University of North Texas at Dallas

Biosafety Manual

(After UNTD Biosafety Manual, July 2019, ITR)

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I. Introduction

Biosafety encompasses the knowledge, techniques, equipment, and facilities necessary to prevent or minimize an exposure to, or release of, a biohazard. The University of North Texas at Dallas (UNTD) Biosafety Manual is intended to be a resource for information, guidelines, policies, and procedures that will enable and encourage safe teaching and research and to reduce, or eliminate, the potential for exposure to biohazards. The information presented here also reflects the requirements and guidelines of federal, state, and local regulations (see Appendix A – Guidelines and Regulations). The most current version of the UNTD Biosafety Manual is maintained on the Institutional Biosafety Committee website.

This UNTD Biosafety Manual is applicable to all laboratory teaching, research, and support activities that may involve biohazards. Biohazards include any microorganism (including, but not limited to, bacteria and their phages and plasmids, viruses, fungi, mycoplasmas, rickettsia, protozoa, parasites, or prions) or infectious substance; human and non-human primate tissues, body fluids, blood, blood byproducts, and cell lines; animal remains and insects that may harbor zoonotic pathogens; or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing death, disease, or other biological malfunction in a human, animal, plant, or another living organism; deterioration of food, water, equipment, supplies, or material of any kind; or deleterious alteration of the environment. Biohazards are often referred to as infectious agents or etiological agents (see Appendix B – Definitions and Acronyms).

In order to conduct animal research, UNTD will be required to have an occupational health and safety program that addresses potential hazards associated with the conduct of animal research; **such kind of research is not currently conducted at UNTD.** All laboratory animal facilities, their operational practices, and the quality of animal care are required to meet applicable standards and regulations (refer to *Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations* and the publication by the Institute for Laboratory Animal Research –ILAR- *Occupational Health and Safety in the Care and Use of Research Animal*).

In order to ensure that all activities involving potentially biohazardous materials are conducted in compliance with federal, state, and local regulations and applicable University policies, all research protocols must be reviewed and approved by the Institutional Biosafety Committee (IBC) prior to beginning work if they involve the use of any of the following:

- Select agents and toxins.
- Agents that can infect and/or cause disease in humans, animals, or plants.
- Experimentally infected animals and those naturally harboring zoonotic infectious agents.
- Recombinant and synthetic nucleic acid molecules.
- Genetically modified organisms.
- Transgenic plants, animals, and other organisms.
- Environmental/field samples (e.g., water, soil, and air samples).
- Human/nonhuman primate cell lines and other materials of human/nonhuman primate origin.
- Archaeological samples (e.g., bones, clothing fragments, and pottery).
- Biohazardous waste.

II. Biosafety Oversight

Guidance documents from the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) form the basis for the biosafety practices included in this manual. There are additional guidance documents and regulations imposed by various funding agencies that individual principal investigators must be aware of and incorporate into a Laboratory-Specific Biosafety Manual. Biosafety requirements must be followed not only to ensure the continuation of grant funding from federal agencies, but for health and safety purposes.

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*) detail safety practices and containment procedures for basic and clinical research involving recombinant or synthetic nucleic acid molecules, including the creation and use of organisms and viruses containing recombinant or synthetic nucleic acid molecules (https://osp.od.nih.gov/biotechnology/nih-guidelines/).The *NIH Guidelines:*

- Mandate the establishment of an Institutional Biosafety Committee for the review and oversight of biological research;
- Outline roles and responsibilities for biosafety; and
- Establish the practices, procedures, and conditions under which recombinant and synthetic nucleic acid activities must be conducted.

All institutions, including UNTD, receiving NIH funding for recombinant or synthetic nucleic acid molecules activities must comply with the *NIH Guidelines*. Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if NIH does not fund the individual project. **Non-compliance with the *NIH Guidelines* may result in suspension, limitation, or termination of financial assistance** for the research project and of NIH funds for other recombinant or synthetic nucleic acid activities at UNTD or the requirement for prior NIH approval of any and/or all recombinant or synthetic nucleic acid projects at UNTD.

The CDC/NIH manual, *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*), describes the appropriate measures and facilities for work with all microbial agents, including bacterial, viral, fungal, parasitic, rickettsial, and prion agents, as well as toxins of biological origin (https://www.cdc.gov/labs/BMBL.html). This publication, along with the University of North Texas (UNT) Biosafety Manual (https://riskmanagement.unt.edu/environmental-risk/biosafety), are the main sources of information utilized in this UNTD Biosafety Manual.

The requirements described in the Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogens regulation (29 CFR § 1910.1030; https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030) apply to work with human blood, tissue, organs, body fluids, and cell cultures. Special training, medical surveillance, procedures, and equipment must be in place for protection against bloodborne pathogens, needle sticks, and other sharps injuries. Handling and disposal of biohazardous waste is also regulated by this OSHA regulation and by other federal and state laws.

Packaging and shipment of biohazards are regulated by the Department of Transportation Hazardous Materials Regulations (49 CFR § 171-180; https://www.ecfr.gov/cgi-bin/text-idx?SID=028795e5b4a0b194cc473338c7237c13&mc=true&tpl=/ecfrbrowse/Title49/49CIsubchapC.tpl). In addition, permits may be required to ship biological materials; refer to the CDC Import Permit Program (https://www.cdc.gov/cpr/ipp/index.htm) and the Animal and Plant Health Inspection Service (APHIS) Permits and Certifications program (http://www.aphis.usda.gov/aphis/resources/permits).

Specific requirements for handling biological toxins are found in the *BMBL* and OSHA's Occupational Exposure to Hazardous Chemicals in Laboratories standard (29 CFR § 1910.1450 - https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1450).

Teaching and research activities involving the use of animals are regulated by the United States Department of Agriculture (USDA) Animal Welfare Act (https://www.nal.usda.gov/awic/animal-welfare-act), which covers all mammals used in research except rats of the genus *Rattus* and mice of the genus *Mus* that are bred for use in research. There are additional exceptions for agricultural research and teaching activities. Likewise, animal care and use facilities must be built and operate in compliance with the recommendations of the Institute for Laboratory Animal Research (ILAR) published in the *Guide for the Care and Use of Laboratory Animals*. Yearly reports by the institutions on the status of their animal care program are required. The Institutional Animal Care and Use Committee (IACUC) will oversee all research and teaching activities involving vertebrate animals; **such kind of activities are not currently conducted at UNTD.**

III. Roles and Responsibilities

The University of North Texas at Dallas (UNTD), its Department of Risk and Management Services (RMS), and the Institutional Biosafety Committee (IBC) promote a safe academic and work environment that complies with regulations and recommendations for biosafety, biosecurity, and the humane treatment of animals in research and teaching activities, as well as the health and safety of students, staff, researchers, the community, and the environment. Roles and responsibilities for biosafety and biosecurity include the following:

A. University of North Texas at Dallas (UNTD)

The University of North Texas at Dallas, through the Department of Risk and Management Services,has instituted and maintains a biosafety program for personnel who may be exposed to biological hazards (biohazards) during the performance of their duties. The biosafety program is designed to achieve regulatory compliance and to provide a means for employees to be informed about and protected from biohazards. To maintain regulatory compliance and to protect personnel from biohazards, UNTD must:

- Ensure appropriate training is provided to personnel conducting research with biohazards or recombinant or synthetic nucleic acid materials;

- Ensure that research conforms to the provisions of the *NIH Guidelines* and *BMBL*;
- Establish an Institutional Biosafety Committee (IBC) with adequate expertise and training;
- Establish an Institutional Animal Care and Use Committee (IACUC) to provide an animal care and use program that provides a humane and compliant environment for vertebrate animals that support the research and teaching programs of our researchers, teachers, and students;
- Appoint a Biological Safety Officer for the institution, **if it engages in large-scale research or production activities involving viable organisms containing recombinant or synthetic nucleic acid molecules and/or if it engages in recombinant or synthetic nucleic acid molecule research at BL3 or BL4 (*NIH Guidelines*);**
- Implement policies for safe conduct of biological and recombinant or synthetic nucleic acid research; and
- Report any significant problems, violations or significant research-related accidents or illnesses to the NIH Office of Biotechnology Activities within 30 days.

B. Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) is a standing committee established to ensure that all activities involving potentially biohazardous materials are conducted in compliance with federal, state, and local regulations and applicable university policies. The IBC seeks to reduce the risk of potential biohazardous threats to the UNTD community, the public, and the environment. The Institutional Biosafety Committee serves as defined in the *NIH Guidelines* and performs the following activities:

- Develops and implements policies, procedures, and guidelines related to the use of potentially biohazardous materials in university activities;
- Has the authority to review and approve procedures regarding teaching, research, and other activities at UNTD involving potentially biohazardous materials, including reviewing applications for research involving recombinant DNA (rDNA) to ensure that the research conforms to the *NIH Guidelines*;
- Notifies principal investigators (PIs), laboratory managers, the Office of Research and other UNTD committees of the results of the IBC's review of initial and renewal applications;
- Directs inspections of facilities where biohazardous materials are used and/or stored, and take appropriate actions (i.e., require modifications, disapprove, suspend, or terminate activities);
- Assists and advises the UNTD community on incorporating good biological safety practices into all activities involving potentially biohazardous materials, in compliance with applicable regulations and guidelines;
- Informs the UNTD administration of developments and practices regarding the use of potentially biohazardous materials and, upon request, provide an overall safety, health and environmental review of the university's activities involving potentially biohazardous materials;
- Files an annual report with the NIH Office of Science Policy, when necessary; and
- Reviews the UNTD Biosafety Manual every year and recommends revisions.

C. Principal Investigator (PI) and/or Laboratory Manager

The principal investigator (PI) or laboratory manager shall be a scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling biohazards, and must be responsible for the conduct of work with any biohazards or materials within the laboratory. The PI or laboratory manager is directly and primarily responsible for the safety of operations under his/her control. His/her knowledge and judgment are critical for assessing and controlling risks associated with the handling of potentially biohazardous materials, training laboratory personnel and students, and responding to emergency situations. The Pi or laboratory manager shall:

- Accept direct responsibility for the health and safety of those working with biohazardous materials, and/or select agents and toxins;
- Make available to all laboratory staff and students the protocols that describe the potential biohazards and the precautions to be taken;
- Instruct and train laboratory staff and students in the practices and techniques required to ensure safety, and the procedures for dealing with accidents;
- Inform the laboratory staff and students of the reasons and provisions for the Occupational Health and Safety Program, possible symptoms of illness relating to materials used, and provisions for any precautionary medical practices advised or required;
- Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
- Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Department of Risk Management Services, the Institutional Biosafety Committee, NIH OSP, and other appropriate authorities (if applicable);
- Correct work errors and conditions that may result in the release of recombinant or synthetic nucleic acid molecule materials;
- Adhere to approved emergency plans for handling accidental biohazardous spills and personnel contamination;
- Comply with permit and shipping requirements for recombinant or synthetic nucleic acid molecules, transgenic, or biohazards. This includes permits, material transfer agreements, and other documentation for international, interstate, and intrastate transport of genetically modified and biohazardous material;
- Develop specific biosafety standard operating procedures for biohazards used in the laboratory;
- Ensure compliance by laboratory personnel with relevant regulations, guidelines, and policies;
- Ensure all appropriate personal protective equipment is provided and used;
- Ensure and document proper training, including refresher training, and instruction for laboratory personnel in safe practices and protocols, including, at a minimum, training in aseptic techniques and characteristics of the material(s) used. Please refer to the UNTD Laboratory-Specific Biosafety Training Checklist. Maintain these documents within the lab and easily accessible at all times;

- Ensure the integrity of the safety equipment (e.g., biological safety cabinets), maintain biological containment (e.g., purity and genotypic and phenotypic characteristics), and ensure correct procedures or conditions are followed to prevent a release of or exposure to recombinant or synthetic nucleic acid molecules and/or biohazards, select agents or toxins;
- Ensure proper decontamination of the laboratory and the equipment as necessary to ensure safety during any required inspection, calibration, and recertification activity;
- Ensure proper treatment and disposal of all potentially biohazardous materials;
- Ensure and document proper functioning of decontamination equipment through monthly verifications using approved methodologies, when necessary;
- Propose appropriate microbiological practices and laboratory techniques to be used for the research;
- Maintain an inventory of all chemicals, vectors, microbial strains, viruses and other potentially biohazardous materials used or stored in their laboratory and make it available for inspection and submit annually to RMS/IBC;
- If the research activities submitted require approval by EPA, NIH, CDC, and/or USDA, the PI must obtain such approval prior to the approval of the protocol by the IBC. PIs shall not initiate or modify research involving potentially biohazardous materials that requires IBC approval until that research or the proposed modification has been approved by the IBC; and
- Obtain Institutional Biosafety Committee approval prior to initiating or modifying any research involving biohazardous materials, and/or select agents and toxins; and maintain that approval through timely submission of annual reviews.

Principal investigators are also responsible for full compliance with the *NIH Guidelines* during the conduct of recombinant or synthetic nucleic acid research. The Principal Investigator shall:

- Initiate or modify no recombinant or synthetic nucleic acid molecule research until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;
- Make the initial risk assessment and determination of biological containment levels in accordance with the *NIH Guidelines* when registering research with the Institutional Biosafety Committee;
- Develop specific biosafety standard operating procedures for recombinant or synthetic nucleic acid molecules or biohazards used in the laboratory;
- Obtain Institutional Biosafety Committee approval before initiating recombinant or synthetic nucleic acid molecule research subject to the *NIH Guidelines;*
- Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;
- Select appropriate microbiological practices and laboratory techniques to be used for the research;
- Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, III-B, III-C, III-D, or III-E (see *Experiments Covered by the NIH Guidelines*), to the Institutional Biosafety Committee for review and approval or disapproval;

- Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project and submit annual registration updates;
- Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination; and
- Immediately report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents, illnesses, or releases to the Institutional Biosafety Committee, the Department of Risk Management Services, NIH Office of Biotechnology Activities, and other authorities, as appropriate.

D. Laboratory Personnel and/or Students

The responsibilities laboratory personnel and/or students working in a lab include but are not limited to the following:

- Participate in appropriate training and instruction to ensure that they are adequately trained and fully understand the instructions, which include taking refresher courses as applicable;
- Fully comprehend all biohazards and select agents and toxins being used in the lab and the potential risks associated with exposure, as well as fully understanding the associated emergency response procedures;
- Follow all laboratory practices, protocols, and comply with all applicable policies, procedures, and guidelines;
- Complete any necessary medical surveillance;
- Report thefts, security incidents, accidents, spills, or contamination incidents to supervisor and/or RMS/IBC.

E. Other Organizations

Other committees, including the Institutional Review Board and the Department of Public Safety must consult and coordinate with the Institutional Biosafety Committee and RMS on any proposals under their purview, which involve the use of biohazardous materials at UNTD.

F. Contractors, Vendors, and Visitors

Contractors must ensure that appropriate personal protective equipment is available for their own workers.

All contractors, vendors, and visitors must:

- Use personal protective equipment provided for them by the laboratory or employer; and
- Comply with all security requirements and procedures.

IV. Training for Working Safely with Biohazards

The principal investigator and/or laboratory manager is responsible for providing or arranging for site-specific and/or online training of all personnel and his/her students. All training

must be documented. Refer to the UNTD Laboratory-Specific Training Checklist for more information and contact RMS or IBC for more information on scheduling and finding training, when necessary.

V. Research Project Registration

Each principal investigator is responsible for the preparation of the Institutional Biosafety Committee disclosure for all research involving potential biohazards, including the assignment of the required Biological Safety Level (BSL), as determined by a risk assessment. The Institutional Biosafety Committee will review all submitted registration documents, confirm, where applicable, that exempt status is appropriate for certain recombinant or synthetic nucleic acid work, and consider approval for those registration documents that are complete and that provide for safe handling of potential biohazards under the appropriate biosafety level. **Projects that are exempt from IBC oversight must still be registered with the IBC using the IBC registration form for exempted research.** Registration information and forms can be found on the Institutional Biosafety Committee website.

A. Research Requiring Registration

1. Select Agents and Toxins

Select agents are certain microorganisms and toxins specifically identified in federal regulations (https://www.selectagents.gov/). Select agents also include nucleic acids that encode for any select agent or toxin. Certain select agent toxins are not regulated as select toxins if the amount under the control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the following table:

PERMISSIBLE TOXIN AMOUNTS - HHS Toxins [§73.3(d)(7)]		
Select Agent Toxin	**CAS #**	**Amount**
Abrin	1393-62-0	1,000 mg
Botulinum neurotoxins	93384-43-1	1 mg
Diacetoxyscirpenol (DAS)	2270-40-8	10,000 mg
Ricin	96638-28-7	1,000 mg
Saxitoxin	35523-89-8	500 mg
Short, paralytic alpha conotoxins	76862-65-2 / 156467-85-5	100 mg
Staphylococcal enterotoxins (Subtypes A, B, C, D, and E)	11100-45-1	100 mg
T-2 toxin	21259-20-1	10,000 mg
Tetrodotoxin	4368-28-9	500 mg

Any research above these amounts or with any select agents must get prior approval. Contact the RMS/IBC for additional information.

The UNTD Biosafety Manual, when used in combination with the Laboratory-Specific Biosafety Manual, is designed to meet the federal requirements of the Department of Health and Human Services (HHS) Standard, 42 CFR § 73 (https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=8a4be60456973b5ec6bef5dfeaffd49a&r=PART&n=42y1.0.1.6.61) and the Department of Agriculture (USDA) Standards, 7 CFR § 331 (https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=b9126e9fba23e3e7933354a1d2630d72&ty=HTML&h=L&n=7y5.1.1.1.9&r=PART) and 9 CFR § 121 (https://www.ecfr.gov/cgi-bin/text-idx?SID=8208a7227c20f12dfb5b9273b2cef234&mc=true&node=pt9.1.121&rgn=div5).

2. Toxins of Biological Origin

Any biological toxin with a median lethal dose (LD_{50}) of less than 100 micrograms per kilogram body weight in vertebrates, must be approved by the Institutional Biosafety Committee prior to beginning research. Research with recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight requires pre-approval from the National Institutes of Health's Office of Biotechnology Activities. Examples of biological toxins with an LD_{50} of less than 100 nanograms per kilogram include the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin.

3. Human or Non-Human Primate (NHP) Blood and Tissue

In any laboratory where work involves the use of and/or exposure to human or non-human primate blood, body fluids, or unfixed human tissue, including cell cultures, there is the danger of exposure to bloodborne pathogens (disease-causing microorganisms) that may be found in such material. Research with material of human origin (e.g., blood, tissue, organs, and cell lines) is regulated by the Occupational Safety and Health Administration (OSHA). Work with this material must follow the Bloodborne Pathogens Exposure Control Plan included in this manual. In addition, when human blood or tissue donors are involved, the principal investigator must determine whether a human subject Institutional Review Board application is required. Work done with NHP blood or tissue must also be registered with the Institutional Biosafety Committee.

4. Recombinant and Synthetic Nucleic Acid Molecules

The use of recombinant and synthetic nucleic acid molecules is regulated by the NIH, as outlined in the *NIH Guidelines*. At UNTD this research must be reviewed by the Institutional Biosafety Committee prior to initiation of the work. Guidelines include registration of the recombinant or synthetic nucleic acid molecules, understanding the classification of the use of work, and safe work practices/proper disposal of material (including whole plants and animals) containing recombinant or synthetic nucleic acid molecules. The use of more than 10 liters of organisms containing recombinant or synthetic nucleic acid requires special practices and approval from the Institutional Biosafety Committee.

5. Environmental Samples

Environmental samples, such as water, air, soil, or plants, may contain pathogens (e.g., bacteria, viruses, and spores) that could present a health hazard to people, animals, or the environment. Using appropriate personal protective equipment when collecting environmental samples will reduce exposure to potential pathogens and minimize transfer of pathogens in the environment. Use care when handling environmental samples, especially if the sample will be enhanced or cultured in the laboratory or a greenhouse. Techniques used to enhance and/or culture environmental samples should be conducted at BSL-2 or higher levels in an appropriate containment device, such as a biological safety cabinet or fume hood and must be approved by the Institutional Biosafety Committee. If the environmental sample is sterilized prior to experimentation, then the sample may be manipulated in a BSL-1 rated laboratory and registered as an IBC exempt project.

6. Animals

In any laboratory where work involves the use of and/or exposure to live animals (including invertebrates/insects), there is a risk of physical hazards and injuries, including, but not limited to, bites and scratches, sharps injuries (needle sticks), chemical hazards, animal allergies, and zoonoses. Animal work done with controlled substances (for uses other than anesthesia or euthanasia), biological agents (bacteria, viruses, protozoa), or toxins must be approved by the IBC prior to initiation of work. **Activities involving vertebrate animals are not currently conducted at UNTD.**

7. Hazardous or Potentially Hazardous Biological Agents

The *NIH Guidelines* list the most commonly encountered infectious agents by risk group. The principal investigator is responsible for reviewing the *NIH Guidelines* and specifying in the IBC application the appropriate category for the proposed research or educational activity, subject to approval of such classification by the IBC. Agent risk groups may also be searched at the ABSA Risk Group Database (https://my.absa.org/tiki-index.php?page=Riskgroups). Inactivated biological samples derived from BSL-2 and above agents or attenuated pathogens derived from BSL-2 and above agents must also be registered.

8. Dual Use Research of Concern (DURC)

Dual use research (DUR) is research conducted for legitimate purposes that generates knowledge, information, technologies, and/or products that can be utilized both for benevolent and harmful purposes. Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security. Any DURC research must be submitted and approved by the IBC and other governing bodies prior to initiation.

9. Other Biological Research

Other biological research conducted at UNTD that does not meet the criteria of numbers 1-8, but uses biological materials (such as animal cells or tissues), must be registered with the IBC as an exempt project. If you are uncertain, or if you have questions, contact RMS/IBC.

B. Registration/Approval Process

1. The Principal Investigator or Laboratory Manager will obtain and complete an IBC Registration Form or an IBC Registration for Exempt Projects form. These forms are available online.

2. The completed and signed registration forms will be submitted to the IBC website.

3. The submitted form will be assigned a unique IBC file number.

4. Depending upon the level of risk presented by the proposed research and the requirements of any granting agency supporting it, the proposed activity may be initiated immediately following review by the IBC Chair, or it may not be initiated until formal approval is issued by the full IBC and any other required agency (e.g. NIH, USDA).

5. Registrations requiring full IBC committee approval must be received at least two weeks prior to an IBC committee meeting to be considered for a vote at that meeting. Expedited reviews or approvals by a subgroup of the IBC on behalf of the entire IBC for research subject to the *NIH guidelines* is not in keeping with the requirements of the *NIH guidelines* and is therefore not permitted. Reviews and approvals may only be conducted when a quorum of the IBC is present at a convened meeting.

6. The protocol approval will be granted for 3 years. Upon the completion of 3 years, a new registration form must be submitted to IBC for approval.

7. The PI is also required to submit an amendment form /new registration for registering any changes to the protocol. The amendment form is available online.

VI. Controlled Substances

The Controlled Substances Act (Title II of the Comprehensive Drug Abuse Prevention and Control Act of 1970; https://www.govinfo.gov/content/pkg/STATUTE-84/pdf/STATUTE-84-Pg1236.pdf) places all substances regulated by federal law into one of five schedules or categories based on the medicinal value and the potential for abuse. The Drug Enforcement Administration (DEA), part of the U.S. Department of Justice, has control and enforcement authority for controlled substances. Many drugs used for medical treatment, anesthesia, analgesia, or euthanasia are considered controlled substances. In order to legally purchase, store, use, dispense, and dispose of these drugs a DEA license is required. The following table lists the five schedules of controlled substances:

DEA Controlled Drug Schedule			
	Potential for Abuse	**Medical Use**	**Examples**
Schedule I	High	None	Heroin Hydromorphinol Marijuana Lysergic Acid Diethylamide
Schedule II	High	With restrictions	Fentanyl Methadone Oxymorphone Pentobarbital
Schedule III	Less than I or II	Currently accepted medical use	Euthanasia solutions Nalorphine Buprenorphine Ketamine
Schedule IV	Low	Currently accepted medical use	Chloral Hydrate Phenobarbital Butorphanol
Schedule V	Lower than IV	Currently accepted medical use	Codeine

Principal investigators who use controlled substances in their laboratory must obtain a Researcher DEA license. Information on how to apply and further details and requirements can be found on the DEA registration website (https://www.deadiversion.usdoj.gov/drugreg/index.html#regapps).

All persons possessing controlled drugs must maintain specific records for a minimum period of 2 years per DEA requirements. Further, inventory records, usage logs, destruction/disposal logs, and transfer forms must be provided to RMS/IBC.

Any person who is registered with the DEA who violates record keeping requirements or abandons controlled substances will be subject to the civil penalties outlined in the United States Code (USC): 21 USC Sec. 842 (https://www.deadiversion.usdoj.gov/21cfr/21usc/842.htm).

NOTE: Abandoning substances is equivalent to distributing a controlled substance to an unauthorized person.

VII. Incident Response

A. Injury or Sudden Illness

Safety is an intrinsic part of each laboratory and/or other biohazardous operations;

work is planned so that exposures to potentially hazardous agents will not occur. However, accidents creating exposure to hazards do occur. These may involve spills or releases of potentially hazardous infectious or chemical agents. Also, failure of equipment and facility safeguards may place workers at a high risk of accidental exposure. Likelihood of severe injury or infection can be reduced if plans for emergencies are established and well known to all who need to know. For this reason, various regulations, standards, and the *NIH Guidelines* require the preparation of emergency plans for laboratories and facilities involved in biohazardous activities. It is not possible to recommend a single plan of action that would be applicable in all situations, therefore, **each principal investigator and laboratory manager is responsible for developing appropriate emergency procedures for his/her work area, limiting access to authorized individuals only, and make sure that these personnel and/or students are trained with regards to these emergency procedures.**

Principal investigators must be aware of the provisions for emergency procedures and preparedness, which must be incorporated into the Laboratory-Specific Biosafety Manual and used in the laboratory. Each laboratory should have a written emergency plan specifying the appropriate response to potential emergencies. Accidents and spills of infectious materials will be discussed in Emergency Procedures below. In addition, each principal investigator will submit to RMS/IBC the following:

- A completed Responsible Party Information Sheet (annually).
- Annual chemical and biological inventories.
- A Risk Assessment (see Appendix C - How to Conduct a Biological Risk Assessment) for each project and/or biological agent and toxin stored or used in the laboratory.

The following **basic principles** should be used in developing specific procedures for dealing with accidental spills or releases of potentially hazardous materials:

1. Call 911 when special first aid, resuscitation, transport, or rescue service is required. Clearly describe the situation and your location.

2. Place all contaminated materials in either a biological safety cabinet or appropriate containment so that medical help can enter the facility.

3. Notify the PI and/or laboratory manager.

4. Emergencies may include, but are not limited to, a biohazardous or hazardous chemical spill, fire, BSC malfunction, or a total power failure. **The primary objective in an emergency is preservation of personal safety and health.** Protecting the facility and the experiment are secondary to personal safety. If there is a hazardous spill in your work area, call 911 immediately, isolate the spill, and leave the area. Contact RMS (972-338-1829) as soon as possible for help with cleaning manageable spills.

5. Immediate personal safety overrides maintenance of containment. Evacuation takes priority. Get people out of the emergency area. If possible, biohazardous materials should be covered and contained. All equipment should be turned off. RMS must be

informed as soon as possible and will take charge of re-entry, clean-up, and other corrective measures.

6. The PI or laboratory manager is responsible for deciding whether to override containment procedures in case of serious injury or sudden illness.

7. It is essential that the authorized users of the laboratory familiarize themselves with the procedures detailed in this manual. Personnel should be aware of the location and operation of all exits, fire extinguishers, fire alarms, eyewash stations, safety showers, spill and first aid kits. Questions about these procedures should be directed to the PI, the laboratory manager, or RMS/IBC.

KNOW WHAT TO DO BEFORE AN EMERGENCY OCCURS!

All accidents and sudden illnesses shall be reported as follows:

Each person involved in or supporting biohazard work shall report to his/her principal investigator or laboratory supervisor:

- Each accident (both injury causing and those without injury).
- Each accident resulting in damage to University or other property.
- Each situation or condition observed on the job that has the potential for either injuring or endangering the health of people and/or causing damage to property.

In case of injury, illness, disease, or exposure to infectious material or disease, the person involved or someone on his/her behalf, must report it to his/her department within 24 hours. Incidents involving injuries resulting in lost time, medical expenses, or resulting in a laboratory-acquired illness are immediately reportable to RMS (972-338-1829 using the Incident Report form.

Each PI and/or laboratory manager is responsible for reporting all biosafety accidents to the IBC within 48 hours. A Biohazard Incident Report form must be used and to properly document the accident; additional reports may be required. The IBC may be contacted for clarification and assistance with this requirement.

Medical evaluation is necessary if recognition of a disease early symptom developed. Treatment for non-emergencies should be obtained at the closest health care facility. Call 911 for emergencies, serious illnesses, or accidents. These events are those that result in:

- Fatality;
- Hospitalization or medical treatment (beyond first-aid) of any person;
  NOTE: This includes non-UNTD personnel or any other person;
- First-aid treatment of five (5) or more persons;
- Property damage exceeding $1,000.00; or
- Biohazard exposure resulting in lost time or accidental release of biohazards with a potential for involving the public or exposure of non-involved persons.

B. Exposures to Biohazards

In the event of an exposure to a biohazard, the following guidelines should be used:

1. Intact Skin

1. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur.
2. **Vigorously wash contaminated skin for 1 minute with soap and water.**
3. Call 911 or seek medical attention at the nearest medical clinic.
4. Inform the laboratory's principal investigator and/or laboratory manager immediately.
5. Submit an Incident Report form to RMS within 24h in case of an injury.
6. Submit a Biohazard Incident Report Form to the IBC within 48h.

2. Broken, Cut or Damaged Skin, or Puncture Wound

1. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur.
2. **Vigorously wash contaminated skin for 5 minutes with soap and water.**
3. Call 911 or seek medical attention at the nearest medical clinic.
4. Inform the laboratory's principal investigator and/or laboratory manager immediately.
5. Submit an Incident Report form to RMS within 24h in case of an injury.
6. Submit a Biohazard Incident Report Form to the IBC within 48h.

3. Eye

1. **Immediately flush eyes for at least 15 minutes with water, using an eyewash.** Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.
2. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur.
3. Call 911 or seek medical attention at the nearest medical clinic.
4. Inform the laboratory's principal investigator and/or laboratory manager immediately.
5. Submit an Incident Report form to RMS within 24h in case of an injury.
6. Submit a Biohazard Incident Report Form to the IBC within 48h.

4. Ingestion or Inhalation

1. Move to fresh air immediately and **do not induce vomiting unless advised to do so** by a health care provider.
2. Call 911 or seek medical attention at the nearest medical clinic.
3. Inform the laboratory's principal investigator and/or laboratory manager immediately.
4. Submit an Incident Report form to RMS within 24h in case of an injury.
5. Submit a Biohazard Incident Report Form to the IBC within 48h.

C. Spills of Biohazards

UNTD does not have a centralized biological spill response team. Therefore, each

laboratory working with potentially hazardous biological material must be prepared and trained to handle its own biological spills. RMS/ IBC are available for assistance if necessary.

Performing all work on plastic-backed absorbent liners to absorb spills can minimize the consequences of a spill of a biohazard. The quantities of these materials should be limited so they can be easily contained, cleaned, or destroyed. A simple spill kit with the following supplies should be made available by the PI and/or laboratory manager and used by trained personnel:

- Bleach or other EPA-registered disinfectant (see https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants and Appendix D - Disinfection Tables)
- Biohazard bag
- Disposable lab coat
- Disposable shoe covers
- Hand sanitizing wipes
- Nitrile gloves (4 pairs – one per each size)
- Mini brush and dustpan (or something to scoop spilled materials)
- Paper towels
- Safety goggles
- Tong or forceps to pick up broken glass
- Spray bottle (to make fresh bleach solution)
- "Biohazard Spill" sign (stating: "DO NOT ENTER, BIOHAZARD SPILL, contact [name and phone#] for information"

1. Spills Inside a Biological Safety Cabinet

1. Remain calm and secure research samples.
2. Alert the other laboratory employees of the spill.
3. Leave the Biological Safety Cabinet (BSC) turned on.
4. While wearing gloves, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
5. Soak up disinfectant and spill with paper towels.
6. Pick up any pieces of broken glass with forceps or tongs and place in sharps container. Never use hands to pick broken glass up.
7. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
8. Dispose cleanup materials in the biohazard waste container.
9. Wash hands and any exposed surfaces thoroughly after the cleanup procedure.
10. Report the spill to the laboratory's principal investigator, laboratory manager, or supervisor if there was a potential for any biohazardous material escaping the Biological Safety Cabinet.
11. Resume work if deemed safe by supervisor/manager.

2. Small Spill (<500 mL) Outside a Biological Safety Cabinet

1. Remain calm and make note of whether your person has been contaminated.
2. Alert other laboratory employees in the area and block off the area.
3. Wearing gloves, safety glasses, and a lab coat, cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
4. Pick up the towels and discard into a biohazard container.
5. Pick up any pieces of broken glass with forceps or tongs and place in sharps container. Never use hands to pick broken glass up.
6. Re-wipe the spill area with disinfectant and thoroughly wash hands after glove removal.
7. Report the spill to the laboratory's principal investigator, laboratory manager, supervisor, and to RMS/IBC immediately.
8. Resume work if deemed safe by supervisor/manager.
9. Submit Biohazard Incident Report to RMS/IBC.

3. Large Spill (>500 ml) Outside a Biological Safety Cabinet

1. Remain calm and hold your breath and leave the room immediately if no other workers are present. Otherwise,
2. Warn others to stay out of the spill area to prevent spread of contamination.
3. Post a sign stating: "DO NOT ENTER, BIOHAZARD SPILL, contact [name and phone#] for information" and block off area as possible.
4. Remove any contaminated clothing, ensuring that clothing is not pulled over the face, and put into a biohazard bag for later autoclaving.
5. Wash hands, eyes and exposed skin.
6. Notify the principal investigator, laboratory manager, supervisor, and RMS/IBC immediately.
7. Wait 30 minutes before re-entering the contaminated area to allow for dissipation of aerosols.
8. Meanwhile, put on protective clothing (lab coat, gloves and, if indicated, respirator, eye protection, and shoe covers) and assemble clean-up materials.
9. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
10. Collect all treated material and discard in a biohazard container.
11. Pick up any broken glass with forceps and place them into a sharps container. Never use hands to pick broken glass up.
12. Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of clean-up.
13. Submit Biohazard Incident Report to RMS/IBC.

4. Small Spill (<500 ml) of r/sNA Molecules

1. Put on gloves and eye protection if you are not already wearing them.
2. Cover spilled material with an absorbent paper towel or Kimwipe. Once the absorbent material is in place over the spill, wet the material with a 10% solution of bleach or other EPA-registered disinfectant.
3. Let stand 15 minutes, wipe up and wash surface with appropriate disinfectant.

4. Wipe down all equipment and surfaces that may have been splashed.
5. Dispose of contaminated paper towels as infectious waste.
6. Submit Biohazard Incident Report to RMS/IBC.

5. Large Spill (>500 ml) of r/sNA Molecules in a Biological Safety Cabinet

1. Biological safety cabinets must run during cleanup to contain aerosols and to filter exhaust air.
2. Put on appropriate personal protective gear before initiating cleanup.
3. Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is not acceptable.
4. If the spill is contained on a bench pad, remove the contaminated bench pad and discard it as infectious waste.
5. If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time, then remove the contaminated towels and discard them as infectious waste.
6. Wipe down the interior of the cabinet and any splatter on items within the cabinet with a disinfectant-soaked towel.
7. Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
8. Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
9. Place contaminated re-usable items in biohazard bags, autoclavable pans with lids, or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use.
10. If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required, as follows:
    a. Ensure the drain valve under the cabinet is closed.
    b. Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
    c. Absorb spilled fluid-disinfectant from work surface with paper towels and discard in biohazard bag.
    d. Prepare to empty drain pan. Place disinfectant solution in a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.
    e. Open the drain valve and empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water and remove the flexible tubing. Manage contaminated materials as if they are infectious.
11. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands when gloves are removed.
12. Notify principal investigator, laboratory manager, supervisor, and RMS/IBC if there was a potential for any material escaping the Biological Safety Cabinet. Consult with RMS/IBC to determine whether formaldehyde decontamination of the

cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.

13. Run the biological safety cabinet at least 10 minutes after cleanup, before resuming activity in the cabinet.
14. Submit Biohazard Incident Report to RMS/IBC.

6. Large Spill (>500 ml) of r/sNA Molecules Outside a Biological Safety Cabinet

1. If a spill of a biohazard occurs outside the biological safety cabinet, notify other individuals in the laboratory to evacuate.
2. Exit the laboratory to the hallway, closing the door behind you.
3. Remove any contaminated clothing (turn contaminated portion inward) and place it in an autoclave bag.
4. Wash all exposed skin.
5. Place signs on door(s) to the laboratory warning individuals who may want to enter that a spill occurred and access is denied.
6. Allow aerosols to settle for 30 minutes before re-entering the laboratory.
7. Notify the principal investigator, laboratory manager, supervisor, and RMS/IBC prior to proceeding with cleanup.
8. Assemble supplies (e.g., disinfectant, sharps containers, towels, tongs, and autoclave bags) before entering the laboratory.
9. Put on appropriate personal protective equipment (e.g., disposable gown, protective eyewear, gloves, shoe coverings, and respiratory protection if needed).
10. Clean up spill with a suitable disinfectant as follows:
    a. Surround spill area with disinfectant or diking material that is soaked in disinfectant.
    b. Place paper towels soaked in a disinfectant over the entire spill area.
    c. Allow 20-minute contact time with the disinfectant to ensure adequate germicidal action.
    d. Wipe down non-autoclavable materials with germicidal disinfectant.
    e. Place items designated as contaminated used sharps in an appropriate infectious waste sharps container. Place other disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
    f. Place contaminated re-usable items in biohazard bags or autoclavable pans with lids. Sterilize preferably by autoclaving, and then clean for re-use.
    g. Remove protective clothing used during cleanup then place in a biohazard bag for autoclaving.
    h. Wash hands when gloves are removed.
11. Submit Biohazard Incident Report to RMS/IBC.

7. Spill of Biohazards (Including r/sNA Molecules) in a Centrifuge

A single centrifuge spill or release can lead to multiple infections in a laboratory. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. Therefore, whenever opening a centrifuge, it must be performed slowly. Using adequate personal protective equipment (PPE), follow these procedures, depending on the type of buckets:

a. Unsealed Buckets

1. If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening.
2. Unplug centrifuge before initiating clean up.
3. Put on two pairs of nitrile gloves and other PPE before proceeding with clean up.
4. Flood centrifuge bowl with a disinfectant (e.g., 10% bleach solution or other EPA-registered disinfectant).
5. Place paper towels soaked in a disinfectant over the entire spill area. Allow 20 minutes contact time.
6. Remove broken tubes and glass fragments using tongs or forceps. Place fragments in a sharps container for autoclaving and disposal as infectious waste.
7. Remove buckets, trunnions, and rotor and place in disinfectant for 20 minutes or autoclave.
8. Unbroken, capped tubes may be placed in disinfectant and recovered after 20 minutes contact time or autoclaved.
9. Remove remaining disinfectant soaked materials from centrifuge bowl and discard as infectious waste.
10. Place paper towels soaked in a disinfectant in the centrifuge bowl and allow it to soak overnight, wipe down again with disinfectant, wash with water and dry. Discard disinfectant soaked materials as infectious waste.
    NOTE: Household bleach is a corrosive. Use caution when immersing or having metal components in contact with bleach (sodium hypochlorite) for extended periods of time.
11. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands whenever gloves are removed.
12. Notify principal investigator, laboratory manager, supervisor, and RMS/IBC.
13. Submit Biohazard Incident Report to RMS/IBC.

b. Sealed Buckets (Safety Cups)

1. If breakage is suspected, remove the sealed bucket to a biological safety cabinet before opening.
2. If breakage occurred, replace the cap on the safety cup loosely and autoclave.
3. Notify principal investigator, laboratory manager, supervisor, and RMS/BSO if there was a potential for any material escaping the centrifuge.
4. Submit Biohazard Incident Report to RMS/IBC.

VIII. Incident Reporting

A. Reporting Exposures

In the event of an exposure to a biohazard:

1. Report to the closest health care facility (contact RMS at 972-338-1829 for assistance).
2. Complete an Accident/Illness Report form and submit to RMS within 24 hours of incident.

3. Complete a Biohazard Incident Report form and submit to IBC within 48 hours of incident.
4. If exposure or incident occurs with s/r NA, work with the principal investigator, laboratory manager, supervisor, and IBC to report accident to the NIH Office of Biotechnology Activities as required by the *NIH Guidelines.*

B. Reportable Incidents and Violations

Incidents or problems involving biohazards and/or recombinant or synthetic nucleic acid molecules must be immediately reported to the principal investigator, laboratory manager, and/or supervisor. Examples of reportable significant incidents include, but are not limited to, any overt exposure, such as a needle stick, splash, and contamination due to equipment failure, and any potential exposure to biohazards. A significant event may also occur from a containment breach, which may be subsequently determined to pose either an overt or potential exposure to individuals.

It should be noted that waste from recombinant or synthetic nucleic acid molecule research is considered biohazardous and incidents involving improper disposal of recombinant or synthetic nucleic acid molecules must also be reported. Questions regarding reportable incidents should be directed to the RMS/IBC.

Failure by research personnel to follow federal and institutional regulations, guidelines, policies and/or procedures may also require reporting to the appropriate institutional, local, state and/or federal agencies. Violations may include but are not limited to conduct of new or ongoing research without appropriate federal or institutional registration, review, approval, or oversight.

C. Principal Investigator/Laboratory Manager Responsibilities

The principal investigator or laboratory manager and their personnel must report any significant incident, violation of the *NIH Guidelines*, or any significant research-related accidents and illnesses immediately by contacting RMS/IBC. Examples of incidents and violations include:

- Overt exposures, which are defined as exposures that result in direct personnel exposure to biohazards such as injection, spills, splashes, or aerosol inhalation.
- Potential exposures, which are defined as exposures that have a high risk of exposing personnel to biohazards such as spills, containment failure while working with the agent, or equipment failure that may produce aerosols.
- Overt or potential exposures in BSL-1 or BSL-2 laboratories.
- Any illness that may be caused by the agents used in the laboratory.
- Any incident involving the improper disposal of recombinant or synthetic nucleic acid molecules.

D. Institutional Biosafety Committee Responsibilities

The Institutional Biosafety Committee is required, by the *NIH Guidelines*, to report to

the appropriate University Official and to the NIH OSP within 30 days any significant incidents, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses. The Institutional Biosafety Committee will be responsible to determine what actions, if any, are necessary. For example the Institutional Biosafety Committee may choose to change the frequency of lab inspections, or change the biosafety level of the disclosure, based on results of the incident. Other Institutional Biosafety Committee reporting requirements (to the NIH OSP and other agencies) include but are not limited to:

- ***ANY*** research involving recombinant or synthetic nucleic acid molecules or biohazards without prior Institutional Biosafety Committee approval.
- Relaxed security, unsafe procedures used in a laboratory setting, and improper disposal of recombinant waste.
- Significant changes to proposed research risk without prior notification and approval by the Institutional Biosafety Committee.

Some incidents must be reported to the NIH OSP on an expedited basis. Spills or accidents in BSL-2 laboratories (involving recombinant or synthetic nucleic acid molecules) resulting in an overt exposure must be immediately reported to the NIH OSP. The Institutional Biosafety Committee will report to the Institutional Official, who, in turn will direct the reporting process to the NIH OSP of any of the above-described incidents.

Institutional violations that will be reported to the appropriate college or department head may include, but are not limited, to:

- Lapses in disclosure approval.
- Failure to comply with institutional and federal regulations, guidelines, and policies.
- Unsafe work practices.

E. Institutional Official Responsibilities

Upon receiving a report from the Institutional Biosafety Committee, the Institutional Official will directly report:

- In writing, any problems with or violations (non-compliance) of the *NIH Guidelines*, or any significant incident, accidents, or illnesses related to recombinant or synthetic nucleic molecules, to the NIH OSP within 30 days or immediately for overt exposure to a BSL-2.
- Any significant research-related illness or accident that may be hazardous to the public health and cooperate with state and local public health departments.

IX. Risk Group Classifications

According to the *BMBL*, the three primary hazardous characteristics associated with biological agents include:

- The capability of an agent to infect and cause disease in a susceptible human or animal

host;
- The virulence of an agent as measured by the severity of disease; and
- The availability of preventive measures and effective treatments for the disease.

By taking the route of transmission of the disease into consideration, a standardized methodology was developed to classify biological agents into four different risk groups (see Table below). Knowing the risk group of an agent assists researchers and safety professionals in determining the appropriate safety protocols to be followed. The risk group, in conjunction with several other factors, helps to determine the biosafety level that should be utilized in the laboratory. PIs must perform a Risk Assessment (see Appendix C - How to Conduct a Biological Risk Assessment) for all projects.

Risk Group (RG) Classifications			
RG-1	**RG-2**	**RG-3**	**RG-4**
Agents not associated with disease in healthy adult humans.	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

X. Biological Safety Levels

Based on the degree of hazard associated with a microbial agent, CDC and NIH have established four levels of biosafety to describe the combination of laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure (see *BMBL* for more information). The *BMBL* outlines four different biological safety levels that are appropriate for the operations performed in a laboratory, the documented or suspected routes of transmission of the biological agent, and the laboratory function or activity. These four biosafety levels (BSL) require successively more stringent practices and facilities as work moves from the least restrictive, BSL-1, to work with the highest hazard level of BSL-4. Exposure to biohazards may be prevented or limited by establishing and following the appropriate biosafety level practices and conditions. The requirements for each laboratory biosafety level can be found in the *BMBL*. UNTD currently only has facilities appropriate for BSL-1 and BSL-2 level work.

The following bullets provide a brief summary of the four biological safety levels:

- **BSL-1** is required for work involving well-characterized agents not known to

consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment.

- **BSL-2** is required for work involving agents that pose moderate hazards to personnel and the environment.
- **BSL-3** is required for 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.
  NOTE: No research or any other activities with biohazards at BSL-3 are currently permitted in UNTD facilities.
- **BSL-4** is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.
  NOTE: No research or any other activities with biohazards at BSL-4 are currently permitted in UNTD facilities.

Personal protective equipment varies depending upon the biological safety level. The table below contains the basic requirements for each of the four biological safety levels. Refer to section XV - Personal Protective Equipment for detailed information and UNTD requirements.

Biological Safety - Personal Protective Equipment (PPE) Requirements*			
BSL-1	**BSL-2**	**BSL-3**	**BSL-4**
Protective laboratory coats, gowns, or uniforms recommended for preventing contamination of personal clothing. **Protective eyewear** worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses in laboratories should also wear eye protection. **Gloves** must be worn to protect hands from exposure to hazardous materials.	**Protective laboratory coats, gowns, smocks, or uniforms** must be worn while working with hazardous materials. **Eye and face protection** (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms are handled outside the Biological Safety Cabinet (BSC) or physical containment device. Personnel who wear contact lenses in laboratories should also wear eye protection. **Gloves** must be worn to protect hands from exposure to hazardous materials. **Eye, face, and respiratory protection** should be used in rooms containing infected animals.	**NOT CURRENTLY PERMITTED AT UNTD.** **Protective laboratory clothing with a solid-front**, such as tie-back or wrap- around gowns, scrub suits, or coveralls must be worn. **Eye and face protection** (goggles, mask, face shield or other splash guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials. [All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices.] Personnel who wear contact lenses in laboratories must also wear eye protection. **Gloves** must be worn to protect hands from exposure to hazardous materials. **Eye, face, and respiratory protection** must be used in rooms containing infected animals.	**NOT PERMITTED AT UNTD.** Refer to the CDC/NIH document, "Biosafety in Microbiological and Biomedical Laboratories" (*BMBL*) for PPE requirements.

* Safety is improved when PPE is used in combination with physical containment devices or equipment, such as Biological Safety Cabinets (BSCs).

XI. Animal Biosafety Levels

Similar to the BSL, there are four animal biosafety levels (ABSL). These four animal biosafety levels are required for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and also in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. **Such kind of research and activities are not currently permitted at UNTD.** Refer to *BMBL* (https://www.cdc.gov/labs/BMBL.html) for animal biosafety guidelines and the Guide for the Care and Use of Laboratory Animals (https://doi.org/10.17226/12910) for standards and regulations.

XII. Biohazardous Waste

Biohazardous waste is defined as materials containing:

- Infectious agents (to human, plants, animals)
- Biological toxins
- Materials derived from humans and primates (blood, body fluids, tissues)
- Human and primate tissue or cell lines (including recombinant)
- Non-human primate tissue or cell lines
- Recombinant plant and animal cell lines
- Recombinant microorganisms
- Transgenic animals (vertebrate and invertebrate)
- Materials derived from transgenic animals (body fluids, tissues)
- Transgenic plants
- Recombinant materials such as plasmids, DNA/RNA, and synthetic DNA
- Microbiological waste

The National Institutes of Health's "Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules" (*NIH Guidelines*) requires UNTD to manage discarded preparations made from genetically altered living organisms and their products as biohazardous waste. For example, recombinant or synthetic nucleic acid waste materials used in research laboratories is considered biohazardous waste. **All waste containing recombinant or synthetic nucleic acid molecules must be inactivated prior to disposal.**

A. Animal Biohazard Waste

Wastes unique to the animal facility include animal bedding and animal carcasses. These are generated along with the sharps and other biologically contaminated equipment that typically need to be discarded in all laboratories. All animal waste must be treated prior to disposal. **There is no animal facility at UNTD; nor is vertebrate animal research currently conducted at UNTD.**

B. Liquid Biohazard Waste

Autoclaving is the method of choice for disinfection of the following:

- Animal blood/body fluids from animals infected with BSL2 agents;
- Human tissue culture, human cell lines (primary or established);
- Human body fluids; and
- Liquid growth media removed from human tissue cultures.

Autoclaved liquid wastes may be discharged directly to the sanitary sewer.

Chemical disinfection may be an acceptable alternative to autoclaving liquid biohazard waste generated in research laboratories at UNTD such as bleach treatment. When this is done, care must be taken to avoid splash and the drains must be flushed with generous amounts of water.

Waste rules do not allow chemical disinfection of regulated liquids followed by disposal to the sanitary sewer unless approval has been obtained from RMS. To obtain approval for chemical treatment of infectious liquids, you must provide information demonstrating the effectiveness of the chemical being used to treat the specific microbiological agents, taking into account factors such as temperature, contact time, pH, concentration, penetrability and reactivity of organic material. All requests for approval must be submitted through RMS/IBC, and documented in the Laboratory-Specific Biosafety Plan.

Regulated liquids include the following:

- Liquid waste media from cells/tissue used for propagating risk group 1, 2, or 3 pathogens or toxins, including those produced in recombinant DNA procedures;
- "Microbiological waste": e.g. cultures and stocks of infectious agents; and
- Waste from animals intentionally infected with microbes, viral vectors, or toxins.

C. *Drosophila*

An alternative to autoclaving *Drosophila* is dumping anesthetized flies directly into a container with a small amount of mineral oil or a bottle containing either ethanol or isopropanol. If you do not plan to re-use the material, these bottles must be labeled as ethanol, isopropanol, or mineral oil waste to be picked up by RMS. If you are going to reuse the material you are dumping the *Drosophila* into, then you will label the bottle recycled ethanol, isopropanol, or mineral oil. **These bottles of chemicals cannot be poured down the sink or sanitary sewer. They must be discarded through RMS.**

D. Human Tissues/Body Parts

Recognizable human anatomical remains or tissues and large tissues must be disposed of by incineration. Remains contaminated with hazardous chemical or radioactive substances require special disposal and RMS must be contacted for disposal.

Unrecognizable human tissues can be autoclaved and disposed of in regular trash. If the tissues have been chemically preserved, they can be disposed of as chemical hazardous waste.

E. Handling and Disposal of Sharps

Sharps are objects that can penetrate an individual's skin, such as hypodermic needles, glass Pasteur pipettes, scalpel blades, pipette tips, broken vials and glassware, slides, and coverslips. If human blood or other potentially infectious materials, as defined in the OSHA Bloodborne Pathogens standard (29 CFR 1910.1030), is present or may be present on the sharp, it is a contaminated sharp and appropriate personal protective equipment must be worn.

An accident or injury involving a contaminated sharp may result in an individual being infected with human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), or other bloodborne pathogens. Careful handling of contaminated sharps can prevent injury and reduce the risk of infection. The UNTD Bloodborne Pathogens Exposure Control Program specifies measures to reduce these types of injuries and the risk of infection.

Safer Medical Devices

Wherever possible, departments are required to use safer medical devices, such as self-sheathing or retractable needles. These devices have built-in protection to guard workers against contact with the contaminated sharp. All individuals who may be potentially exposed to injuries from sharps are encouraged to provide input to their management and Risk Management Services (RMS) regarding the identification, evaluation, and selection of safer medical devices.

Sharps Containers

Used sharps must be discarded immediately or as soon as feasible into sharps containers. These containers must be puncture-resistant and the sides and the bottom must be leak-proof. Biohazardous sharps containers must be appropriately labeled and color-coded red to warn everyone that the contents are biohazardous. They must be closable (i.e., have a lid, flap, door, or other means of closing the container), and they must be kept upright to keep the sharps and any liquids from spilling out of the container.

During use, containers for used sharps must be easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found. Sharps containers must also be maintained upright throughout use, replaced routinely, and **not be allowed to overfill**. When moving sharps containers from the area of use, the containers must be:

- Closed immediately prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping; and
- Placed in a secondary container if leakage is possible. The second container must be:
  - constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping;

- closable;
- appropriately labeled or color-coded; and
- autoclaved prior to being disposed of as regular waste.

Sharps containers must not be opened, emptied, or cleaned manually or in any other manner that would expose individuals to the risk of accident or injury.

Recapping Needles

Contaminated sharps must never be sheared or broken. **Recapping, bending, or removing contaminated needles is prohibited.** However, in rare circumstances, recapping is permissible if it can be demonstrated by the department that no alternative is feasible or that such action is required by a specific procedure. Procedures that describe the recapping process must be written and included in the laboratory-specific safety plan. If recapping is necessary, individuals must use either a mechanical device or a one-handed technique. The cap **must not be** held in one hand while guiding the sharp into it or placing it over the sharp. A one-handed "scoop" technique uses the needle itself to pick up the cap, and then the cap is pushed against a hard surface to ensure a tight fit onto the device. The cap may also be held with tongs or forceps and placed over the needle. Immediately (or as soon as possible) after use, these sharps must be placed into appropriate containers until properly reprocessed or disposed.

Reporting an Accident or Injury

In the event of a needlestick, sharps injury, or exposure to human blood or other body fluid, immediately follow these steps:

1. Wash cuts and/or other needlestick injury with soap and water;
2. If there is exposure to the nose, mouth, or mucous membranes, flush with water;
3. If there is exposure is to the eyes, irrigate with clean water, saline, or sterile irrigants;
4. Report the incident to your supervisor; and
5. Immediately seek medical treatment.

It is highly recommended that post-exposure treatment, if indicated, be started as soon as possible following an exposure incident. If an exposure occurs, and an **employee** needs medical treatment, (s)he must be seen by a workers' comp in-network provider. If treatment is received by an out-of- network primary care physician, this will be at the expense of the injured employee and will not be covered by workers' compensation. For a life-threatening emergency, call 9-1-1 or seek medical treatment at the nearest Emergency Room. For non-emergency injuries, contact Risk Management Services (RMS) for assistance in obtaining authorization for medical care. The supervisor must complete the **Workers' Comp Employee Injury Report Form** and if the employee determines they need medical treatment at a later date, contact RMS for authorization of treatment.

If the injured person is a **student,** the individual should immediately tell the instructor and contact Risk Management Services (RMS). For a life-threatening emergency, call 9-1-1 or seek medical treatment at the nearest Emergency Room.

Supervisors must report all accidents and injuries to RMS and submit the Biohazard Incident Report Form within 48h. Federal, state, and local agencies may also need to be notified depending on the nature of the accident/injury. If the project involves recombinant or synthetic nucleic acids, the Institutional Biosafety Committee will be required to report any significant problems with or violations of the National Institutes of Health (NIH) Guidelines for Research with Recombinant or Synthetic Nucleic Acid Molecules and any significant research- related accidents or illnesses to the NIH within 30 days.

XIII. Disinfection and Decontamination

A. Decontamination

Decontamination is a process or treatment that renders an instrument or environmental surface safe to handle. A decontamination procedure can be as simple as clean-up with detergent and water or as thorough as sterilization. Sterilization, disinfection, and antisepsis are all forms of decontamination.

- **Sterilization** is the use of physical or chemical processes to destroy all viable forms of microbial life, such as bacterial spores.
- **Disinfection** is a chemical or physical treatment that destroys the most resistant vegetative microbes or viruses, but not the spores, in or on inanimate surfaces. Effectiveness is influenced by a number of factors, including the type and number of organisms, amount of organic matter, the object being disinfected, the disinfectant being used, concentration, temperature, and exposure time.
- **Antisepsis** is the application of a liquid antimicrobial to skin or other living tissue to inhibit or destroy microorganisms. Examples include hand washing with germicidal solutions or swabbing skin with alcohol before an injection.

B. When to Decontaminate

In most UNTD laboratories, it is recommended that decontamination be accomplished by steam heat sterilization in an autoclave or by surface application of or placement in a chemical disinfectant solution, such as 1:10 bleach solution or an EPA-registered disinfectant and applied per manufacturer instructions.

All material and equipment contaminated with or containing potential biohazards should be decontaminated:

- Upon completion of procedures involving the use of biohazardous material;
- In the event of spills of biohazards;
- Before being washed, stored, or discarded; and
- At least daily.

Regulated Medical Waste Disposal Chart

Blood and body fluids (**regulated medical waste**)	Treated with bleach or **autoclaved at 123°C for 70 minutes**.
Microbiological waste including Biosafety Level 1 and 2 organisms (**regulated medical waste**)	**Autoclaved at 123°C for 70 minutes** or chemically treated and sent to landfill.
Pathological waste (animal carcasses infected with human BSL1 and BSL2, this includes transgenic mice) (**regulated medical waste**)	Animals are incinerated by vendor (TBD)
Uninfected animal carcasses	Animals are incinerated by vendor (TBD)
Biohazardous sharps	**Red plastic sharps containers are autoclaved at 123°C for 70 minutes** then sent to landfill
Non-hazardous sharps	White plastic sharps containers sent to landfill

C. Autoclave Use

Autoclaving (saturated steam under pressure of approximately 15 pounds per square inch (psi) to achieve a chamber temperature of at least 121°C for a designated time) is the preferred and most convenient method to rapidly destroy all forms of microbial life. However, to do this, the autoclave process must reach proper temperature, pressure, and time, and also prevent the entrapment of air in the bag or container of treated material.

- Material to be sterilized must come into contact with steam.
- Bags or containers should be left open during autoclaving.
- Heat indicator tape is to be placed in an X outside the bag or container with each autoclave load to indicate that sterilization has been completed.
- Autoclave sterility monitoring must be conducted on a monthly using biological indicators (such as *G. stearothermophilus* spore strips) placed among treated materials and at locations throughout the autoclave. The spores, which are more resistant to heat than most other biological materials, provide validation of general microbial destruction when they are effectively inactivated by autoclave operation.

1. Autoclavable Waste Bags/Containers

Red polypropylene, preprinted, biohazard autoclave bags (Fisher Scientific 01-828 A through E) should be utilized in UNTD autoclaves. Do not use polyethylene bags, as these will melt at higher temperatures. **DO NOT** enclose the cardboard boxes used for gathering sharps/glass within an autoclave bag. This will prevent steam penetration during autoclaving. Steam penetration is crucial during the decontaminating process. **DO NOT** tape bag shut prior to autoclaving. Remember to line the boxes with a red autoclave bag marked with an "x" over the biohazard symbol before lining the box. **RED BIOHAZARD BAGS MUST NOT BE USED FOR ANYTHING OTHER THAN BIOHAZARDOUS WASTE.**

The outer collection container must be durable, leak proof, have a lid and be of such a design so as not to be mistaken by housekeeping personnel for regular trash. This container must be labeled with a biohazard sticker. Wire cages cannot be used as the outer container.

The marked biohazard container must be lined with a red autoclavable biohazard bag. Before lining the container with the red biohazard bag, crisscross the bag's biohazard symbol and/or markings with heat sensitive autoclave tape, (available from Fisher Scientific as stock number 15-903). The lid should be kept on the biohazard container when not in use. Remove bags at 2/3 full. Never place glass in these containers.

Research Lab/Clinic Pipetting

For **large-scale** collection **outside** the biosafety cabinet of glass (Pasteur) and plastic pipettes contaminated under the definition of biohazard waste, line a puncture-resistant outer container (such as the box the pipettes came in) with a red autoclave bag marked with a heat sensitive autoclave tape "x" (available from Fisher Scientific catalog #15-903) over the biohazard symbol. To avoid possible exposure, place the indicator tape "x" over the bag's biohazard symbol prior to loading the bag with pipettes. The universal biological hazard symbol must also be displayed on the outer container. When the box is full, close the inner bag leaving an opening for the steam to penetrate. Tape the outer box closed with autoclave tape. Do **not** use colored tape to close box.

Inside the Biological Safety Cabinet

For frequently removed **small scale** collection, such as sterile pipetting in a biological safety cabinet, line a small red autoclave bag inside a hard-walled collection container inside the cabinet. When the bag is 2/3 full, close it loosely, spray with proper disinfectant and transfer it to a larger scale pipette collection container located outside of the cabinet.

Another alternative for collecting biohazardous pipettes is to place them in a long, hard walled cylindrical container filled with an effective (EPA approved) disinfectant. The pipettes should be allowed to remain in the disinfectant for the recommended contact time to ensure decontamination.

2. Loading and Unloading the Autoclave Safely

Contaminated materials should **never** be left in hallways or other public spaces prior to autoclaving. Biohazard bags should never be left lying on the floor within labs. Biohazard bags should remain in the laboratory until they are ready to be placed in the autoclave. Never leave bags sitting on the floor next to the autoclave. Bags that are closed and ready for autoclaving must be placed in secondary containment. If the bags are being transported to the autoclave, they must be contained in closed, hard-walled secondary containers.

Minimize contact with biohazard waste as much as possible. Never crush or push down biohazard waste. Biohazard waste containers should be removed for autoclaving when they are

less than 2/3 full. Indicator tape should be applied when placing the new autoclave bag into the hard walled outer container; this will reduce handling of the biohazard waste during removal. The heat sensitive autoclave tape should be placed in an "X" pattern over the biohazard symbol. The heat sensitive tape is to be of the type that changes color, such as the type that the word "autoclaved" appears after treatment. This tape is available from Fisher Scientific catalog# 15-903. Once the autoclave disinfection is complete, the tops of the bags may be sealed tightly with lab tape.

After the proper autoclave waste decontamination steps are followed, the decontaminated waste is then placed in a 44 gallon or 32 gallon white Rubbermaid Brute container (with a drum dolly), lined with black plastic garbage bags, and located in the vicinity of the autoclave. These containers are to be labeled "AUTOCLAVED/DECONTAMINATED WASTE ONLY". Biohazard bags placed in the white Brute containers and marked with the heat sensitive tape signal to housekeeping personnel that the waste is safe and ready to be removed from the laboratory for disposal in the dumpster.

Each department is responsible for providing an adequate number of these containers which are available from Fisher Scientific. **Housekeeping personnel will not remove or otherwise handle overflowing waste or waste in untreated biohazard bags**.

3. Autoclaving Precautions

Autoclaving, or steam sterilization, is the most dependable procedure for the destruction of all forms of microbial life. Proper temperature and exposure time are critical factors in ensuring the reliability of this method. These critical factors are dependent upon steam penetration to every part of the waste load. Therefore, the autoclave user must be mindful to prevent the entrapment of air. If all the air is not allowed to escape from the waste during the cycle, it cannot be replaced by steam. Saturated steam is employed under pressure (at least 15 pounds per square inch) to achieve a chamber temperature of at least 121°C (250°F) for a minimum of 15 minutes. This time is measured **after** the temperature of the steam saturated material being sterilized reaches 121°C.

The hazards associated with autoclaves include extreme heat and high pressure and large, heavy doors and loading carriage. When operating an autoclave the following safety procedures must be followed:

- Become familiar with the autoclave's owner's manual. Though the principle is the same for each, manufacturer recommendations for use can vary widely.
- Firmly lock autoclave doors and gaskets in place before you run the autoclave to prevent a sudden release of high-pressure steam. Some autoclaves do not have safety interlocks that prevent the autoclave from running if the door isn't closed properly. If your autoclave does not have safety interlocks, you will need to take additional precautions to ensure that the doors are closed.
- If you have an older autoclave that has little or no heat shielding around the outside, attach signs warning of "Hot Surfaces, Keep Away" on or next to the autoclave to remind people of the hazard.

- Do not stack or store combustible materials (cardboard, plastic, volatile or flammable liquids, compressed gas cylinders) next to an autoclave.
- Do not autoclave toxic, volatile or radioactive material. If you have biohazard waste that contains any of these materials, please contact RMS for guidance.
- When a cycle is complete, wait approximately 1-2 minutes after the pressure gauge reads zero before opening the door of the autoclave.
- Wait at least 30 seconds after opening the door before reaching or looking into the autoclave.
- Open the door slowly, keeping head, face, and hands away from the opening.
- Allow contents to cool before removing them from the autoclave.
- Remove solutions from the autoclave slowly and gently; some solutions can boil over when moved or when exposed to room temperature. Thick, heat-resistant gloves, safety goggles or face shield and a rubber apron must be worn when removing hot liquids from the autoclave. Liquids should stand for over 1 hour before being handled without heat-resistant gloves.
- Clean up any spills immediately.
- Report any malfunctions or accidents immediately to your supervisor.

Training

All employees that use an autoclave must complete the online autoclave safety training. To ensure that infrequent users do not neglect proper operating techniques, autoclave operating instructions should be posted in close proximity to the autoclave.

4. Autoclave Waste Decontamination Procedures

The autoclave is to be operated at 121°C or higher for a minimum of 70 minutes for most biohazard waste (see chart below). The time and temperature used for each type of waste in the laboratory must be validated using biological indicators to ensure effective sterilization (see procedure below). Some autoclaves are equipped to operate at higher temperatures, which would allow for shorter exposure times.

Criteria for Autoclaving Typical Materials		
Material	**Temperature**	**Time**
Laundry	121 C°	30 minutes
Biohazard bags, <2/3 full, containing infectious waste	**123 C°**	**70 minutes**
Glassware	121 C°	60 minutes
Liquids	121 C°, each gallon	60 minutes
Animals	121 C°	8 hours

Use the appropriate autoclave settings. Autoclaves may have settings for "LIQUIDS" to be used for liquid materials. "LIQUID" settings run for longer periods at lower temperatures to minimize liquid evaporation and spills. For solid materials, the "DRY GOODS WITH VACUUM" should be used for infectious waste as it is the

most effective at moving steam and heat into the deepest parts of large bags producing the best conditions for killing persistent organisms. "DRY GOODS WITHOUT VACUUM" should only be used for clean items that need to be sterilized. Exhaust settings should also be appropriate for the type of waste being autoclaved. FAST exhaust should be used for solid items and SLOW exhaust should be used for liquids.

Solid waste

Do not overfill waste bags or the autoclave. This will interfere with steam penetration. Keep the waste bags slightly open to allow for steam penetration. Bags are placed into stainless steel or polypropylene trays prior to autoclaving.

Liquid waste

Liquids should be placed in borosilicate (Kimax or Pyrex) or polypropylene containers for autoclaving. The containers should not be filled to more than 75% capacity. The caps or stoppers on the containers should be loosened. **Never autoclave sealed containers of liquid. This could result in an explosion of superheated liquid**. Liquid containers should be placed in a stainless steel or polypropylene tray with ¼ to ½ inch of water in the bottom of the tray. The tray should be placed on a shelf in the autoclave and not on the bottom of the chamber.

Medical waste rules state that autoclaves are to be provided with a chart recorder which accurately records time and temperature for each cycle.

5. PI Autoclave Waste Decontamination Cycle Testing and Verification

Geobacillus stearothermophilus biological indicators must be used **monthly** with waste using average spore populations of 10^4; to 10^6; organisms. There are many commercially available biological indicators with a choice of spore ampoules or spore strips with growth media.

Follow the instructions provided by the manufacturer of the biological indicators. Many require refrigeration when kept in storage.

Place the indicator in the middle of the waste bag or material to be autoclaved. It is best to put the indicator in the waste bag before it is filled completely. To aid recovery of the indicator after sterilization, tape it to a brightly colored sheet of paper or to a long string allowed to protrude from the bag. Indicators can also be placed in test waste bags filled with materials that simulate full loading for the test.

Autoclave the waste following normal procedures. Once the cycle is complete and contents have cooled, remove the indicator from the waste bags wearing appropriate protective equipment. Prepare and incubate the indicator and a control indicator that was not autoclaved as recommended by the manufacturer.

Check for signs of growth at regular intervals during the incubation period (8, 12, 24 and 48 hours). There should be signs of growth on the control indicator that was not autoclaved or the test is invalid. If there are signs of growth on the indicator placed in the waste, the waste was not sterilized properly. The time, temperature and autoclave procedures should be re-evaluated. If an autoclave problem is suspected, Facilities Services or the service contractor must be contacted immediately for repair.

A log of each test must be maintained for 3 years (Texas Administrative Code Title 30 Chapter 326), which includes the type of indicator used, date, time, and result of the test. An Autoclave Testing log is available for download at the RMS website. Submit the log annually to RMS/BSO.

The waste does not have to be held until the results of the testing confirm effectiveness. If test results indicate that the autoclave is not sterilizing properly, the autoclave should not be used for waste until it has been repaired. The first load run in the autoclave should be tested with a biological indicator to insure proper functioning of the autoclave.

6. Autoclave Preventative Maintenance

Autoclave operators should perform the following preventative maintenance on their autoclave to preserve the autoclave's effectiveness:

- Remove the plug screen or drain strainer to make sure it is free of dirt, dust, or sediment that may collect in it and it should be cleaned as necessary.
- Clean the interior surfaces of residues collected from the steam or materials being sterilized as needed.
- Visually inspect the gaskets, doors, shelves and walls for residue buildup or wear regularly.
- Report any problems with your autoclave to Facilities Services and/or the service contractor.

D. Chemical Disinfectant Use

The most practical use of chemical disinfectants is for surface decontamination and, when used in sufficient concentration, as a decontaminant for liquid wastes prior to final disposal. General recommendations are:

1. Liquid Decontamination

- Add liquid chlorine bleach to provide a final 1:10 (made within two weeks of use);
- Let stand at least 20 minutes; and
- Discard the solution appropriately.

NOTE: No waste down the drain unless approval has been obtained from RMS.

2. Surface Decontamination

- Wipe with 1:10 dilution of chlorine bleach; or
- Wipe with iodophor disinfectant (per label concentration); or
- Wipe with another EPA registered disinfectant following manufacturer guidelines.

See Appendix D. Disinfection Tables, for additional information on disinfectants.

E. Decontamination in Animal Facilities

In UNT animal facilities, decontamination is accomplished by use of the provided disinfectants applied to surfaces and equipment; by chemical sterilants; by steam heat sterilization in an autoclave (particularly for surgical equipment); or by use of the cage-washing machine. All animal users should be familiar with the safe and proper use of all chemical decontamination materials and equipment that they need to use as part of their animal lab responsibilities.

XIV. Laboratory Procedures and Equipment

A. Exposure Control

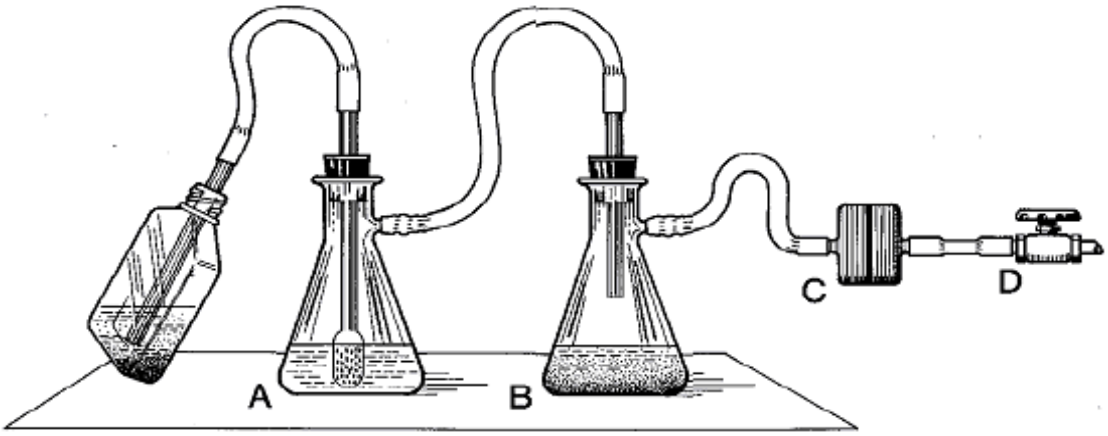
The term "containment" is used in describing safe methods for managing biohazardous in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The three elements of containment include laboratory practices and techniques, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements. Each principal investigator or lab manager is required to complete a risk assessment for each biological agent and toxin stored in his or her laboratory. Copies of the risk assessments must be available for inspection.

B. Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for handling such material safely. The principal investigator or the lab manager of each laboratory is responsible for providing or arranging the appropriate training of personnel and for verifying each person's competence. In addition, each principal investigator of BSL-2 laboratories must develop a Laboratory- Specific Biosafety Manual to address the use, handling, and disposal of biohazardous material (including select agents and toxins) in the laboratory. While not required of BSL-1 labs, it is highly recommended that BSL-1 labs also have laboratory-specific safety manuals.

The Laboratory-Specific Biosafety Manual must identify specific hazards that will or may be encountered and consider procedures needed to minimize or eliminate risks. Personnel should be advised of special hazards and are expected to follow the required practices and procedures.

1. Proper aspiration vacuum flask set up



A. Primary flask that is used to collect liquid.
B. Secondary flask (overfill flask) minimizes splash.
C. In line filter between secondary flask and vacuum source (Fisher Scientific 09-744-75).
D. Vacuum line that is occasionally serviced by lab workers or UNTD support personnel.

The primary and secondary flasks should contain a 10% bleach solution. The flask solution should be changed at least once a week to insure the killing strength of the bleach solution. Flask waste solution can be disposed of down the sink drain only after all potentially infectious material has had at least 20 minutes of contact time.

NOTE: If using a disinfectant other than a bleach solution, it may not be approved for sink disposal and you should contact the RMS.

C. Safety Equipment (Primary Barriers)

Safety equipment includes biological safety cabinets, enclosed biohazardous containers, and other engineering controls designed to eliminate or minimize exposures to biohazards and toxins. The biological safety cabinet is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Primary safety barriers may also include personal protection equipment (PPE) such as gloves, lab coats, safety glasses or goggles, face shields, andrespirators. Personal protective equipment is often used in combination with biological safety cabinet and other containment devices. In some situations in which it is impractical to work in a biological safety cabinet, personal protective equipment may formthe primary barrier between the worker and the infectious materials.

1. Biological Safety Cabinets (BSC)

Biological safety cabinets are classified as Class I, Class II, or Class III cabinets. When properly maintained and operated, they effectively contain and capture microbial contaminants

and infectious agents using HEPA (High Efficiency Particulate Air) filters (See Figure 1; source: Biosafety in Microbiological and Biomedical Laboratories 5th Edition, Appendix A). Biosafety cabinets should not be confused with clean benches or PCR cabinets, which only protect the material being worked with and are not suitable for work with infectious or toxic material (although clean benches, like biological safety cabinets, have HEPA-filtered air, in clean benches the air flows over the experimental material toward the user rather than being drawn away). Biological safety cabinets should also not be confused with conventional fume hoods that do not filter microorganisms.

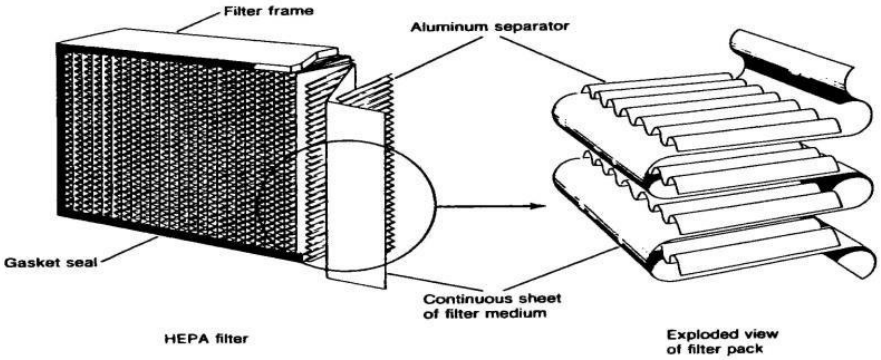
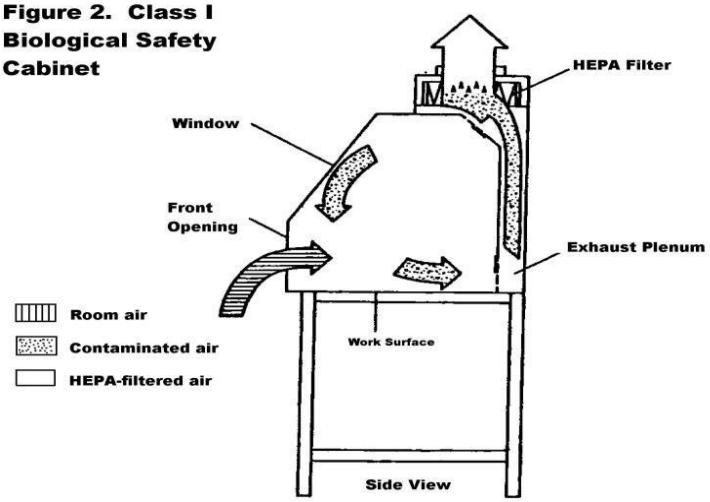


Figure 1. Diagram of HEPA filter. These filters are typically constructed of continuous sheets of paper-thin filter medium, pleated to increase surface area, divided by aluminum separators, and affixed to a frame.

Class I Biological Safety Cabinets

Class I biological safety cabinets provide personnel and environmental protection, but not product protection (See Figure 2; source: Biosafety in Microbiological and Biomedical Laboratories 5th Edition, Appendix A).

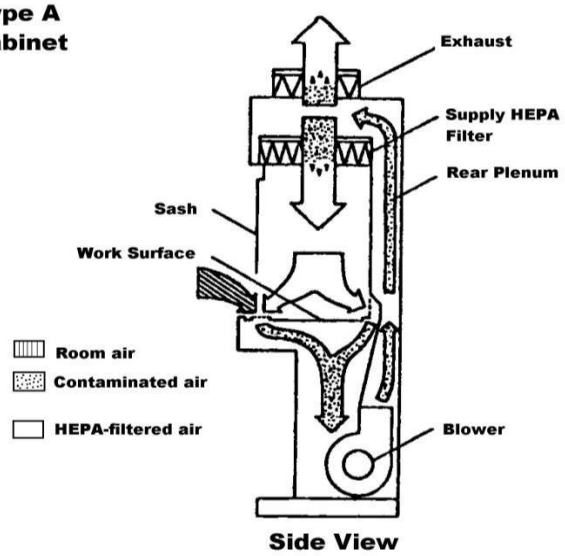
Figure 2. Class I Biological Safety Cabinet



Class II Biological Safety Cabinets

Class II biological safety cabinets are the most commonly used biological safety cabinets for biohazards. These cabinets provide personnel, environmental, and product protection (See Figure 3; source: Biosafety in Microbiological and Biomedical Laboratories 5th Edition, Appendix A). Only those cabinets that are hard ducted to the outside and provide a face velocity of 80 to 125 feet per minute should be used when working with volatile chemicals. Additionally, cabinets are not designed to prevent ignition of volatile flammable chemicals, such as ethanol and isopropanol.

Figure 3. Class II, Type A Biological Safety Cabinet



Guidelines for Working in a Biological Safety Cabinet

1. Turn off the ultraviolet lamp if one is in use. Turn on the fluorescent lamp.
2. Make sure the biological safety cabinet is certified.
3. Inspect the air intake grilles for obstructions and foreign material and remove if necessary.
4. Turn on cabinet fan at least 10 minutes before beginning work, if not left running
5. Put on appropriate PPE: rear-fastening, long-sleeved gown with tight- fitting cuffs, safety glasses and a pair (or two pairs) of high quality nitrile gloves.
6. Disinfect work surface with an appropriate EPA registered disinfectant.
7. Place items into the BSC, at least 6 inches from the front grill and approximately 2-4 inches from the rear grill, without unnecessary disruption of the airflow. Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.
8. Items used for surface decontamination and cleanup of a small spill should be included inside the BSC. Ensure there are biohazard waste containers directly outside of the BSC, but not attached to the unit as it can disrupt airflow.
9. Adjust the working height of the stool so that the worker's face is above the front opening.

10. Work as far to the back (beyond the air split) of the BSC work space as possible.
11. Minimize the movement (e.g., sweeping) of arms and reduce the frequency of placing hands/arms into the BSC and taking them out.
12. Employ good microbiological practices; work with materials from the clean to the dirty side.
13. Always use mechanical pipetting aids.
14. Avoid using open flames inside BSCs. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.
15. Do not work in a BSC while a warning light or alarm is signaling.
16. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper airflow and the level of protection provided.
17. Keep the front and rear grilles clear.
18. When work is completed, remove equipment and supplies from the cabinet. Wipe the bottom and side surfaces with disinfectant and allow cabinet to run for 15 minutes.
19. Some BSCs are equipped with ultraviolet (UV) lights. If one is used, the tube should be wiped with 70% ethanol **every two weeks**, while turned off, to remove dust. UV radiation should not take the place of disinfectant for disinfection of the cabinet interior.
20. The UV lamp should never be on while an operator is working in the cabinet.
21. Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.

NOTE: Be very careful when using small pieces of materials such as paper tissues in the hood, these can be blown into the hood and disrupt the motor operations. **Open flames are NOT permitted for use in BSCs**.

Certification of the Biological Safety Cabinet

Biological safety cabinets provide a partial containment system for the safe handling of pathogenic microorganisms, environmental samples, and other biohazardous materials. To ensure safety, biological safety cabinets must be used correctly with good microbiological techniques and be in proper mechanical working order. Cabinets must be certified for performance upon installation using National Sanitation Foundation (NSF) Standard #49. Certification is a series of performance tests on the biological safety cabinet to confirm that it will provide the user and experimental material the protection for which it is designed. The airflow, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards.

Biological safety cabinets intended for research with biohazards must be certified:

- After they are received and installed (before use with infectious materials).
- After filter changes.
- After being moved (even a few feet).
- After a mechanical failure.
- Annually.

Biological safety cabinet decontamination (using formaldehyde gas, chloride dioxide gas,

or other approved method) may be provided (e.g., by an outside vendor) and needs to be done:

- Before any maintenance work requiring disassembly of the air plenum, including filter replacement.
- Prior to cabinet recertification.
- Before moving the cabinet to a new laboratory.
- Before discarding or salvaging.

The production of formaldehyde gas is a health concern. Biological safety cabinets at UNTD are not ducted to the outside; therefore, consideration of a temporary "cease work" order may be implemented and extreme caution must be used when having the procedure performed.

RMS/BSO must maintain the certifications for the BSCs at UNTD.

D. Facility Design (Secondary Barriers)

The design of a facility is important in providing a barrier to protect those working inside and outside the laboratory and to protect people, plants, or animals in the community from biohazards and toxins, which may be accidentally released from the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The secondary barriers required will depend on the risk of transmission of specific agents. In working with agents at Biosafety Level 2 (BSL-2), the exposure risks involve direct contact with the agents or inadvertent contact through contaminated work environments. Recommended secondary barriers in these laboratories include separation of the laboratory work area from public access, hand washing facilities, and availability of a decontamination facility such as an autoclave.

XV. Personal Protective Equipment

UNTD requires the use of gloves, lab coat, and eye protection at all times when handling potential biohazards. Gloves are required when manipulating **any** microbiological culture in a teaching lab and goggles are also required when manipulating **any** liquid in a teaching lab, regardless of risk group or biosafety level.

Personal Protective Equipment (PPE) is used to protect personnel from contact with recombinant and potentially biohazardous materials. Appropriate clothing may also protect the experiment from contamination. PPE must be provided without cost to personnel. The following PPE is recommended for regular use.

A. Laboratory Clothing

Laboratory clothing includes: laboratory coats, smocks, scrub suits, and gowns. Long sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect skin from contamination. If the garment is not disposable, it must

be capable of withstanding sterilization in the event it becomes contaminated. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables must be available for visitors and maintenance and service workers entering the lab if they are required. All protective clothing must be either discarded in the laboratory or laundered by the facility. Personnel must not launder laboratory clothing at home.

B. Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with recombinant, potentially biohazardous, and microbiological material. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment must be overlapped by the glove. A long sleeved glove or disposable arm shield may be worn for further protection of the garment. Double gloving may be appropriate or required. However, if a medical condition dictates that only a single pair is worn, then that is acceptable. If a spill occurs, hands will be protected after the contaminated outer gloves are removed. Gloves must be disposed of when contaminated or removed when work with recombinant and/or biohazardous materials is completed. Gloves must never be reused. Gloves must not be worn outside the laboratory. Disposable gloves must not be washed or reused. Always wash hands after removing gloves.

C. Face Protection

Goggles or safety glasses with solid side shields in combination with masks or chin length face shields, or other splatter guards, are required for anticipated splashes, sprays or splatters of recombinant and/or potentially biohazardous materials. Application or removal of contact lenses is not permitted in the laboratory setting. Persons who wear contacts must wear eye protection when in areas with potentially aerosolizable agents.

D. Footwear

Open-toed shoes are not permitted in the laboratory. Protective footwear such as shoe covers may be necessary to minimize contamination of the laboratory and prevent the accidental release of recombinant and potentially biohazardous materials from a laboratory. If disposable shoe covers are used in the laboratory, waste containers must be available to dispose of used shoe covers. Shoe covers must not be reused.

E. Respirators

Additional respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Respirators must be carefully fitted to the individual and fit tested before use. Personnel who require respiratory protection must contact Risk Management Services for assistance in selection of equipment, training in proper usage, and enrollment in the RMS Respiratory Protection Program.

XVI. Protective Clothing Beyond the Laboratory

The improper use or lack of protective clothing and equipment in a laboratory can lead to chemical burns, biological exposures, or other potential dangers. To help reduce the risk of exposure, personnel in UNTD laboratories are required to wear gloves, safety glasses, lab coats and other personal protective clothing. However, in public areas, such as hallways and lounges, wearing personal protective clothing and equipment is not recommended. This is because contaminated clothing may present a hazard, and the perception of contaminated protective clothing and equipment in a public area may project a careless image to both colleagues and visitors.

Wearing gloves outside the laboratory should be minimized, except to move hazardous materials between laboratories. Chemicals should be transported from place to place on a cart, in a clean secondary container, or in a bottle carrier with secure handles. When this is not an option, personnel should use a clean, ungloved hand to touch common surfaces and a gloved hand to carry the items: the one-glove rule. Alternatively, the material should be packaged so the outer container may be transported without the need for personal protective equipment.

Protective gloves should never come into contact with door handles, elevator buttons, telephones, lavatory faucets, vending machines, bottled water dispensers, ice-making machines, or other surfaces outside the laboratory.

Also, be aware that strict federal and state regulations address the transport of hazardous (e.g., biological, chemical, radiological) materials on public roads.

For the sake of safety, appearances, and courtesy, personnel are asked not to wear contaminated, stained, or potentially contaminated lab coats and other research clothing and equipment in any public area, especially dining areas, lounges, auditoriums, conference rooms, or other non- hazardous areas.

XVII. Laundering Laboratory Clothing

Laboratory coats/gowns and contaminated clothing or clothing suspected to be contaminated with chemicals or biohazards are never be taken home or to a public laundry facility.

A. UNTD Laundry Facilities

Laundry facilities **DO NOT** exist at UNTD. *Mild to moderately* contaminated clothing shall laundered by a professional laundry service or autoclaved prior to laundering at home.

B. Professional Laundering Services

A professional service company may be used if the department does not have the capability to wash *mild to moderately* contaminated clothing. It is each laboratory's responsibility to determine if the cleaning company is capable and willing to launder the

contaminated clothes. Laboratory managers shall ensure that all laundry sent off-site is containerized in leak-proof bags or boxes marked with the biohazard symbol and shall advise the vendor that the laundry is contaminated with blood and/or potentially infectious bodily fluids for textiles that are mildly contaminated.

C. Laundering of Personal Clothing

Clothing contaminated with biohazardous material must be autoclaved prior to laundering at home. Documentation of effective autoclaving must be maintained.

NOTE: Personal laundering is not acceptable for clothing contaminated with chemicals, blood, blood products, or other bodily fluids.

D. Overtly Contaminated Clothing

Clothing that is overtly contaminated with chemicals must be disposed as hazardous waste. Clothing contaminated with radiological material must be disposed as radiological waste. Clothing that is contaminated with blood, blood products, or other bodily fluids must be removed and containerized in leak-proof bags or boxes at the location where it was used. Containers or bags must be marked with the biohazard symbol.

XVIII. Food and Beverages in the Laboratory

In order to reduce potential exposures and to ensure compliance with prudent laboratory operations, regulations, and other best management practices, UNT prohibits the storage and consumption of food and drink in all designated laboratory space. The only exception is for food and beverages used in research and teaching projects. These materials must be labeled, "Not for Human Consumption." In order to prevent potential exposure to hazardous materials:

- Do not eat, drink, smoke, chew gum, apply cosmetics, or take medicine in laboratories where hazardous materials are handled or stored.
- Do not store food, beverages, cups, or other drinking and eating utensils in areas where hazardous materials are handled or stored.
- Do not use glassware for laboratory operations to prepare or consume food or beverages.
- Do not use laboratory refrigerators, ice chests, cold rooms, and ovens for food storage or preparation.
- Do not use laboratory water sources or deionized laboratory water for drinking water.

Important: Food and beverages must never be stored in any laboratory refrigerator where chemicals, biological, and radioactive materials are kept unless they have been labeled: "Not for Human Consumption."

XIX. Nails and Jewelry

Principal Investigators (PIs) at UNTD are responsible for ensuring that laboratory personnel maintain appropriate hand and nail hygiene. Hands should be kept clean and washed

frequently (e.g., after completing work, after removing gloves, and before leaving the laboratory). Jewelry should be kept to a minimum to prevent puncturing or otherwise compromising protective gloves or limiting dexterity. CDC, NIH, and WHO recommend nail length should be no longer than 0.25 inch beyond the end of fingertips. Artificial nails (e.g., nail extensions, nail wraps, and nail jewelry) are not recommended when working in the laboratory.

XX. Transfers, Packaging, and Shipping of Biological Materials

The transferring, packing, and shipping of select agents and toxins is HIGHLY regulated. Please contact the RMS or IBC for more information.

A. Transfers

For materials that are **non- select agents**, each principal investigator must develop procedures for transferring or shipping from the laboratory. The principal investigator must ensure the following:

1. Personnel who package, handle, and ship non-select agents and biohazardous materials (including import and export) are subject to all applicable training.
2. Standard operating procedures should be in place for all import and export activities.
3. Package, label, and transport biohazards in compliance with all applicable local, federal, and international transportation and shipping regulations, including U.S. Department of Transportation (DOT) regulations. Materials that are transported by airline carrier should also comply with packaging and shipping regulations set by the International Air Transport Association (IATA).
4. Required permits (e.g., granted by the U.S. Public Health Service, USDA, DOT, U.S. Department of Commerce, and IATA) should be obtained before biohazards are prepared for transport.
5. Decontaminate contaminated or potentially contaminated materials before they are removed from the laboratory area.
6. Avoid hand-carrying biohazards when transferring them to other external facilities. If biohazards are to be hand-carried on common carriers, all applicable packaging, transport, and training regulations should be followed.
7. Develop and follow a protocol for intra-facility transfer (or between laboratories on UNT System campuses) of all biological and biohazards. Contact RMS/IBC for assistance.
8. Packaging and shipping of biological materials must be completed in a way that ensures the contents will not leak and that the package will arrive in good condition.

B. Packaging

All biological materials including diagnostic specimens and biological products that may contain an etiologic/biohazardous agent must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions possible with ordinary handling and transportation (e.g., passage through cancellation machines, sorters, and conveyors). Contents should not leak to the outside of the shipping container even if leakage of the primary container occurs.

NOTE: Special training is required to ship Category A or B substances.

Specific packaging requirements apply to materials that are known to contain, or reasonably believed to contain certain etiologic agents. For such materials the following procedures apply (See Figure 4; source: Biosafety in Microbiological and Biomedical Laboratories 5th Edition, Appendix C):

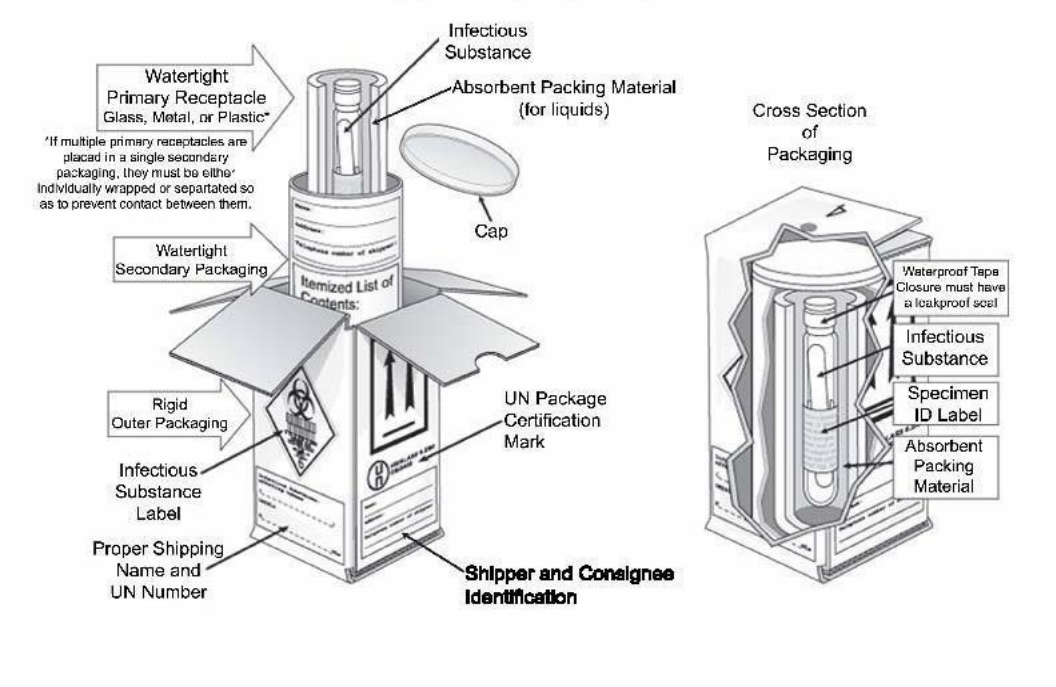


Figure 4. Packaging Diagram for Biohazards

1. Packaging Volumes

a) Volume not exceeding 50 milliliters (ml)

1. Place material in a securely enclosed, watertight primary container (e.g., test tube, vial). Enclose this primary container in a secondary, durable, watertight container. Several primary containers may be enclosed in a single secondary container as long as the total volume of material in all the primary containers enclosed does not exceed 50 ml.
2. Place absorbent non-particulate material (e.g., paper towels, **not** sawdust or vermiculite) in the spaces at the top, bottom, and sides between the primary and secondary containers. Use enough absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage.
3. Enclose each set of primary and secondary containers in an outer shipping container constructed of corrugated fiberboard, cardboard, wood or other material of equal strength. Do not use bags, envelopes, or similar materials.
4. If you package the material with dry ice, see the Packaging with Dry Ice section in this document.

b) Volume greater than 50 ml

1. Follow requirements for lesser volumes outlined above.
2. Place shock absorbent material at the top, bottom, and sides between the secondary container and the outer shipping container. (This material should at least equal the amount of absorbent material placed between the primary and secondary containers).
3. Ensure single primary containers contain no more than 1000 ml of material; however, two or more primary containers (combined volumes not exceeding 1000 ml) may be placed in a single secondary container. The maximum amount of etiologic agent that may be enclosed within a single outer shipping container must not exceed 4000 ml.

c) Packaging with Dry Ice

1. If used, place dry ice between the secondary and outside containers.
2. Place shock absorbent material so as to prevent the secondary container from becoming loose inside the outer container as the dry ice sublimates.
3. Use the DOT dry ice label. Guidelines for shipping are available by contacting RMS.

C. Labeling

The outer shipping container of all materials containing etiologic/biohazards that are being shipped or transported must bear a special labels. Please contact RMS for more information about shipping labels.

1. Shipping and Transportation Methods and Requirements

a) Registered Mail or the Equivalent

For a list of etiologic agents that use registered mail or an equivalent system, which provides the sender with immediate notification of receipt refer to the CDC Select Agent website at https://www.selectagents.gov/.

b) Federal Express or UPS

For Federal Express/UPS shipments, internationally or domestically, follow the International Air Transport Association (IATA) Dangerous Goods Regulations. (Receipt of shipment notice is not required since the shipment is traceable through the specific carrier.)

- Apply appropriate labels to the outer shipping container for packages containing dry ice and/or biohazard as shown in Figures 5 and 6, respectively.
- Contact the specific carrier's dangerous goods agent prior to shipment for any additional packaging and labeling requirements.

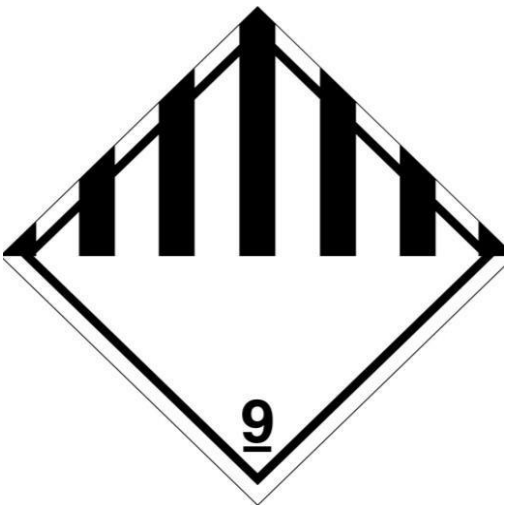


Figure 5. **Figure 6.**

D. Damaged Packages

When evidence of leakage or any other damage to packages bearing an Etiological Agents/Biomedical Material label is discovered, the carrier must promptly isolate the package and notify the Director, Centers for Disease Control and Prevention (CDC), 404.633.5313, 1600 Clifton Road NE, Atlanta, Georgia 30333.

E. Notice of Delivery

In the event that a package sent from UNTD is not received by the recipient within 5 days following the anticipated delivery of the package, the sender must notify the Director, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, Georgia 30333 or by telephone 404.633.5313.

F. Importation/Exportation of Etiologic Agents

Importation of biohazards, etiologic agents, and vectors that may contain such agents is governed by federal regulation. In general, an importation permit is required for any infectious agent known to cause disease to humans. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts, and molds. In some instances, an agent that is suspected of causing human disease also requires a permit.

There are two main import permit types for biologically hazardous agents and vectors: U.S. Public Health Service (USPHS); and Centers for Disease Control and Prevention (CDC). You can contact RMS for additional information regarding export controls.

XXI. Safety Audits

Principal Investigators/Laboratory managers should perform annual laboratory self-assessments. A BSL-1/2 Assessment tool is available to help laboratories comply with biosafety

and biosecurity requirements and make the facility a safer place to work. UNTD Risk Management Services (RMS) will conduct regular (e.g., semi-annual) inspections of each laboratory to ensure compliance with the procedures and protocols of this manual. Any significant concerns will be reported to the Institutional Biosafety Committee.

The safety audit typically includes an evaluation of the autoclave, biological safety cabinet, microbiological techniques, emergency and safety equipment, storage of biohazardous material, general housekeeping, and review of the Laboratory-Specific Biosafety Manual. Please refer to the UNTD Biosafety Inspection Checklist, for more information about the biosafety audit form used by RMS.

RMS will make every attempt to schedule safety audits with the principal investigators or lab managers. However, if the principal investigator or lab manager is unavailable or is unresponsive, RMS will proceed with the safety audit. RMS may also conduct unannounced inspections. Please be aware that federal, state, and local inspectors may also conduct unannounced inspections.

Following the biological safety survey, a report listing the safety concerns is sent to the principal investigator responsible for the laboratory. The principal investigator is responsible for correcting the hazards. If the principal investigator fails to correct the hazard, a second notice is sent to the department head with a copy to the principal investigator. Follow-up audits may be conducted in laboratories with extremely hazardous conditions and/or numerous concerns.

XXII. Security

A. Physical Security

Laboratory security is an integral part of an effective safety program. Follow these steps to ensure a secure working environment in your laboratory:

- Do not prop doors open.
- Do not give out door codes to unauthorized users.
- Keep laboratory doors closed and locked when unoccupied.
- Keep stocks of organisms and hazardous chemicals locked when the laboratory is unoccupied.
- Keep an accurate record of chemicals, stocks, cultures, project materials, growth media, and those items that support project activities.
- Notify UNTD police if materials are damaged or missing from laboratories.
- Notify UNTD police of any threats made to the laboratory or its workers.
- Inspect all packages arriving into the laboratory.
- When research is completed for the day, ensure that chemicals and biological materials have been stored properly and securely.
- Decontaminate materials and work surfaces after completing work and at least daily.
- Turn off equipment, flames, steam supply, and electrical appliances after completing work.
- Ask strangers (someone you do not recognize as a co-worker or support staff person) to exit the room if they are not authorized to be there.

- Discuss other security-specific requirements with your supervisor and colleagues.

B. Information Security

Refer to the Information Security Handbook and Information Security Policy for IT-specific guidelines and regulations.

XXIII. Emergency Preparedness

Emergency guidelines can be found online at the UNTD Police Department (https://police.untdallas.edu/safety-resources).

XXIV. Working Alone

All faculty, staff, students, and visitors working in an area (e.g., laboratory) where hazardous conditions exist should have knowledge of the following:

- Emergency Contacts
- Emergency Response Procedures
- Evacuation Routes
- First Aid Procedures
- Health and Safety Training Requirements
- Personal Protective Equipment Requirements
- Procedures to Report Unhealthy and Unsafe Conditions
- Safety Policies and Procedures
- Spill Response Equipment and Procedures

All personnel working alone (according to the National Safety Council, the term "alone" means that a person is beyond the visual or auditory range of any other individual for more than a few minutes at a time) in a laboratory where hazardous conditions exist should:

- Obtain written permission (e.g., e-mail, letter) from the Principal Investigator or Laboratory Supervisor to work alone in the laboratory;
- Ensure that a means to contact emergency response personnel is available when working alone in the laboratory; and
- Require that individuals working alone contact their supervisor before beginning work and upon completion.

XXV. Recordkeeping

A. Inventory

Detailed inventory records must be maintained by the PI or lab manager for all agents or biological materials used and/or maintained in their lab areas. These records must include the full identity of the strains, their origins and the vendor/originator of the material, their storage location, and the assigned biosafety level. These must be submitted to RMS/IBC annually.

B. Additional Records

The principal investigator or lab manager must maintain the following records and be prepared to present these at the annual laboratory inspection:

- Laboratory specific SOPs/biosafety manuals (as appropriate).
- A risk assessment for each project, biological agent, or toxin stored in that room.
- A current Responsible Information Party Sheet.
- Training Documentation Forms.
- Safety, security, and emergency response plans.
- Safety and security incident reports.
- Annual laboratory self-assessments
- Monthly autoclave testing logs (if applicable).

XXVI. Program Evaluation

The review of the elements as noted in the Recordkeeping sections of this document will constitute an evaluation of the UNTD Biosafety and Biosecurity Program.

Appendix A - Guidelines and Regulations

This Biosafety Manual (or Biological Safety Manual) has been adopted by the University of North Texas at Dallas (UNTD) to be a resource for information, guidelines, policies, and procedures that will enable safe teaching and research and to help eliminate, or reduce, the potential for exposure to biohazards in the laboratories, the university campus, and the community. Substantial information included in this manual derives from the UNT Biosafety Manual (https://riskmanagement.unt.edu/sites/default/files/01_unt_biosafety_manual.pdf).

The Institutional Biosafety Committee (IBC) adopted this manual to help ensure compliance with the following guidance materials and regulations:

* Centers for Disease Control and Prevention/National Institutes of Health. ***Biosafety in Microbiological and Biomedical Laboratories.*** HHS Publication No. (CDC) 21-1112, 5th ed., Revised Dec. 2009. (***BMBL***) - https://www.cdc.gov/labs/BMBL.html
* National Institutes of Health. ***Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.*** NIH, April, 2016. (***NIH Guidelines***) - https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/
* 7 Code of Federal Regulations § 331
* 9 Code of Federal Regulations § 121
* 18 United States Code § 175b
* 29 Code of Federal Regulations § 1910.1030 - https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030
* 29 Code of Federal Regulations § 1910.1450 - https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1450
* 42 Code of Federal Regulations § 72-73
* 42 Code of Federal Regulations § 1003
* 49 Code of Federal Regulations, § 171-180 - https://www.ecfr.gov/cgi-bin/text-idx?SID=028795e5b4a0b194cc473338c7237c13&mc=true&tpl=/ecfrbrowse/Title49/49CIsubchapC.tpl
* United States Department of Agriculture (USDA) Animal Welfare Act
* Texas Administrative Code 25 TAC §1.136
* Texas Administrative Code 30 TAC §326.39
* Texas Administrative Code 30 TAC §326.41

This Biosafety Manual should not be considered the only reference for health and safety concerns. It is intended that the principal investigator and supervisory personnel will supplement this information with instruction and guidance regarding specific practices and procedures unique to the work being done in their areas by completing a Lab-Specific Biosafety Manual and including all relevant documentation available to laboratory users.

Appendix B – Definitions and Acronyms

Animals: Any member of the animal kingdom except a human including an animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws).

Arthropods: Any living insect including crustaceans, spiders, scorpions, etc., capable of being a host or vector of human disease.

Autoclave: A device designed to sterilize equipment or biological waste by means of heat and pressure within a chamber.

Biohazard: Any microorganism (including, but not limited to, bacteria and their phages and plasmids, viruses, fungi, mycoplasmas, rickettsia, protozoa, parasites, or prions) or infectious substance, human and non-human primate tissues, body fluids, blood, blood byproducts, and cell lines, animal remains and insects that may harbor zoonotic pathogens, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing death, disease, or other biological malfunction in a human, animal, plant, or another living organism; deterioration of food, water, equipment, supplies, or material of any kind; or deleterious alteration of the environment.

Biohazardous activity: Any activity involving the use of potentially biohazardous agents.

Biological product: A biological prepared and manufactured in accordance with regulations that govern the manufacture of vaccines, reagents, etc.

Centers for Disease Control and Prevention (CDC): The Centers for Disease Control and Prevention of the United States Department of Health and Human Services.

Diagnostic specimen: Any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, etc., which is reasonably believed to contain an etiologic agent and is being shipped for purposes of diagnosis.

Etiologic agent: A viable microorganism or its toxin that causes, or may cause, human disease.

Field study: Any intentional release of a potentially biohazardous, genetically-modified or artificially-engineered living agent or their toxins to the environment, or the use of a chemical potentially capable of changing the environment for some biological control purpose (e.g., pesticide).

GMO: Any organism that has had gene(s) and/or a recombinant DNA construct introduced into its genome in a heritable fashion.

Human materials: Human blood, blood components, blood products, body fluids, tissues, or organs.

Infectious substance: Any material that is known or reasonably expected to contain a biohazard.

Interstate shipping: Transporting across state lines within the continental United States.

Intrastate shipping: Transporting within the State of Texas.

Personal protective equipment (PPE): Specialized clothing or equipment worn by a person for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) not intended to function as protection against a hazard are not considered to be PPE.

Principal investigator (PI): Any UNTD faculty member, staff employee, or student responsible of conducting research or other educational activities utilizing UNTD facilities.

Recombinant or synthetic nucleic acid (r/s NA) molecules: Molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or molecules that result from the replication of those described above, and synthetic nucleic acid segments, which are likely to yield a potentially harmful polynucleotide or polypeptide.

Toxin: The toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes: any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or any poisonous isomer or biological product, homolog or derivative of such a substance.

Vector: Any animals (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal products (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are known to transfer or are capable of transferring an infectious biological agent to a human.

Acronyms

AAALA	Association for Assessment and Accreditation of Laboratory Animal Care International
AC	Animal Care
APHIS	Animal and Plant Health Inspection Service
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	Biological Safety Cabinet
BSO	Biological Safety Officer
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DEA	Drug Enforcement Administration
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
NIH	National Institutes of Health
PHS	Public Health Service
PI	Principal Investigator
PPE	Personal Protective Equipment
RMS	Risk Management Services
SDS	Safety Data Sheet
UNTD	University of North Texas at Dallas
USDA	United States Department of Agriculture

Appendix C - How to Conduct a Biological Risk Assessment

Adapted from the "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories: Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel" (https://www.cdc.gov/mmwr/preview/mmwrhtml/su6101a1.htm).

A risk assessment should always be conducted prior to initiating any work in a laboratory. For work that needs to be registered with and approved by the Institutional Biosafety Committee (IBC), a risk assessment is a key part of the registration process. The Principal Investigator (PI)/Laboratory Director is responsible for identifying potential hazards, assessing risks associated with those hazards, and establishing precautions and standard procedures to minimize employee exposure to those risks. These should be documented in a Laboratory-Specific Biosafety Manual and made available to all staff working in the laboratory. The risk assessment conducted by the PI will be reviewed by the IBC who may require changes prior to the approval of the work.

Qualitative biological risk assessment is a subjective process that involves professional judgments. Because of uncertainties or insufficient scientific data, risk assessments sometimes are based on incomplete knowledge or information. Inherent limitations and assumptions made in the process also exist, and the perception of acceptable risk differs for everyone. The risk is never zero, and potential for human error always exists.

Identifying potential hazards in the laboratory is the first step in performing a risk assessment. A comprehensive approach for identifying hazards in the laboratory will include information from a variety of sources. No one standard approach or correct method exists for conducting a risk assessment; However, several strategies are available, such as using a risk prioritization matrix, conducting a job hazard analysis, or listing potential scenarios of problems during a procedure, task, or activity. The process involves the following five steps:

1. Identify the hazards associated with an infectious or biohazardous agent or material, including human pathogens, recombinant viral vectors, and acute biological toxins.

2. Identify the activities that might cause exposure to the agent or material.

3. Consider the training, competencies, and experience of laboratory personnel.

4. Evaluate and prioritize risks (evaluate the likelihood that an exposure would cause a laboratory-acquired infection [LAI] and the severity of consequences if such an infection occurs).

5. Develop, implement, and evaluate controls to minimize the risk for exposure and establish plans for how to deal with an exposure, should it occur.

Step 1. Identify the hazards associated with an infectious or biohazardous agent or material

- The potential for infection, as determined by the most common routes of transmission (i.e., ingestion by contamination from surfaces/fomites to hands and mouth; percutaneous inoculation from cuts, needle sticks, non-intact skin, or bites; direct contact with mucous membranes; and inhalation of aerosols; see Table 1).
- The volume and concentration of organisms handled.
- Intrinsic factors (if agent is known):

    - Pathogenicity, virulence, and strain infectivity/communicability
    - Mode of transmission (mode of laboratory transmission may differ from natural transmission)
    - Infectious dose (the number of microorganisms required to initiate infection can vary greatly with the specific organism, patient, and route of exposure) or LD_{50} for toxic materials
    - Genetic modifications that alter the risk, such as expression of oncogenes or siRNAs to knockdown tumor suppressors
    - The risk of the formation of replication competent viruses when using recombinant viral vectors
    - Form (stage) of the agent (e.g., presence or absence of cell wall, spore versus vegetation, conidia versus hyphae for mycotic agents)
    - Invasiveness of agent (ability to produce certain enzymes)
    - Origin of the material being handled (e.g., human tissues or cell lines make harbor pathogens; see Table 2)
    - Availability of vaccines and/or prophylactic interventions
    - Resistance to antibiotics

Step 2. Identify activities that might cause exposure to the agent or material

- The facility (e.g., BSL-2, BSL-3, open floor plan [more risk] versus separate areas or rooms for specific activities [less risk], sufficient space versus crowded space, workflow, equipment present).
- The equipment (e.g., uncertified Biological Safety Cabinets [BSCs], cracked centrifuge tubes, improperly maintained autoclaves, overfilled sharps containers, Bunsen burners).
- Potential for generating aerosols and droplets. Aerosols can be generated from most routine laboratory procedures but often are undetectable. The following procedures have been associated with generation of infectious aerosols:

    - Manipulating needles, syringes and sharps

        - Subculturing positive blood culture bottles, making smears
        - Expelling air from tubes or bottles
        - Withdrawing needles from stoppers

- Separating needles from syringes
- Aspirating and transferring body fluids
- Harvesting tissues

➢ Manipulating inoculation needles, loops, and pipettes

- Flaming loops
- Cooling loops in culture media
- Subculturing and streaking culture media
- Expelling last drop from a pipette (including Eppendorff pipettes)

➢ Manipulating specimens and cultures
- Centrifugation
- Setting up cultures, inoculating media
- Mixing, blending, grinding, shaking, sonicating, and vortexing specimens or cultures
- Pouring, splitting, or decanting liquid specimens
- Removing caps or swabs from culture containers, opening lyophilized cultures, opening cryotubes
- Spilling infectious material
- Filtering specimens under vacuum
  - Preparing smears, performing heat fixing, staining slides
  - Performing serology, rapid antigen tests, wet preps, and slide agglutinations
  - Throwing contaminated items into biohazardous waste
  - Cleaning up spills

- Use of animals.
- Use of sharps.
- Production of large volumes or concentrations of potential pathogens or agents.
- Improperly used or maintained equipment.
- Decreased dexterity or reaction time for workers wearing gloves, reduced ability to breathe when wearing N95 respirators, or improperly fitting personal protective equipment (PPE).
- Working alone in the laboratory. No inherent biologic danger exists to a person working alone in the laboratory; however, the supervisor is responsible for knowing if and when a person is assigned to work alone. Because assigning a person to work alone is a facility-specific decision, a risk assessment should be conducted that accounts for all safety considerations, including type of work, physical safety, laboratory security, emergency response, potential exposure or injury, and other laboratory-specific issues.

Step 3. Consider the competencies and experience of laboratory personnel.

- Age (younger or inexperienced employees might be at higher risk).
- Genetic predisposition and nutritional deficiencies, immune/medical status (e.g., underlying illness, receipt of immunosuppressive drugs, chronic respiratory conditions, pregnancy, non-intact skin, allergies, receipt of medication known to reduce dexterity or

reaction time).
- Education, training, experience, competence.
- Stress, fatigue, mental status, excessive workload.
- Perception, attitude, adherence to safety precautions.
- The most common routes of exposure or entry into the body (i.e., skin, mucous membranes, lungs, and mouth; see Table 1).

Step 4. Evaluate and prioritize risks.

Risks are evaluated according to the likelihood of occurrence and severity of consequences.

- Likelihood of occurrence:

  - Almost certain: expected to occur
  - Likely: could happen sometime
  - Moderate: could happen but not likely
  - Unlikely: could happen but rare
  - Rare: could happen, but probably never will

- Severity of consequences:

Consequences may depend on duration and frequency of exposure and on availability of vaccine and appropriate treatment. Following are examples of consequences for individual workers:

  - Colonization leading to a carrier state
  - Asymptomatic infection
  - Toxicity, oncogenicity, allergenicity
  - Infection, acute or chronic
  - Illness, medical treatment
  - Disease and sequelae
  - Death

Step 5. Develop, implement, and evaluate controls to minimize the risk for exposure.

- Engineering controls. If possible, first isolate and contain the hazard at its source.

  - Primary containment: BSC, sharps containers, centrifuge safety cups, splash guards, safer sharps (e.g., autoretracting needle/syringe combinations, disposable scalpels), and pipette aids
  - Secondary containment: building design features (e.g., directional airflow or negative air pressure, hand washing sinks, closed doors, double door entry)

- Administrative and work practice controls.

- Strict adherence to standard and special microbiological practices
- Adherence to signs and standard operating procedures
- Frequently washing hands
- Wearing PPE only in the work area
- Minimizing aerosols
- Prohibiting eating, drinking, smoking, chewing gum
- Limiting use of needles and sharps, and banning recapping of needles
- Minimizing splatter (e.g., by using lab "diapers" on bench surfaces, covering tubes with gauze when opening)
- Monitoring appropriate use of housekeeping, decontamination, and disposal procedures
- Implementing "clean" to "dirty" work flow
- Following recommendations for medical surveillance and occupational health, immunizations, incident reporting, first aid, post-exposure prophylaxis
- Training
- Implementing emergency response procedures

- PPE (as a last resort in providing a barrier to the hazard).

  - Gloves for handling all potentially contaminated materials, containers, equipment, or surfaces
  - Face protection (face shields, splash goggles worn with masks, masks with built-in eye shield) if BSCs or splash guards are not available. Face protection, however, does not adequately replace a BSC. At BSL-2 and above, a BSC or similar containment device is required for procedures with splash or aerosol potential.
  - Laboratory coats and gowns to prevent exposure of street clothing, and gloves or bandages to protect non-intact skin
  - Additional respiratory protection if warranted by risk assessment

- Job safety analysis.

One way to initiate a risk assessment is to conduct a job safety analysis for procedures, tasks, or activities performed at each workstation or specific laboratory by listing the steps involved in a specific protocol and the hazards associated with them and then determining the necessary controls, on the basis of the agent/organism. Precautions beyond the standard and special practices for BSL-2 may be indicated in the following circumstances:

- Organisms transmitted by inhalation
- Work with vectors expressing oncogenes or toxins
- Work with large volumes or highly concentrated cultures
- Compromised immune status of staff
- Training of new or inexperienced staff
- Technologist preference

- Monitoring effectiveness of controls

Risk assessment is an ongoing process that requires at least an annual review because of changes in new and emerging pathogens and in technologies and personnel.

- Review reports of incidents, exposures, illnesses, and near-misses.
- Identify causes and problems; make changes, provide follow-up training.
- Conduct routine laboratory inspections.
- Repeat risk assessment routinely.

TABLE 1. Laboratory activities associated with exposure to infectious agents	
Routes of exposure/transmission	**Activities/practices**
Ingestion/oral	• Pipetting by mouth • Splashing infectious material • Placing contaminated material or fingers in mouth • Eating, drinking, using lipstick or lip balm
Percutaneous inoculation/nonintact skin	• Manipulating needles and syringes • Handling broken glass and other sharp objects • Using scalpels to cut tissue for specimen processing • Waste disposal (containers with improperly disposed sharps)
Direct contact with mucous membranes	• Splashing or spilling infectious material into eye, mouth, nose • Splashing or spilling infectious material onto intact and nonintact skin • Working on contaminated surfaces • Handling contaminated equipment (i.e., instrument maintenance) • Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material • Bites and scratches from animals and insects • Waste disposal • Manipulation of contact lenses
Inhalation of aerosols	• Manipulating needles, syringes, and sharps • Manipulating inoculation needles, loops, and pipettes • Manipulating specimens and cultures • Spill cleanup
Source: Sewell DL. Laboratory-associated infections and biosafety. Clin Micobiol Rev 1995;8:389–405 (*18*).	

TABLE 2. Selected adventitious agents associated with cell cultures, organs and tissues that could be used to generate cell cultures, and cell culture reagents

Infectious agent	Source
Adenovirus	Human kidney, pancreas, some adenovirus transformed cell lines, rhesus monkey kidney cells
Bovine viruses: Bovine rhinotracheitis virus Bovine diarrhea virus Parainfluenza type 3 Bovine enterovirus Bovine herpesvirus Bovine syncytial virus	Bovine serum, fetal bovine serum (substantially lower risk today due to ultrafiltration of bovine serum)
Cytomegalovirus	Kidney, human foreskin, monkey kidney cells
Epstein-Barr virus (EBV)	Some lymphoid cell lines and EBV-transformed cell lines, human kidney
Hepatitis B virus	Human blood, liver
Herpes simplex virus	Human kidney
Herpesvirus group	Monkey kidney cells
Human or simian immunodeficiency virus	Blood cells, serum, plasma, solid organs from infected humans
Human papilloma virus (HPV)	HeLa cell lines
HTLV-1	Human kidney, liver
Lymphocytic choriomeningitis virus	Multiple cell lines, mouse tissue
Mycoplasmas	Many cell cultures
Myxovirus (SV5)	Monkey kidney cells
Porcine parvovirus	Fetal porcine kidney cells, trypsin preparations
Rabies virus	Human cornea, kidney, liver, iliac vessel conduit
Simian adenoviruses	Rhesus, cynomologous, and African green monkey kidney
Simian foamy virus	Rhesus, cynomologous, and African green monkey kidney
Simian virus 40 (SV40)	Rhesus monkey kidney cells
Simian viruses 1–49	Rhesus monkey kidney cells
Swine torque teno virus	Trypsin, swine-origin biological components
Squirrel monkey retrovirus	Multiple cell lines, commercial interferon preparations
West Nile virus	Human blood, heart, kidney, liver, lung, pancreas

Appendix D - Disinfection Tables

DISINFECTANT ACTIVITY											
Disinfectants		**Practical Requirements**					**Inactivates**				
Type	Category	Use Dilution	Contact Time (min)	Contact Time (min) Broad Spectrum	Temperature (C^O)	Relative Humidity (%)	Vegetative Bacteria	Lipoviruses	Nonlipid Viruses	Mycobacteria	Bacterial Spores
Liquid	Quaternary Ammonia Compound	0.1%-2.0%	10	NE			+	+			
	Phenolic Compounds	1.0%-5.0%	10	NE			+	+	B		
	Chlorine Compounds	500 ppm*	10	30			+	+	+	+	+
	Iodophor	25-1600 ppm*	10	30			+	+	+		
	Alcohol, Ethyl	70%-85%	10	30			+	+	B		
	Alcohol, Isopropyl	70%-85%	10	30			+	+	B		
	Formaldehyde	0.2%-8.0%	10	30			+	+	+	+	+
	Glutaraldehyde	2%	10	30			+	+	+	+	+
Gas	Ethylene Oxide	8-23g/ft^3	60	60	37	30	+	+	+	+	+
	Paraformaldehyde	0.3 g/ft^3	60	60	>23	60	+	+	+	+	+

NE=not effective B=Variable results dependent on virus *=Available halogen (1:100)

DISINFECTANT												
Disinfectants		**Important Characteristics**										
Type	Category	Effective Shelf Life >1 week (A)	Corrosive	Flammable	Explosion Potential	Residue	Inactivated by Organic Matter	Compatible for Optics (D)	Skin Irritant	Eye Irritant	Respiratory Irritant	Toxic (E)
Liquid	Quaternary Ammonia Compound	+					+	+	+	+		+
	Phenolic Compounds	+	+			+			+	+		+
	Chlorine Compounds		+			+	+		+	+	+	+
	Iodophor	+	+			+	+		+	+		+
	Alcohol, Ethyl	+		+						+		+
	Alcohol, Isopropyl	+		+						+		+
	Formaldehyde	+				+			+	+	+	+
	Glutaraldehyde	+				+		+	+	+	+	+
Gas	Ethylene Oxide	N/A		+(B)	+(B)			+	+	+	+	+
	Paraformaldehyde	N/A		+(C)	+(C)			+	+	+	+	+

N/A=not applicable (A)=Protected from light and air (B)=Neither flammable nor explosive in 90% CO_2 or fluorinated hydrocarbon, the usual form (C)=At concentrations of 7%-73% by volume in air, solid exposure to open flame (D)=Usually compatible, but consider interferences from residues and effects on associated materials such as mounting (E)=By skin or mouth, or both. Refer to manufacturer's literature and the MSDS.

DISINFECTANT APPLICATIONS												
Disinfectants		**Important Characteristics**										
Type	Category	Work Surface	Dirty Glassware	Large Area	Air Handling	Portable Equip. Surface Decon	Portable Equip. Penetrating Decon	Fixed Equip. Surface Decon	Fixed Equip. Penetrating Decon	Optical and Electronic Inst.	Liquid and Discard	Book, Paper
Liquid	Quaternary Ammonia Compound	+	+			+		+				
	Phenolic Compounds	+	+			+		+				
	Chlorine Compounds	+	+			+		+			+	
	Iodophor	+	+			+		+				
	Alcohol, Ethyl	+	+			+		+				
	Alcohol, Isopropyl	+	+			+		+				
	Formaldehyde	+	+			+		+				
	Glutaraldehyde	+	+			+		+				
Gas	Ethylene Oxide					+	+			+		+
	Paraformaldehyde			+	+	+	+		+	+		

REVISION HISTORY

This document shall be reviewed at least annually or when significant changes to the NIH guidelines, BMBL, or other pertinent changes occur.

Date of Review	Changes Made	Signature
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