transgenic/knock-in/knock-out animals, and genetically modified plants
7.1.5 Biological toxins
7.1.6 DHS Select Agents (Here is a list of exclusions related to select agent activities)

7.2 PIs shall complete the rDNA registration portion of the IBC protocol as appropriate.
7.2.1 All protocols involving rDNA activities shall follow the requirement of the National Institutes of Health as presented in the latest edition of the NIH Guidelines for Research Involving Recombinant DNA Molecules and all supplements published thereafter in the Federal Register.
7.2.2 Non-exempt BSL-1, BSL-2, and exempt rDNA protocols not requiring review by federal agencies can be approved by the IBC.

7.3 Areas to address when rDNA protocols are being prepared for submittal
7.3.1 Synthetic and associated sequence(s)
7.3.2 rDNA insert
7.3.3 Potential protein product
7.3.4 Vector
7.3.5 Carrier used to introduce rDNA into a host system facilitating replication
7.3.6 Types of Containment
- Biological Containment - limiting the infectivity of a vector or vehicle for specific hosts, limiting the dissemination and survivability of a host and/or vector in the environment.
- Physical Containment - specifically designed equipment, facilities used to physically contain microbes, and limiting access to the BSL-2 space.

7.3.7 Good Laboratory Practices
- Design practices and procedures specifically to physically contain microbes.
- Mechanisms for inactivation and disposal of microbes.

7.3.8 When appropriate, standard operating procedures (SOPs) shall be developed for lab activities that include biohazards and/or rDNA using the University of Notre Dame Biohazard SOPs Template.

7.4 Review of IBC protocols and Registration Documents may include:
7.4.1 Independent assessment of the risks associated with the research
7.4.2 Verification of containment levels assigned by the PI
7.4.3 Verification of training
7.4.4 Assessment of facilities, equipment, procedures, practices, training and all other elements associated with the research

7.4.5 The PI will be sent a letter once the protocol/registration document has been approved

7.4.6 Approved IBC Protocols are valid for 3 years from approval date. Renewals shall be submitted no less than 30 days prior to expiration.

8. LABORATORY BIOSAFETY MANUAL

8.1 BSL-2 or greater laboratories are required to have a laboratory biosafety manual.

8.2 PI shall adopt this Biosafety Manual or develop one including the following:

8.2.1 Approved IBC protocol

8.2.2 Safety protocols for the process or agent in question. The protocols can serve as an addendum to this manual.

8.2.3 All special practices required for process or agent.

8.2.4 Emergency action plan for

- spill response
- needle sticks
- lab personnel exposure

8.3 Staff members and students shall read and adhere to all lab specific protocols.

9. BIOLOGICAL RISK MANAGEMENT PROCESS

9.1 Biological Risk Assessment

9.1.1 Is a component of the biological risk management process (see steps 1 and 2 in Section 9.2 and 9.3) focusing on biological agent hazards and laboratory procedures hazards.

9.1.2 Provides a standardized approach to hazard identification process

- The Agent Summary Statements in Section VIII of the BMBL, 6th Edition is designed to assist with risk assessments.
  - Focuses on agents associated with lab acquired infections (LAIs) and those that are of increased public concern.
  - Identifies known and suspected routes of transmission of LAIs, available infective dose, host range, agent stability in the environment.

9.2 NIH Biological Agent Risk Groups

9.2.1 Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans and whether preventative or therapeutic interventions are available (Appendix A for the definitions of each risk group).

- 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,

9.2.2 Is designed to be an on-going process where all personnel have a role in its success. The goal of your risk assessment is to address all realistic, perceivable risks to protect personnel, the community, and the environment.

9.3 Step 1: Identify biological agent(s) hazard(s).

9.3.1 Wild-Type Agents

- Characteristics to consider include:
  - Capability to infect and cause disease in a susceptible host.
  - Severity of the disease it causes.
  - Availability of preventative measures and effective treatments.
  - Routes of transmission of infection.
  - Infectious dose.
  - Stability in the environment.
  - Host range.
  - Whether the agent is indigenous or exotic to the local environment.
  - Genetic characteristics of the agent.

9.3.2 Genetically Modified Agents

- Characteristics to consider include:
  - Those listed in 9.2.1 for wild-type agents.
  - How the genetic modification affects the agent's pathogenicity.
  - How the genetic modification affects the agent's virulence.
  - How genetic modification affects the agent's susceptibility to antibiotics and other effective treatments.

9.3.3 Cell Cultures

- Characteristics to consider include:
  - Those listed in 9.2.1 for wild-type agents for known pathogens.
  - Potential of unanticipated pathogens being present.
  - Potential of viral latency.
  - Origin of cells, tissues, etc.
  - Origin of agent(s) present.

9.4 Step 2: Identify laboratory procedure hazards.

9.4.1 The principal laboratory procedure hazards are the following:

- Agent concentration.
- Suspension volume.
- Equipment and procedures generating aerosols and larger airborne particles / droplets.
  - Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are

extremely pervasive, placing all laboratory personnel at risk of exposure.
    - Procedures and equipment that impart energy into a microbial solution will produce aerosols.
    - Procedures with a potential to create aerosols or splashes include, but not limited to, pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers without care, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    - Equipment known to generate aerosols include, but not limited to, pipettes, blenders, centrifuges, sonicators, vortex mixers, cell sorters, and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometers.
- Use of sharps.
- Working with Animals.
    - Bites and scratches.
    - Exposure to zoonotic agents,
- Handling of experimentally generated infectious aerosols.
- Facility Control Hazards also need to be considered.
    - Overall integrity of HVAC systems.
    - Quality of work for installations.
    - Consistency of facility preventative maintenance and up-keep programs.
    - Non-adherence to biosafety level containment requirements.

9.5 Step 3: Determine appropriate biosafety level and select additional precautions indicated by the risk assessment.

9.6 Step 4: Before implementation of controls, review the risk assessment and selected safeguards with the University's Biosafety Officer, subject matter expert, the IBC, or equivalent resource.

9.7 Step 5: Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment (an ongoing process).

9.7.1 Lab personnel at all skill levels need to know how to identify hazards in the laboratory and how to obtain assistance in protecting themselves and others in the laboratory.

9.7.2 Evaluate lab personnel for the following:

- Training.
- Experience in handling biological agents.
- Proficiency in following good microbiological practices.
- Proficiency in following equipment use requirements.
- Consistency using SOPs for specific lab activities.
- Ability to respond to adverse conditions and events.

- Willingness to accept responsibility for protecting one's self and others.
- History of by-passing safety controls and mechanisms.
- Attentiveness.

9.7.3 Competency assessments can be used to address deficiencies identified in the Section 9.6.2 evaluation

9.8 Step 6: Revisit the risk management process regularly and verify risk management strategies and determine if any changes are necessary.

10. MEDICAL SURVEILLANCE AND VACCINATIONS

10.1 Medical surveillance may be required for both those workers who use biohazardous agents as well as any animal handler who shall tend to animals inoculated with etiologic agents. The Wellness Center shall work with the Animal Facility Director to identify animal handlers who may be at risk for occupational exposure to infectious microorganisms in the course of their duties.

10.1.1 Staff and lab personnel with access to risk group 3 biological agent or animals inoculated with risk group 3 biological agents shall complete the medical surveillance questionnaire.

10.2 Vaccinations are available for many etiologic agents used in the laboratory. The Wellness Center medical staff, in conjunction with the IBC and the BSO, will make the recommendation for the use of vaccinations on a case-by-case basis. Refer to the Occupational Health Category A/B Process.

11. PERSONAL PROTECTIVE EQUIPMENT (PPE)

11.1 Purpose of personal protective equipment (PPE) is to protect employees from risk of injury or death by creating a barrier against workplace hazards.

11.2 The PPE Hazard Assessment Tool shall be completed for work conducted in the laboratory to determine any PPE requirements.

11.3 Personal protective equipment is not a substitute for good engineering or administrative controls or good work practices, but shall be used in conjunction with these controls to ensure the safety and health of employees.

11.4 PPE that is required when working in BSL-1 and BSL-2 labs:

11.4.1 Eye Protection – Safety glasses, goggles, or face shield meeting the rating standards of ANSI Z87.1 2015. The ANSI designation is imprinted on the equipment by the manufacturer.

11.4.2 Hand Protection – Gloves compatible with the chemicals used with biohazards.

11.4.3 Body Protection

- Long pants or skirt (to the ankle)
- Lab coats (cotton or disposable)
- Foot protection – Closed-toe, closed-heel shoes and booties as required.
- Additional PPE may be required per the PPE Hazard Assessment.

11.5 PPE required when working in a BSL-3 lab:

11.5.1 Eye protection – Safety glasses, goggles, or face shield meeting the rating standards of ANSI Z87.1 1989. The ANSI designation will be imprinted on the equipment by the manufacturer.

11.5.2 Hand protection – Gloves compatible with the chemicals used with the biohazards. These shall be taped around wrists to provide complete seal. Double gloves as required.

11.5.3 Body protection – Tyvek suit with attached booties or Tyvek suit with loose booties taped at ankle.

11.5.4 Foot protection – Closed-toe / closed-heel shoes and booties attached to a Tyvek suit or loose booties taped to Tyvek suit at ankle.

11.5.5 Additional PPE may be required per the PPE Hazard Assessment..

11.6 Respirator Use

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

11.6.2 The OSHA Respiratory Protection Standard at 29 CFR 1910.134 shall be complied with for all personnel who are required or volunteer to wear a respirator, see the University's Respiratory Protection Plan.

11.6.3 RMS shall be contacted before purchasing or using respiratory protection.

11.6.4 Dust masks may not require participation in the University's Respiratory Protection Plan.

11.6.5 When selecting a mask for the first time, contact RMS to determine if participation is required.

11.6.6 Even if exempt from the Respiratory Protection Plan, the Respiratory Protection Plan's Appendix E: "Information to Employees Who Wear Respirators for Voluntary Use" form shall be completed and submitted into the OnBase system.

12. TRAINING

12.1 All IBC members, PI's, and laboratory staff members conducting activities involving microorganisms or biotoxins, shall complete training in biosafety, regardless of the level of activity they propose to use (BSL-1, BSL-2, or BSL-3) initially and annually thereafter.

12.1.1 BSL-1 and BSL-2 training is completed on complyND and meets the University of Notre Dame's biosafety training requirements.

12.1.2 BSL-3 training is available from the Lab Manager of the BSL-3 laboratory.

12.2 The PI is responsible for the development and administration of training on lab specific biosafety SOPs to their laboratory staff members and students. This training shall include but not limited to:
12.2.1 Procedures and techniques
12.2.2 Laboratory safety rules
12.2.3 Laboratory emergency action plans
12.2.4 Spill containment and disinfection / cleanup
12.2.5 Instructions on the safe operating perimeters and procedures for use of laboratory equipment (chemical fume hood, biosafety cabinets, autoclaves, centrifuges, etc.) See Appendix B for more information on centrifuge safety. See the Autoclave Safe Use and Validation Procedure for more information on autoclave safety.
12.2.6 Laboratory Fundamentals, Biosafety and Biocontainment and, as appropriate, Bloodborne Pathogen training shall be given to all new laboratory personnel and all laboratory members working in or around biohazards shall receive annual refresher training which is available online.

12.3 Record of attendance and the content of the training provided shall be maintained by the PI per the University Record Retention requirements.

13. CONTAINMENT LEVELS

13.1 Biosafety Containment Types

13.1.1 Primary (p): methods to protect the internal laboratory environment, e.g., microbiological techniques and appropriate safety equipment
13.1.2 Secondary (s): methods to protect the environment external to the laboratory, e.g., facility design and operational practices
13.1.3 The IBC, in its review of protocols, will consider for the need of additional containment.

13.2 Biosafety Level 1 (BSL-1) Containment
13.2.1 Facility Requirements
- Laboratory is designed to be easily cleaned.
  - Work surfaces are non-porous, impervious to water, and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Carpets and rugs are inappropriate.
  - Spaces between benches, cabinets, and equipment are accessible for cleaning.

- Chairs used in the lab are constructed of non-porous materials, which are easily cleaned and decontaminated with appropriate disinfectants.
- Sink and eye wash station(s) are readily available and in the lab.
- Doors to secure containment area.
- Biohazard warning signage posted at the laboratory entry.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Lab windows that open to the exterior are fitted with screens.
- Laboratory furniture can support anticipated loads and uses.

13.2.2 Special containment equipment is generally not required.

13.2.3 Laboratory safe practices shall include:

- PPE (lab coats, gloves, face/eye protection)
- Restraining hair so it cannot contact hands, specimens, containers, equipment, or be itself a hazard.
- Work may be completed on an open bench top
- Sharps program
- Integrated Pest Management

13.3 Biosafety Level 2 (BSL-2) Containment

13.3.1 Facility Requirements

- Laboratory is designed to be easily cleaned.
  - Work surfaces are non-porous, impervious to water, and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Carpets and rugs are inappropriate.
  - Spaces between benches, cabinets, and equipment are accessible for cleaning.
  - Chairs used in the lab are constructed of non-porous materials, which are easily cleaned and decontaminated with appropriate disinfectants.
- Sink and eye wash station(s) are readily available and in the lab.
- Laboratory doors are self-closing and have locks in accordance with institutional policies.
- Biohazard warning signage posted at the laboratory entry
  - RMS will supply signage after lab has been approved for BSL-2.
  - See Section 14 for more information.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Lab windows that open to the exterior are not recommended, but if present, need to be fitted with screens.
- Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters.
  - Filters are replaced as determined by application, frequency of use, etc. or whenever needed.

- Biosafety cabinets (BSCs) and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
    - BSCs are installed so fluctuations of the room air supply do not interfere with proper operations.
    - Biosafety cabinets are located away from doors, windows that can be opened, heavily traveled laboratory areas, or other possible airflow disruptions.
    - BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III).
    - BSCs are certified at least annually to ensure correct performance.

13.3.2 Laboratory safe practices include BSL-1 plus:

- Lab specific Biosafety Manual (see Section 10 for more information)
- SOPs for the handling of agents.
- A biological safety cabinet for material manipulations where there is a potential for aerosol generation.
- Limited access to the lab by locking the lab door.
- Class I or II biosafety cabinet or other physical containment devices are required for all manipulations of agents that cause splashes or aerosols of infectious materials (see Section 9.3.1 for information on aerosol generation).
    - If a procedure is not possible to perform in a BSC or other containment device, a combination of appropriate PPE and administrative controls are used, based on a risk assessment.
- Autoclave availability.
- Centrifuging infectious agents in the open laboratory setting only if sealed rotors and safety cups are used, and if they are loaded and unloaded in a BSC or another containment device.
- Animals and plants not associated with the scope of work are not permitted in the laboratory.
- Laboratory equipment is decontaminated routinely, after spills, splashes, or other potential contamination, and before repair, maintenance, or removal from the laboratory.
- Reporting all incidents that may result in exposure to biological agents so they are immediately evaluated.

13.4 Biosafety Level 3 (BSL-3) Containment

13.4.1 Laboratory safe practices shall include BSL-1 and BSL-2 plus the following:

- A double-door entry (anteroom) where the first door must be fully closed prior to the next door opening. Laboratory is under negative pressure from the outer hallway.
- Air movement from areas of lesser contamination to areas of higher contamination, such as from the corridor into the laboratory
- Air movement is a single pass; exhaust air is not recirculated to other rooms or spaces.
- All work with the potential to create aerosols or splatter is conducted inside a biological safety cabinet.
- Sealed wall, ceiling and floor penetrations to keep aerosols in, while containing gaseous decontaminants. The floor shall be monolithic and continuous cove moldings extending at least 4" up the wall.
- Waterproof ceiling for ease of cleaning.
- Training shall include hands-on donning and doffing of PPE.

14. CONTAINMENT SIGNAGE AND MATERIAL LABELING

14.1 Signs are managed by RMS and can be requested by emailing labsafety@nd.edu.

14.2 BSL-1 Signage Requirements

14.2.1 A biohazard warning sign (see Appendix E) incorporating the universal biohazard symbol and biosafety level shall be posted on the access door to the laboratory work area.

14.3 BSL-2 Signage Requirements

14.3.1 A biohazard warning sign (see Appendix E) shall be posted on the access door to the laboratory work area and shall have the following information.

- the universal biohazard symbol and the lab's biosafety level
- the PI, supervisor, and lab manager name and contact information.
- PPE requirements
- general occupational health requirements.
  - Whether immunizations are required and if so, which ones.
  - Whether respiratory protection is required.
  - Whether medical surveillance is required.
- any required laboratory entering and exiting procedures.
- biological agents information.

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14.4 BSL-3 Signage Requirements

14.4.1 A biohazard warning sign (see Appendix E) shall be posted on the access door to the laboratory work area and shall have the following information.

- the universal biohazard symbol and the lab's biosafety level

- the PI, supervisor, and lab manager name and contact information.
- PPE requirements
- general occupational health requirements.
  - o whether immunizations are required and if so, which ones.
  - o whether respiratory protection is required.
  - o whether medical surveillance is required.
- any required laboratory entering and exiting procedures.
- biological agents information.

14.5 Material Labeling Requirements

14.5.1 All human tissue, body fluid, or other potentially infectious materials shall be stored in a compliant container (see Sections 19 and 21). labeled with a biohazard symbol (see Appendix F).

14.5.2 Refrigerators, freezers, incubators, or other pieces of equipment where potentially infectious materials are stored or handled shall be labeled with the biohazard symbol (see Appendix F).

14.5.3 Refrigerators, freezers, microwaves and blenders shall be labeled with a biohazard symbol and a "No food or drink" label.

14.5.4 Any food products (dry milk, fruit juices, etc.) used for research activities shall be labeled "Not for human consumption".

15. BIOSAFETY CABINETS

15.1 Biosafety Cabinet Types and Selection by Risk Assessment information can be found in Appendix C.

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

15.2.1 Lab personnel protection from material inside the cabinet.

15.2.2 Protection for the material from contaminants.

15.2.3 Protection for the environment from the material inside the cabinet.

15.3 A biosafety cabinet shall generally not be used for work with hazardous chemicals.

15.4 Most biosafety cabinets exhaust the contaminated air through high efficiency particulate air (HEPA) filters back into the laboratory.

15.5 There are three types of BSCs

15.5.1 Class I

- Provides protections to personnel and environmental only. The material (research experiment) inside the cabinet is not protected and subject to contamination.
- The use of Class I BSC is not advised at the University of Notre Dame. Contact the BSO if there is a reason to purchase one.

15.5.2 Class II
- Provides protection of personnel, product, and the environment.

15.5.3 Class III
- Gas-tight and designed for use with high-risk (BSL-4) agents.

15.6 The use of alcohol burners cannot only be extremely dangerous in cabinets, but can also void the manufacturer's warranty. There are many alternatives to the use of burners (micro-incinerators, disposable tissue culture supplies, etc.).

15.7 Installation and Maintenance of BSCs

15.7.1 Installation of cabinets shall be done by certified professionals. ND has an agreement with a certified company for installation, cabinet certification, decontamination and any other needs that may arise. Contact the BSO for assistance.

15.7.2 Certifications shall be done annually or whenever a biosafety cabinet has been moved.

15.7.3 Installation or certification are arranged through Maintenance.

15.7.4 Payment for such services is the responsibility of the PI or Department.

16. SHARPS

16.1 All sharps waste shall be placed in an approved sharps container.

16.2 Sharps containers requirements include:

16.2.1 Constructed of rigid, hard plastic,

16.2.2 Labeled with the universal biohazard symbol,

16.2.3 Not overfilled.

16.3 The lid of the sharps container shall be closed and the locking tabs engaged once the sharps container is filled to the full line. A Biohazardous Discard form container labeled with the PI Name and Lab # prior to disposal.

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

16.5 RMS shall pickup all full sharps containers to ensure proper disposal.

17. EXPOSURES

17.1 For any exposure to a biohazard, rDNA, or needle stick incident, the following steps shall be taken:

17.1.1 Seek medical attention as soon as possible. (CDC recommends within 2 hours).
17.1.2 Non-life threatening medical attention is needed, report to the Wellness Center (employees) or University Health Services (students in class).
17.1.3 For emergencies: contact NDPD (911 or 574-631-5555 from a cell phone) for emergency medical assistance.
17.1.4 If there has been a needle stick/puncture wash the affected area with antiseptic soap and warm water for 15 minutes.
- Seek medical assistance.
- All needle sticks involving blood (someone else's) or other potentially infectious materials shall be reported to ND Compliance (compliance@nd.edu) and the University's Biosafety Officer (labsafety@nd.edu).

17.1.5 For a mucous membrane exposure, flush the affected area for 15 minutes using eyewash. Seek medical attention.

17.2 Notify PI, manager, or supervisor to initiate an incident notification report.

17.3 If a spill has occurred, contain and initiate emergency response. See Section 18 for more information.

17.4 Call NDPD at 911 from a campus phone or 574-631-5555 from a cell phone.

18. EMERGENCY RESPONSE

18.1 Spills shall be cleaned as soon as possible. If the spill is considered too large or too dangerous for laboratory personnel to safely clean up:
18.1.1 Secure the entire laboratory and
18.1.2 Call Notre Dame Police (NDPD) immediately for assistance. 911 from a campus phone or 574-631-5555 from a cell phone.

18.2 The following procedures are guidelines to biohazardous or recombinant and synthetic nucleic acid molecule spill cleanup. See Appendix D for more information.
18.2.1 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.
18.2.2 Inside the Biosafety Cabinet
- Wait at least five minutes to allow the BSC to contain aerosols.
- Wear laboratory coat, safety glasses and nitrile gloves during cleanup. (Latex gloves shall not be used when working with ethidium bromide).
- Allow BSC to run during cleanup.
- Apply disinfectant and allow a minimum of 20 minutes contact time.

- 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.
- Wipe the walls, work surfaces and any equipment in the cabinet with disinfectant-soaked paper towels.
- Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures.
- Place contaminated reusable items in biohazard bags or autoclavable pans with lids before autoclaving.
- Expose non-autoclavable materials to disinfectant (20 minutes contact time) before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for removal.
- Run BSC 10 minutes after cleanup (prior to resuming work or turning BSC off).
- 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.

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

- 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 spill's potential aerosols to settle for 30 minutes.
- Assemble clean-up materials (disinfectant, paper towels, biohazard bags and forceps).
- Don appropriate PPE, including lab coat, shoe covers, gloves and eye/face protection.
    - 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.
    - Initiate cleanup with disinfectant as follows:
    - Place paper towels or other absorbent material over the spill area.
    - Carefully pour disinfectant around the edges of the spill and then onto the paper towels. Avoid splashing or generating aerosols.
    - Allow the disinfectant to remain in contact with the spill for at least 20 minutes.
    - Apply more paper towels to wipe up the spill.
    - Clean spill area with fresh towels soaked in disinfectant.
    - 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.

- Remove protective clothing and segregate for disposal or cleaning.
- Wash hands with soap prior to leaving the area.

18.2.4 For a spill inside a centrifuge:

- Clear area of all personnel.
- Unplug the centrifuge.
- Wait 30 minutes for aerosol to settle before attempting to clean up the spill.
- 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.
- Wear a laboratory coat, safety glasses and gloves during cleanup. If there is splash potential, a face shield shall be worn.
- A respirator may be needed if aerosols are present.
  - If you feel you need to use a respirator, STOP clean-up activities and consult RMS for appropriate response.
  - If a respirator is deemed not necessary, initiate clean up.
- Remove rotors and buckets to the nearest BSC for cleanup.
- Thoroughly disinfect the inside of the centrifuge.
- Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures.

18.2.5 For a spill outside the laboratory:

- 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.
- Do not attempt to clean it up without appropriate PPE.
- Secure the area, keeping all people well clear of the spill.
- Call NDPD (Campus line: 911 or 574-631-5555) for assistance.
- Standby during the spill response and cleanup activities to provide assistance as requested or as necessary.

19. STORING AND TRANSPORTING BIOHAZARDS ACROSS CAMPUS

19.1 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.

19.2 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.

20. SHIPPING OF SAMPLES

20.1 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.

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

20.2.1 Training for Biological Substance Category B Shipping and Dry Ice Shipping is available through ComplyND.

20.2.2 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.

21. WASTE

21.1 Types of Waste and Storage Requirements

21.1.1 Animal Carcasses Waste

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

21.1.2 Autoclave Waste

- See Autoclave Safe Use and Validation Procedure for guidelines on safe autoclave use.
- Autoclaves shall be validated using a bio-indicator on a monthly basis. See Autoclave Safe Use and Validation Procedure for more information.
- 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.
- All infectious waste shall be stored in a secure location until it can be autoclaved.
- For wastes that can be autoclaved, the individual generator (researcher, department) shall:
  - Place waste in an autoclavable bag containing the Universal Biohazard Symbol on the outside surface.
  - 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.
  - Ensure the bag used for autoclaving can withstand the autoclave cycle without melting.
  - Once autoclaved, the sterilized waste shall be double bagged in a dark colored bag, sealed and labeled “Safe for Trash Disposal”.

21.1.3 Chemically Treated Waste

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

- Contaminated pipettes/beakers can be treated with bleach, rinsed and then reused or disposed.
- All infectious waste shall be stored in a secure location until it can be disinfected by chemical treatment.

21.1.4 Decontamination / Spill Cleanup – Chemical / Gas Disinfectant Waste

- Place an absorbent material (paper towel, bench diaper) over the contaminated surface,
- Add liquid disinfectant; this will prevent spread of contamination.
- Allow sufficient contact time (20 minutes) after applying the disinfectant.
    - When cleaning a spill of concentrated material or if the disinfectant shall act on an uneven surface, allow extra time for the disinfectant to act.
    - Avoid using concentrated or undiluted solutions of your disinfectant to "speed up" the inactivation process.
    - The surface being disinfected may be adversely affected by strong chemicals.
- Rinse the cleaned area with distilled water to avoid adverse effects on your experiment.
    - Some disinfectants will leave a residue of chemicals behind.
    - This is important in tissue culture rooms where a cell line can be ruined by disinfectant residue left on equipment.
- All disinfected spill cleanup materials shall be containerized for proper disposal. If chemically hazardous, the spill cleanup materials shall be disposed through RMS in accordance with the Hazardous Waste Procedure requirements.

21.1.5 Non-Autoclavable Waste

- Includes biohazardous, medical, and infectious waste not able to be rendered non-hazardous through autoclaving or chemical treatment.
- Includes biohazardous, medical, and infectious waste not able to autoclaved due to chemical contamination.
- A closable container lined with a red bag is required for the storage of non-autoclavable waste. A hands-free step activated red can is preferred.

21.2 Labeling Requirements

21.2.1 Infectious Waste

- All infectious waste shall be properly labeled with a biohazard symbol.

21.2.2 Biohazardous, Medical, and Animal Carcass Waste

- All biohazardous waste shall be properly labeled with a biohazard symbol.

21.2.3 Sharps Waste

- All Sharps containers shall be labeled with a biohazard symbol, the P.I. or supervisor's last name, room/lab number, and department. See Section 16 for more information.

21.3 Waste Disposal

21.3.1 The Biohazardous Waste Discard form is used to contact RMS to request a biohazardous waste pickup.

- The Biohazardous Waste Discard form shall be completed and submitted prior to the facility's scheduled waste pickup date. If the facility is not on the pickup schedule, RMS will contact the submitter to schedule a waste pickup.
- If successfully submitted, a confirmation email containing a link to the completed form will be received.

21.3.2 All infectious waste shall be stored in a secure location until RMS can pick it up for proper disposal.

21.3.3 Red bags must be tied off so nothing is protruding out of the top or sides of red bags.

21.3.4 Used and unused sharps shall be placed in a sharps container with a biohazard symbol. All sharps containers ready for disposal need to be completely sealed so nothing is protruding from the container and the lid and all safety tabs are fully engaged.

21.3.5 Contaminated animal carcasses shall be placed in red bags, frozen and secured by tying off the bag.

22. 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. REFERENCES

2. National Institute of Health publication, Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), January 2005 or as periodically updated
3. Centers for Disease Control (CDC) publication, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition
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5. NIH publication, Laboratory Safety Monograph, A Supplement to NIH Guidelines for Recombinant DNA Research, January 1979
6. Laboratory Safety: Principles and Practices, 2nd Edition, ASM Press, Washington DC, 1995,
7. Risk Management and Safety, Chemical Hygiene Plan, February 2021
8. Occupational Safety and Health Act (OSHA), Part 1910, Subpart Z, Section1910.1030
9. Bloodborne Pathogens, December 1991
10. National Research Council, Biosafety in the Laboratory, National Academic Press, Washington DC (1989).September 1996 B-1
11. 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	October 2019

from procedure body to Appendix, replaced the image versions of appendices with editable text versions.	
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

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.
- **Alternate Responsible Official (ARO)**– The approved alternate 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.
- **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 Safety Officer (BSO)**– the individual responsible for establishing and monitoring workplace safety procedures designed to minimize or prevent injury or loss due to biohazards in accordance with policies established by the institution.
- **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.
- **Entity** – any government agency-Federal, state or local- academic institution, corporation, company, partnership, society, association, firm, sole proprietorship or other legal entity.
- **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.

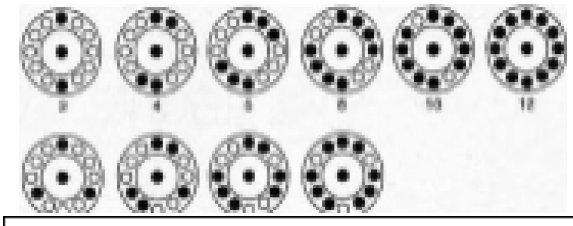


Figure 1. Balanced loading patterns for a 12-position micro centrifuge rotor.

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.

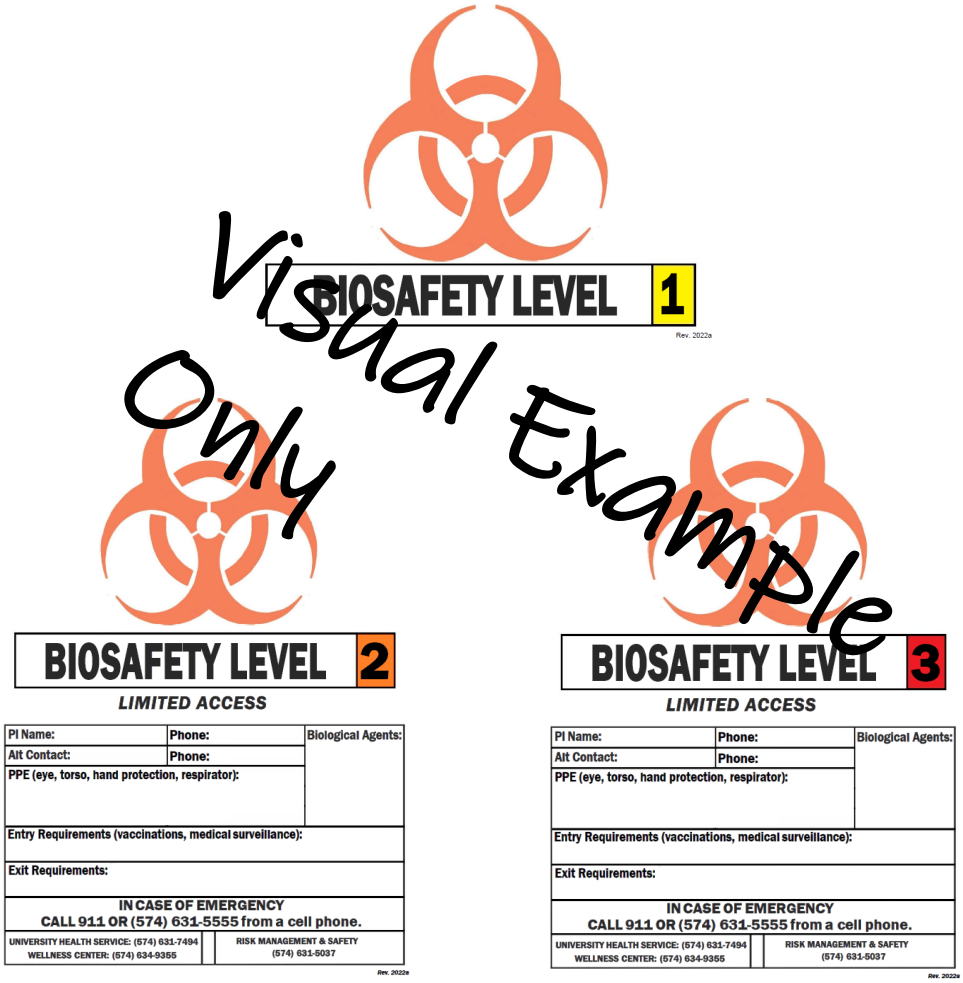
The following two tables provide general information only. Phenolics and quats are available in many formulations with different properties. Follow the manufacturer's recommendations for use.

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 (cationic detergents)	-Dilute according to manufacturer's instructions -Surfaces must be rinsed free of anionic soap or detergents before use -Commonly used to clean walls, floors, etc.	-Strong surface activity -Low toxicity -Non-corrosive -Effective over wide pH range	-Easily inactivated by organic materials, anionic detergents, and salts of metals in water (hard water) -Skin irritant

APPENDIX E – BIOSAFETY LEVEL CONTAINMENT SIGNS



APPENDIX F – INFECTIOUS / BIOHAZARD SYMBOL AND LEGEND