FOREWORD

The Biological Safety Manual (or Biosafety Manual) has been adopted by the University of North Texas (UNT) to be a resource for information, guidelines, policies and procedures that will enable safe research and to help eliminate, or reduce, the potential for exposure to Biohazards. The Institutional Biosafety Committee (IBC) and Biosafety Officer (BSO) developed this manual to help ensure compliance with the following federal and state regulations and guidance materials:

- 7 Code of Federal Regulations § 331
- 9 Code of Federal Regulations § 121
- 18 United States Code § 175b
- 29 Code of Federal Regulations § 1910.1030
- 42 Code of Federal Regulations § 72-73
- 42 Code of Federal Regulations § 1003
- 49 Code of Federal Regulations, § 171-180
- Centers for Disease Control and Prevention/National Institutes of Health, “Biosafety in Microbiological and Biomedical Laboratories”
- National Institutes of Health, “Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules”
- Texas Administrative Code 25 TAC §1.136
- Texas Administrative Code 30 TAC §326.39
- Texas Administrative Code 30 TAC §326.41
- United States Department of Agriculture (USDA) Animal Welfare Act

The UNT Biosafety Manual provides a compilation of suggested work practices, protocols, and systems to work safely at UNT. The UNT 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. In addition, Risk Management Services (RMS)/BSO is always available to address health and safety concerns. The UNT Biosafety Manual is reviewed at least annually by the BSO and IBC and was last approved on XXXX.

____________________________________ __________________________________________
Megan Shoff (Biosafety Officer) Robert Benjamin (IBC Chair)

____________________________________ __________________________________________
Narendra Dahotre (Interim Vice President for Research and Innovation) Doug Welch (Executive Director, Risk Management Services)
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I. Introduction

The University of North Texas (UNT) Biosafety Manual is intended to be a resource for information, guidelines, policies, and procedures that will enable and encourage safe research and to reduce, or eliminate, the potential for exposure to biohazards. The information presented here also reflects the requirements and guidelines of federal and state regulations. The most current version of the Biosafety Manual will be maintained on the RMS Biosafety website.

The UNT Biosafety Manual is applicable to all laboratory, research, teaching, 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.

All research protocols must be reviewed and approved by the Biosafety Officer (BSO) and/or Institutional Biosafety Committee (IBC) prior to beginning work if they involve the use of any of the following:

- 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 and animals.
- Human or non-human primate cell lines and other materials of human or non-human primate origin.
- Select agents and toxins.
- Environmental/field samples (e.g., water, soil, and air samples).
- Archaeological samples (e.g., bones, clothing fragments and pottery).
- Biohazardous waste.

Biosafety encompasses the knowledge, techniques, equipment, and facilities necessary to prevent or minimize an exposure to, or release of, a biohazard. The mission of the RMS Biosafety group is to assure a safe and healthy environment for individuals working with biohazards and to protect the community and environment by preventing the release and exposure to biohazards. For information about field work, please refer to UNT’s Safety Guidelines for Field Researchers.

The UNT Biosafety Manual also requires that all laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. UNT is required to have an occupational health and safety program that addresses potential hazards associated with the conduct of animal research. The publication by the Institute for Laboratory Animal Research (ILAR), Occupational Health and Safety in the Care and Use of Research Animal, is most helpful in this regard.
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 to ensure the continuation of grant funding from federal agencies and for health and safety purposes.

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) detail procedures and practices for the containment and safe conduct of various forms of recombinant or synthetic nucleic acid research. 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 UNT, 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 UNT or the requirement for prior NIH approval of any and/or all recombinant or synthetic nucleic acid projects at UNT.

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.

The requirements described in the Occupational Safety and Health Administration’s (OSHA) Bloodborne Pathogens regulation (29 CFR § 1910.1030) apply to work with human blood, tissue, organs, body fluids, and cell cultures. Special training, medical surveillance, procedures, and equipment that must be in place for protection against bloodborne pathogens, needle sticks, and other sharps injuries, are described in the UNT Bloodborne Pathogen Exposure Control Plan.

Handling and disposal of biohazardous waste is also regulated by OSHA under the OSHA Bloodborne Pathogens regulation and by state and federal statutes. The procedures for biohazardous waste handling are described in the UNT Biological Waste Handling Procedures section.

The requirements for packaging and shipment of biohazards are provided in the Department of Transportation’s hazardous materials regulation 49 CFR § 171-180. In addition, permits may be required to ship biological materials. Please refer to the CDC Etiological Agent Import Permit Program and the Animal and Plant Health Inspection Service (APHIS) permit program.
Information on shipping procedures that comply with these regulations is found in the section on “Shipping and Transportation Methods and Requirements” in this manual. 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. Information regarding UNT’s radiation safety program is found in the UNT Radiation Safety Manual.

Teaching and research activities involving the use of animals are regulated by the United States Department of Agriculture (USDA) Animal Welfare Act. The Animal Welfare Act was signed into law in 1966. It is one of the laws in the United States that regulates the treatment of USDA-covered species in research, exhibition, transport, and by dealers. The USDA Animal Welfare Act 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. In addition, the Institutional Animal Care and Use Committee (IACUC) oversees all research and teaching activities involving vertebrate animals.

The Animal Welfare Act has been amended six times (1970, 1976, 1985, 1990, 2002, and 2007) and may be found in United States Code of Federal Regulations (CFR), Title 7, Chapter 54, and Sections 2131 through 2159. The Act is promulgated and enforced by the USDA, Animal and Plant Health Inspection Service (APHIS), Animal Care (AC). Proposed rules are published in the Federal Register and are open for public comment.

The Public Health Service (PHS) Policy implements the Health Research Extension Act of 1985, which applies to all institutions receiving animal research funds from PHS organizations (such as the National Institutes of Health). This law applies to all vertebrate species. The Health Research Extension Act of 1985 provides the legislative mandate for the PHS Policy. It directs the Secretary of Health and Human Services to establish guidelines for the proper care and treatment of animals used in research and for the organization and operation of animal care committees.

Institutions that receive PHS funds must have an Assurance on file at the Office of Laboratory Animal Welfare (OLAW). The Assurance is the university’s statement to OLAW that they will abide by the PHS Policy. 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.

### III. Roles and Responsibilities

The biological safety program at UNT developed from the University’s commitment to address and comply 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 the staff, researchers, community, and environment. The Institutional Biosafety Committee (IBC) and the RMS department provide oversight of UNT’s biological safety program.

The Animal Care Program consists of the Institutional Animal Care and Use Committee, the Attending Veterinarian, and the vivarium manager. Together they assist the university in achieving its academic mission and commitment to public service by providing for the humane care and use of animals. This, in turn, enables research and training programs to achieve their goals, assuring compliance with applicable federal and state guidelines and regulations.
Roles and responsibilities for biosafety and biosecurity are included in this section.

A. University of North Texas

UNT 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, University of North Texas must:

- Appoint a Biological Safety Officer for the institution.
- 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 and Institutional Animal Care and Use Committee with adequate expertise and training.
- Implement policies for safe conduct of biological and recombinant or synthetic nucleic acid research.
- Report any significant problems, violations or significant research-related accidents or illnesses to the NIH Office of Biotechnology Activities within 30 days.

B. Institutional Animal Care and Use Committee

UNT’s Institutional Animal Care and Use Committee (IACUC) is committed to providing an animal care and use program that provides a humane and compliant environment for animals and supports the research and teaching programs of our researchers, teachers, and students. Research and teaching involving the use of vertebrate animals conducted under the auspices of UNT is reviewed by the IACUC in compliance with federal regulations. Some projects involving animal research require that project description and protocol details be submitted to the IBC and IACUC for approval prior to initiating any work. Additional duties regarding the IACUC are outlined in the Animal Care and Use Policy. IACUC requires that principal investigators and all personnel involved in the care and use of research animals obtain CITI course certifications "Working with the IACUC" basic course for Investigators, Staff, and Students and species-specific course for each species to be used in the protocol, and UNT Animal Biosafety Training. In order to use animals at UNT, PIs must have an approved IACUC, and, when necessary, IBC, protocol, completed all requisite training, and received clearance from the UNT Occupational Health and Safety Program. These requirements must be fulfilled prior to the acquisition and use of laboratory animals.

C. Institutional Biosafety Committee

The Biosafety Committee 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. It is entrusted to assist and advise the UNT
community on incorporating good biological safety practices into all activities involving potentially biohazardous materials. The IBC seeks to reduce the risk of potential biohazardous threats to the UNT community, the public, and the environment.

The Biosafety Committee is vested with the authority to review and approve practices and procedures regarding research and other activities involving potentially biohazardous materials, direct inspection by the Biological Safety Officer of facilities where biohazardous materials are used and/or stored, and take appropriate action. The Biosafety Committee serves as the Institutional Biosafety Committee (IBC) as defined in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

The Biosafety Committee and Biological Safety Officer develop and implement policies, procedures, and guidelines related to the use of potentially biohazardous materials in the conduct of University activities. The Biosafety Committee, through the Biosafety Officer, reviews and monitors all research, teaching, and other activities conducted at UNT that involve the use of infectious agents, biological toxins, recombinant DNA, and other potentially biohazardous materials. The Biosafety Committee carries out all functions required by the NIH Guidelines, including reviewing applications for research at UNT involving recombinant DNA (rDNA) to ensure that the research conforms to the NIH Guidelines. The Biosafety Committee, through the Biological Safety Officer, assists Principal Investigators and others in the UNT community in meeting their responsibilities for assessing risks, establishing policies and procedures, training personnel, and maintaining facilities and equipment. Immediate authority resides with the Biosafety Officer regarding research and other activities involving potentially biohazardous materials where biohazardous materials are used and/or stored, and has the authority to take appropriate action, including suspending research and implementing corrective actions, in situations that present a risk of harm to life or health and in cases of serious, repeated, or continued non-compliance with regulations or IBC safety guidelines. The Biological Safety Officer will notify the Chair of any biosafety actions immediately, with IBC notification within 10 days. The Biosafety Committee provides guidance and support to the Biological Safety Officer in carrying out the requirements of the University’s Biosafety program. The Biosafety Committee, through the Biosafety Officer, advises the Vice President of Research and Innovation (VPRI) and other members of the UNT administration on all matters related to the use of potentially biohazardous materials in the conduct of University activities.

Responsibilities of the Institutional Biosafety Committee include, in collaboration with the BSO, assessment of facilities, procedures, practices, and training of research personnel to assure compliance with NIH Guidelines, Biosafety in Microbiological and Biomedical Laboratories, (BMBL), and other pertinent guidelines and regulations.

To successfully carry out these responsibilities, the Institutional Biosafety Committee is appointed to achieve sufficient knowledge and expertise in biomedical research and biosafety. The Institutional Biosafety Committee, or the BSO acting on behalf of the IBC, has the authority to approve, require modifications to secure approval, disapprove, suspend or terminate research activities as required to assure compliance with applicable regulations and guidelines.

The IBC, through its chairperson and the BSO, shall keep the VPRI informed 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. The IBC, or the BSO acting on behalf of the IBC, shall be responsible for:

- assessing the containment levels required by the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) as well as other potentially biohazardous materials and organisms;

- the assessment of facilities, procedures, practices, and training and expertise of personnel involved in laboratory activities utilizing potentially biohazardous materials;

- notifying Principal Investigators, laboratory managers, the Office of Research and other UNT committees of the results of the IBC’s review of initial and renewal applications;

- adopting emergency plans covering accidental spills and personnel contamination resulting from laboratory activities. The BSO shall cooperate with state and local public health departments by reporting any significant research or education-related illnesses or accidents that may be hazardous to the public health;

- periodically reviewing laboratory work involving biohazardous agents, human materials, and recombinant DNA molecules and educational activities conducted at UNT to ensure compliance with the latest edition of BMBL, the NIH Guidelines, the OSHA Occupational Exposure to Blood borne Pathogens Standards, and any guidelines adopted by the IBC;

- reporting any significant problems with or violations of the NIH Guidelines and all laboratory accidents or illnesses involving recombinant DNA molecules to the UNT Vice President, Research and to the NIH Office of Science Policy within 30 days as required, unless it is determined that a report has already been filed by the Principal Investigator;

- filing an annual report with the NIH Office of Science Policy (IBC-RMS) ; and

- reviewing the biosafety manual every year and recommending revisions to the biosafety program and Biosafety Manual as needed to the Vice President, Research.

D. Biosafety Officer

The Biosafety officer develops and participates in programs to promote safe microbiological practices, procedures, and proper use of containment equipment and facilities; stimulates responsible activities among workers; and provides advice on laboratory design. The responsibilities of the Biological Safety Officer include, but are not limited to, the following:

- Advise researchers on proper biohazardous waste disposal methods based on federal and state regulations.
- Assist researchers in the development of plans for preventing and handling accidental biohazardous spills and personnel contamination.
- Perform and review the required risk assessment to determine appropriate biosafety level and personal protective equipment (PPE) for handling recombinant and synthetic nucleic
acid molecules or biohazards.

- Investigate laboratory accidents involving recombinant and synthetic nucleic acid molecules and biohazards.
- Develop, implement, and maintain the university’s biosafety program to address issues of biosafety and biosecurity.
- Develop, implement, and maintain the university’s program for select agents and toxins.
- Develop, implement, and maintain the university’s emergency plans for handling and investigating laboratory accidents involving biohazardous agents, human materials, toxins or recombinant DNA molecules
- Perform periodic inspections to ensure that laboratory standards are rigorously followed.
- Promote regulatory compliance and a safe laboratory environment.
- Provide advice on laboratory security.
- Provide oversight of the UNT Bloodborne Pathogen Program and conduct training for laboratory personnel with such exposure.
- Provide training and resources for the safe use and practices for those working with potential biohazards and laboratory equipment.
- Serve as a liaison between UNT and external regulatory agencies concerned with the use of biohazardous agents, human materials, toxins, and recombinant DNA molecules;
- Review all funded grants for compliance with applicable sections of this Manual
- Maintain a list of organisms present in the university facilities and where these agents are used and stored.
- Ensure project specific biosafety plans are in place for each BSL-2 Laboratory
- Serve as a voting member of the IBC, including eligibility for appointment as Chair;
- Provide technical advice to principal investigators and the Institutional Biosafety Committee on research safety procedures.
- Ensure biosafety project registrations are in place
- Report to the Institutional Biosafety Committee and the institution any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware. The Biosafety Officer has the authority to immediately halt research that he/she deems to be an immediate threat to safety of personnel, environment, or the community at large. The Biological Safety Officer must report such action to the Institutional Biosafety Committee Chair immediately.

E. Principal Investigator/Laboratory Manager

The Principal Investigator/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 their 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 responding to emergency situations. This individual should consult with the biosafety officer and other health and safety professionals with regard to completing the risk assessment. The Principal Investigator shall:
• Accept direct responsibility for the health and safety of those working with animals, biohazardous materials, and/or select agents and toxins;
• Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
• Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents;
• Inform the laboratory staff 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 (e.g., vaccinations or serum collection);
• 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 Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), 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;
• Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics);
• 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 animals and 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 UNT 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 or animal facility and the equipment as necessary to ensure safety during any required inspection, calibration, and recertification activity;
• Ensure proper disposal of all potentially biohazardous materials;
• Ensure and document proper functioning of decontamination equipment through monthly verifications using approved methodologies;
• 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/BSO;
• 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 and IACUC approval prior to initiating or modifying any research involving use of animals 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 (IBC) 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 (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 Biological Safety Officer, Greenhouse/Animal Facility Director, Institutional Biosafety Committee, NIH Office of Biotechnology Activities, and other authorities, as appropriate.
F. Laboratory Personnel

The responsibilities of animal care and laboratory personnel 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. This includes 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.
- Obtain necessary and recommended vaccinations, or submit declination forms as permitted.
- Report thefts, security incidents, accidents, spills, or contamination incidents to supervisor and/or RMS/BSO.

G. Other Organizations

Other committees, including the Institutional Review Board, Radiation Safety Committee, IACUC, and the Department of Public Safety must consult and coordinate with the Institutional Biosafety Committee and RMS/BSO on any proposals under their purview which involve the use of biohazards.

H. Visitors, Vendors, and Contractors

Contractors must ensure that appropriate personal protective equipment is available for their own workers. All visitors, vendors, and contractors must:

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

IV. Training for Working Safely with Biohazards

The principal investigator and/or laboratory supervisor is responsible for providing or arranging for site-specific training of all personnel. In addition, each employee must attend biosafety training and chemical safety training. Contact RMS or the Biological Safety Officer for more information on scheduling training. All training must be documented. Please refer to the UNT Laboratory-Specific Training Checklist for more information.

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, to the proposed biological research. The Institutional Biosafety Committee, in conjunction with the Biological Safety Officer, 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. IBC and Registration information 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. 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.

<table>
<thead>
<tr>
<th>Select Agent Toxins</th>
<th>CAS #</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrin</td>
<td>1393-62-0</td>
<td>1,000 mg</td>
</tr>
<tr>
<td>Botulinum neurotoxins</td>
<td>93384-43-1</td>
<td>1 mg</td>
</tr>
<tr>
<td>Diacetoxylicirpenol (DAS)</td>
<td>2270-40-8</td>
<td>10,000 mg</td>
</tr>
<tr>
<td>Ricin</td>
<td>96638-28-7</td>
<td>1,000 mg</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>35523-89-8</td>
<td>500 mg</td>
</tr>
<tr>
<td>Short, paralytic alpha conotoxins</td>
<td>76862-65-2 / 156467-85-5</td>
<td>100 mg</td>
</tr>
<tr>
<td>Staphylococcal enterotoxins (Subtypes A, B, C, D, and E)</td>
<td>11100-45-1</td>
<td>100 mg</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>21259-20-1</td>
<td>10,000 mg</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>4368-28-9</td>
<td>500 mg</td>
</tr>
</tbody>
</table>

Any research above these amounts or with any select agents must get prior approval. Contact the BSO (biosafety@unt.edu) for additional information.


Questions? Email biosafety@unt.edu
2. **Toxins of Biological Origin**

Any biological toxin with a median lethal dose, or LD50, of less than 100 micrograms per kilogram body weight in vertebrates, must be approved by the UNT 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 LD50 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 LD50 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, cell lines) is regulated by the Occupational Safety and Health Administration. Work with this material must follow the UNT Bloodborne Pathogens Exposure Control Plan. 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 IBC.

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 UNT 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 IBC approval.

5. **Environmental Samples**

Environmental samples, such as water, air, soil, or plants, may contain pathogens (e.g., bacteria, viruses, 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 in the laboratory by culturing or other growing mechanisms, including greenhouses. 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. 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. All other work with environmental samples must be approved by the UNT Institutional Biosafety Committee.
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. Any animal work done outside of the vivarium must be registered with the IBC.

7. **Hazardous or potentially hazardous biological agents**

The NIH Guidelines for Research Involving Recombinant DNA Molecules, Appendix B (“Classification of Human Etiological Agents on the Basis of Hazard”), lists the most commonly encountered infectious agents by risk group. This list may be viewed on the National Institutes of Health's Office of Biotechnology Activities website. 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 for here: Risk Group Database. 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. Refer to the DURC guidelines on the RMS website for additional information.

9. **Other Biological Research**

Other biological research conducted at UNT that does not meet the criteria of #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 biosafety@unt.edu.

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 biosafety@UNT.edu.

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

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

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.

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) PI is also required to submit an amendment form/new registration for registering any changes to the protocol. A copy of the amendment form is available online.

8) 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.

VI. Controlled Substances

The Controlled Substances Act (Title II of the Comprehensive Drug Abuse Prevention and Control Act of 1970) 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. Table 5 lists the five schedules of controlled substances.

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Potential for Abuse</th>
<th>Medical Use</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schedule I</td>
<td>High</td>
<td>None</td>
<td>Heroin, Hydromorphinol, Marijuana, Lysergic Acid Diethylamide</td>
</tr>
<tr>
<td>Schedule II</td>
<td>High</td>
<td>With restrictions</td>
<td>Fentanyl, Methadone, Oxymorphone, Pentobarbital</td>
</tr>
</tbody>
</table>
### Schedule III

<table>
<thead>
<tr>
<th>Schedule III</th>
<th>Less than I or II</th>
<th>Currently accepted medical use</th>
<th>Euthanasia solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nalorphine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Buprenorphine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ketamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hydrochloride</td>
</tr>
</tbody>
</table>

### Schedule IV

<table>
<thead>
<tr>
<th>Schedule IV</th>
<th>Low</th>
<th>Currently accepted medical use</th>
<th>Chloral Hydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phenobarbital</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butorphanol</td>
</tr>
</tbody>
</table>

### Schedule V

<table>
<thead>
<tr>
<th>Schedule V</th>
<th>Lower than IV</th>
<th>Currently accepted medical use</th>
<th>Codeine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Investigators who use controlled substances in their laboratory must obtain a Researcher DEA license. Information on how to apply for a Researcher DEA license as well as detailed instructions on how to complete the online application can be found on the biosafety website.

The initial application is submitted on the DEA registration website. Once the online application process has been completed, additional documents that need to be completed will be forwarded to the principal investigator (PI) by the DEA. Once the DEA has completed the application process, the DEA license will be mailed to the PI. The entire process takes approximately 4-6 weeks. The license must be renewed annually.

Once the PI secures a DEA license, they will procure controlled substances through a purchase order. In order to order or obtain Schedule I or Schedule II drugs, a DEA 222 form is required.

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 UNT at UNT.CS@unt.edu. Inventories and records of controlled substances listed in Schedules I and II must be maintained separately from all other records maintained by the registrant. Information that must be on file and available for review includes:

### A. Inventory

After an initial inventory is taken, a new inventory of all controlled substances on hand must be taken annually and submitted to UNT.CS@unt.edu. Complete and accurate Controlled Substance records must provide a complete audit trail, from purchase, receipt or acquisition to their dispensing or disposal and must be readily available for review by authorized regulatory agencies. UNT has an inventory form that complies with 21 CFR § 1304 (https://www.deadiversion.usdoj.gov/21cfr/cfr/1304/1304_04.htm). Records pertaining to Controlled Substances in Schedule I and II must be maintained separately from all other records of the PI. Records for Schedule III, IV, and V controlled substances must be maintained either separately from all other records of the PIs or in such form that the information required is readily retrievable from the ordinary business records (21 CFR § 1304.04).

Each inventory must contain the following information:

- Date the inventory was taken.
- A list of all unopened bottles by drug name, including the drug name, the number of bottles, the drug concentration or unit size (e.g., 100mg/ml or 50 mg tablets), and the amount of drug in the bottle (e.g., ml or tablets). For example: ketamine, 2 bottles, 100
mg/ml, 10 ml per bottle.

- A list of all opened bottles and, for each bottle, the drug name, the drug concentration or unit size (e.g., 100 mg/ml or 50 mg tablets), and the amount of drug in the bottle before opening, (e.g., ml/bottle or tablets/bottle) and the remaining units. For example: ketamine, 100 mg/ml, originally 10 ml per bottle, 4.5 ml remaining.

B. **Transfer form or Controlled Drug Inventory form.**
   If you transfer drugs from your inventory (e.g., for reverse distribution of expired drugs), you must maintain a copy of the Transfer Form and provide a copy to UNT.CS@unt.edu.

C. **Controlled Substance Administration Record (CSAR).**
   When a controlled substance is administered to an animal, its usage must be documented. It is essential that all needed information is included. This includes license holder’s name and DEA number, name of drug, drug schedule number, concentration, starting amount, and bottle ID, and bottle lot number. For each use from the bottle, the following needs to be recorded: date, name of user, amount used, amount remaining, and the initials of the person entering the information.

   As per DEA regulations, expired or unused controlled substances must be disposed of via reverse distribution or destroyed.

   The UNT Controlled Substance Usage Log may be found online, and a copy must be submitted to UNT.CS@unt.edu upon completion.

D. **Reporting Theft, Unauthorized Use, or Significant Loss of Controlled Substances**
   The PI must notify the local DEA office orally within one business day after the discovery of theft or significant loss of any Controlled Substances as prescribed by 21 C.F.R. 1301.76. A written report to the DEA, using DEA Form 106 (https://www.deadiversion.usdoj.gov/21cfr_reports/theft/), must be submitted within 15 days after the discovery. Risk Management Services should be notified along with the DEA when the DEA is initially notified and when the written report is sent to the DEA, as well as a copy of the form being provided to UNT.CS@unt.edu.

   Theft or significant losses must be reported whether or not the Controlled Substances are subsequently recovered and/or the responsible parties are identified and action taken against them.

E. **Storage of Controlled Substances**
   Storage of Controlled Substances must comply with federal requirements. PIs are responsible for establishing and maintaining effective controls and procedures to prevent unauthorized access, theft or diversion of Controlled Substances.

   PIs are directly responsible for:
Establishing adequate security to prevent unauthorized access to Controlled Substances.
Establishing adequate security to prevent the diversion of Controlled Substances.
Storing Controlled Substances listed in Schedules I-V in a secured location and in a securely locked, substantially constructed cabinet, or security cabinet (i.e., not easily broken into or moved; see 21 CFR § 1301.71-1301.76).

**F. Disposal of Controlled Substances**

The PI is responsible for the return or disposal of Controlled Substances in accordance with federal and state requirements. Risk Management Services (biosafety@unt.edu) can provide assistance and guidance in this area.

The PI must document the disposal of Controlled Substances ([https://www.deadiversion.usdoj.gov/21cfr_reports/surrend/](https://www.deadiversion.usdoj.gov/21cfr_reports/surrend/)), and a copy of DEA Form 41 must be maintained with the PI’s records to provide accountability for the disposal of these Controlled Substances and a copy provided to UNT_CS@unt.edu.

**G. Abandoned Controlled Substances**

When a PI leaves the university or rescinds their license, arrangements for disposal and/or transfer of all his/her Controlled Substances must be made prior to departure or license termination.

Under no circumstances are controlled substances to be abandoned by a DEA registrant. However, researchers sometimes leave the university without appropriately disposing of or transferring all controlled substances from their lab.

- In this case, contact RMS for disposal instructions. If the researcher was not registered with the DEA and/or the controlled substance(s) was acquired prior to registration requirements (pre-1970 for many substances), the department must contact the RMS for disposal instructions.

**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. Note that 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 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 National Institutes of Health (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. Laboratory personnel must be trained with regards to these emergency procedures.

Each Principal Investigator and laboratory manager is responsible for developing appropriate emergency procedures for his/her work area and limiting access to authorized individuals only.

Principal investigators must be aware of the provisions for emergency procedures and preparedness. Emergency procedures and preparedness 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/BSO the following:

- A completed Responsible Party Information Sheet (annually).
- An annual chemical and biological inventory.
- A Risk Assessment for each project 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 in this type work.

Dial 911, when special first aid, resuscitation, transport, or rescue service is required. Clearly describe the situation and your location. Place all contaminated materials in either a biological safety cabinet or appropriate containment so that medical help can enter the facility. Notify the PI and BSO.

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 (940-565-2109) as soon as possible or for help with cleaning manageable spills.

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

The Principal Investigator or RMS/BSO is responsible for deciding whether to override containment procedures in case of serious injury or sudden illness.

It is essential that the authorized users of The Lab familiarize themselves with the procedures detailed here. Questions about these procedures should be directed to the PI and RMS/BSO. Personnel should be aware of all exits, fire extinguishers, fire alarms, eyewash stations, safety showers, spill and first aid kits. **KNOW WHAT TO DO BEFORE AN EMERGENCY OCCURS.**

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Questions? Email biosafety@unt.edu
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All accidents 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 Risk Management Services, Insurance & Claims at 940-565-2109 and using the Incident Report form.

Each department is responsible for reporting all biosafety accidents to the BSO (biosafety@unt.edu) within 48 hours. To properly document the accident, additional reports may be required. The BSO may be contacted for clarification and assistance with this requirement. A Biohazard Incident report form can be obtained online.

Call Police at 911 in the event of emergency

Serious accidents for this purpose are those, which result in:

- Fatality;
- Hospitalization or medical treatment (beyond first-aid) of any person; NOTE: This includes non-UNT personnel;
- First-aid treatment of five (5) or more persons;
- Property damage exceeding $1000.00; 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.

Medical Evaluation is necessary if recognition of disease early symptom developed. During regular business hours, treatment for non-emergencies should be obtained at the closest CareNow facility (Emergencies should call 911). Students may receive treatment at:

UNT Denton campus – Student Health & Wellness Center (SHWC)
1800 West Chestnut Street, Denton, TX 76201
Tel: 940-565-2787

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 or, if a student, at the UNT Student Health & Wellness Center, if necessary.
4. Inform the laboratory’s principal investigator and/or RMS/BSO immediately.
5. Submit and Incident Report form within 24h in case of an injury.
6. Submit a Biohazard Incident Form to the BSO (biosafety@unt.edu) 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 or, if a student, at the UNT Student Health & Wellness Center, if necessary.
4. Inform the laboratory’s principal investigator and/or RMS/BSO immediately.
5. Submit and Incident Report form within 24h in case of an injury.
6. Submit a Biohazard Incident Form to the BSO (biosafety@unt.edu) 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 or, if a student, at the UNT Student Health & Wellness Center, if necessary.
4. Inform the laboratory’s principal investigator and/or RMS/BSO immediately.
5. Submit and Incident Report form within 24h in case of an injury.
6. Submit a Biohazard Incident Form to the BSO (biosafety@unt.edu) within 48h.

4. **Ingestion or Inhalation**

1. Move to fresh air immediately.
2. Call 911 or seek medical attention at the nearest medical clinic or, if a student, at the UNT Student Health & Wellness Center, if necessary.
3. Do not induce vomiting unless advised to do so by a health care provider.
4. Inform the laboratory’s principal investigator and/or RMS/BSO immediately.
5. Submit and Incident Report form within 24h in case of an injury.
6. Submit a Biohazard Incident Form to the BSO (biosafety@unt.edu) within 48h.

C. **Spills of Biohazards**

UNT 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/BSO is 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. If respiratory protection is required, the UNT Respiratory Protection Program must be followed. A simple spill kit with the following supplies should be available and used by trained personnel:

- Bleach or other EPA-registered disinfectant
- Biohazard bag
- Disposable lab coat
- Disposable shoe covers
- Hand sanitizing wipes
- Nitrile gloves (4 pair)
- 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

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 cabinet 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.
   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 and the Biological Safety Officer if there was a potential for any 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.
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 and to RMS/BSO immediately.
8. Resume work if deemed safe by supervisor/manager.
9. Submit **Biohazard Incident Report to biosafety@unt.edu**

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, supervisor, and RMS/BSO 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, 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
12. Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of clean-up.
13. Submit **Biohazard Incident Report to biosafety@unt.edu**

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 biosafety@unt.edu**

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. Don appropriate personal protective gear before initiating cleanup.
3. Initiate cleanup 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 discard 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 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 reuse.
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.
   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, supervisor, and RMS/BSO if there was a potential for any material escaping the Biological Safety Cabinet. Consult with RMS/BSO 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 biosafety@unt.edu

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, supervisor, and RMS/BSO prior to proceeding with cleanup.
8. Assemble supplies (e.g., disinfectant, sharps containers, towels, tongs, autoclave bags) before entering the laboratory.
9. Don 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. Remove protective clothing used during cleanup then place in a biohazard bag for autoclaving.
    g. Wash hands when gloves are removed.
11. Submit Biohazard Incident Report to biosafety@unt.edu

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.

Submit Biohazard Incident Report to biosafety@unt.edu

8. **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, supervisor, and/or RMS/BSO.
13. Submit [Biohazard Incident Report to biosafety@unt.edu](mailto:biosafety@unt.edu)

9. **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, supervisor, and RMS/BSO if there was a potential for any material escaping the centrifuge.
4. Submit [Biohazard Incident Report to biosafety@unt.edu](mailto:biosafety@unt.edu)

VIII. **Incident Reporting**

A. **Reporting Exposures**

In the event of an exposure to a biohazard:

1. Report to UNT Student Health & Wellness Center (student) or the closest CareNow facility (contact RMS 940-565-2109 for assistance)
2. Complete an Accident/Illness Report Form and submit to RMS within 24h and complete a biohazard incident report form and submit to BSO (biosafety@unt.edu) within 48 hours of incident.
3. If exposure or incident occurs with s/r NA, work with the principal investigator, supervisor, and Biological Safety Officer to report accident to the NIH Office of Biotechnology Activities as required by the [NIH Guidelines](https://nih不住link).
B. Reportable Incidents and Violations

Incidents or problems involving biohazards and/or recombinant or synthetic nucleic acid molecules must be immediately reported to the Biological Safety Officer. 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 Biological Safety Officer.

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 Responsibilities

The principal investigator and their personnel must report any significant incident, violation of the NIH Guidelines, or any significant, research-related accidents and illnesses immediately by contacting the Biological Safety Officer. 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. Biosafety Officer Responsibilities

The Biological Safety Officer is required, by the NIH Guidelines, to report to the Institutional Biosafety Committee:

- All violations of the NIH Guidelines and significant incidents.
- Any significant research-related accidents or illnesses.

E. Institutional 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, improper disposal of recombinant waste.
- Significant changes to proposed research risk without prior notification and approval by 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, 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.

**F. 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 CDC/NIH document, Biosafety in Microbiological and Biomedical Laboratories, also known as 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 1). 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 F. How to Conduct a Biological Risk Assessment) for all projects.

<table>
<thead>
<tr>
<th>Table 1. Risk Group (RG) Classifications</th>
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<td>RG-1</td>
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<td>Agents not associated with disease in healthy adult humans.</td>
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X. Biological Safety Levels

CDC and NIH have established four levels of biosafety, based on the degree of hazard associated with a microbial agent, to describe the combination of laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure (see Appendix C 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 CDC/NIH BMBL. UNT 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 with biohazards at BSL-3 is currently permitted in UNT 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 with biohazards at BSL-4 is currently permitted in UNT facilities.

Personal protective equipment varies depending upon the biological safety level. Please refer to the table below for basic requirements for each of the four biological safety levels. Refer to section XV: Personal Protective Equipment for detailed information and UNT requirements.

<table>
<thead>
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<th>Table 2. Biological Safety - Personal Protective Equipment (PPE) Requirements*</th>
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<tr>
<td><strong>BSL-1</strong></td>
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<tr>
<td><strong>Protective laboratory coats, gowns, or uniforms</strong> recommended preventing contamination of personal clothing. <strong>Protective eyewear</strong> 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. <strong>Gloves</strong> must be worn to protect hands from exposure to hazardous materials.</td>
</tr>
</tbody>
</table>

* 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 (see Appendix D. Animal Biosafety Guidelines for more information). As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents in vivo and in vitro are comparable with the animal biosafety level.

The four animal biosafety levels provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to Biosafety Levels 1-4, respectively. Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

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

- **ABSL-1** is required for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

- **ABSL-2** is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment, and also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

- **ABSL-3** is required for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. **Note:** No research with biohazards at BSL-3 is currently permitted in UNT facilities.

- **ABSL-4** is required for work with animals infected 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 with biohazards at BSL-4 is permitted in UNT facilities.

In addition to animal biosafety consideration, laboratory animal facilities, operational practices, and quality of animal care must meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. The USDA has also developed facility parameters and work practices for handling agents of agriculture significance.

Personal protective equipment varies depending upon the biological safety level. Please refer to the following table for specific requirements for each of the four biological safety levels.
## Table 3. Animal Biological Safety - Personal Protective Equipment (PPE) Requirements*

<table>
<thead>
<tr>
<th>ABSL-1</th>
<th>ABSL-2</th>
<th>ABSL-3</th>
<th>ABSL-4</th>
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<tr>
<td><strong>Protective laboratory coats, gowns, or uniforms</strong> recommended to prevent contamination of personal clothing. <strong>Eye, face, and respiratory protection</strong> should be used in rooms containing infected animals. <strong>Protective eyewear</strong> must be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses should also wear <strong>eye protection</strong> when entering areas with potentially high concentrations or airborne particulates. <strong>Gloves</strong> must be worn to prevent skin contact with contaminated, infectious, and hazardous materials, and when handling animals.</td>
<td><strong>Protective laboratory coats, gowns, or uniforms</strong> must be worn while in areas where infectious materials and/or animals are housed or manipulated. <strong>Eye and face protection</strong> (mask, goggles, face shield or other splatter guard) must be worn when performing manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or physical containment device. Personnel who wear contact lenses should also wear <strong>eye protection</strong> when entering areas with potentially high concentrations or airborne particulates. <strong>Gloves</strong> must be worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals. <strong>Eye, face, and respiratory protection</strong> should be used in rooms containing infected animals.</td>
<td>Not currently permitted at UNT. <strong>Disposable personal protective equipment</strong>, such as non-woven olefin cover-all suits, wrap-around or solid-front gowns, should be worn (over uniforms or scrub suits) before entering areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable. <strong>Eye, face, and respiratory protection</strong> must be used in rooms containing infectious materials and in areas where animals are housed or manipulated. [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 <strong>eye protection</strong>. <strong>Gloves</strong> must be worn to prevent skin contact with contaminated, infectious, and hazardous materials and when handling animals. Double-glove practices should be used. <strong>Boots, shoe covers, or other protective footwear</strong>, are used to prevent cross-contamination.</td>
<td>Not permitted at UNT. Please refer to the CDC/NIH document, “Biosafety in Microbiological and Biomedical Laboratories” for PPE requirements.</td>
</tr>
</tbody>
</table>

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

It is the responsibility of institutional management to provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for the laboratory animal. There are unique hazards associated with infected animals that must be understood.
by personnel with animal contact. Animal activity may create aerosols, and bites and scratches can occur.

A. Animal Hazards and Exposures

Good housekeeping practices and sanitation are essential to reducing the risk of physical hazard injuries. It is important to keep work surfaces clean and clear of obstructions, waste, and other materials. All boxes, hoses, or bags of bedding material should be routinely removed from the work area. Mop floors and clean work surfaces with the appropriate cleaning and disinfectant solutions. Keep in mind that poor housekeeping is unprofessional and will increase the risk of accidents and injuries. Appropriate PPE (gloves, eye protection, laboratory coat, etc.) should be utilized based on risk assessment. **When handling animals, gloves must always be worn.**

1. Bites and Scratches

The risk of animal bites and scratches is associated with handling of animals and is best avoided by proper handling techniques and wearing appropriate personal protective equipment (PPE). Knowledge of animal behavior and how animals respond to their immediate physical environment is important in reducing risk of injury to the individual and the animal.

Animals respond to sights, sounds, and smells as people do, but they also may hear, smell, and react to things that people do not detect. For example, if an animal hears a high-pitched sound, it may become frightened and react defensively. Many animals have a flight zone, and, if approached by another animal or the handler, the affected animal may try to escape. Unsuccessful escape may cause the animal to act aggressively. Of course, inappropriate handling of an animal can cause discomfort, pain, and distress and provoke an animal to bite or scratch.

Animal bites and scratches that cause minor skin damage are sometimes disregarded by animal workers who are unfamiliar with the number of diseases that can be spread by such injuries. Even minor bites and/or scratches can result in infections and illnesses if they are not properly treated. Scrapes and injuries from **contaminated equipment** associated with animal care and housing, such as cages, can be as great a risk as direct animal contact and should be addressed similarly.

Most animals used in research are bred specifically for that purpose and do not have the potential for transmitting the kinds of pathogenic organisms that those in the wild do; however, there are some illnesses and infections that can be passed from animals to people (i.e., zoonoses), and these are discussed in more detail later in this document.

With research animals, biological hazards are of most concern when the animals are naturally infected (e.g., macaques may have Simian Herpes B virus, though UNT has no NHPs) or if animals are infected with a bacteria, virus or human cells (e.g., tumorigenic cell lines) as part of the experimental work. Under these conditions and when doing field research with wild species, it is of critical importance that appropriate PPE and other appropriate protective measures be used to prevent infection.

The most important step to prevent infection following any bite, scratch (or puncture
from sharps exposure) is to immediately and thoroughly wash the injury with soap and water. Inform a supervisor and record the injury in the bite and scratch log located in the animal facility. Medical consultation and treatment should be obtained.

2. **Physical Hazards**

Sharps such as needles, broken glass, syringes, pipettes, and scalpels are all commonly found in animal facilities and laboratories and present a physical hazard. Use extra care to avoid inadvertent contact and injury. **Needlestick injuries** represent substantial risk of becoming infected especially when injecting animals with microbial agents or drawing blood.

The animal facility should have puncture-resistant and leak-proof containers for disposal of sharps. To prevent needle sticks, it is critical to **always place used needles directly into the sharps container without recapping or attempting to bend, shear, break, or remove the needle from the syringe.**

Animal care operations involve a number of activities that can cause physical stress when handling and moving heavy loads. The use of proper lifting techniques can help prevent back and shoulder injuries when moving cages, bags of feed and bedding, pieces of equipment, and supplies. Poor physical fitness, obesity, poor posture, smoking, and medical/physical deficiencies are personal factors that may contribute to back pain. When lifting heavy loads, every attempt should be made to avoid sudden movements and use a two-handed lifting technique. Keep your back straight, feet positioned apart with one slightly ahead of the other, and knees bent as the lift is completed. Reduce loads where possible and get help when lifting awkward loads or those that cannot be handled safely by one person.

3. **Chemical Hazards**

Personnel involved in the care and use of research animals must be familiar with the **chemical hazards** associated with the animal care and laboratory environment. Chemical properties may include flammability, corrosiveness, reactivity, or the potential to be explosive. Potentially hazardous chemicals used in animal laboratories include **solvents** (e.g., xylene, acetone, dimethyl sulfoxide), **acids** (hydrochloric, sulfuric), **bases** (e.g., sodium hydroxide, quaternary disinfectants), **fixatives** (e.g., formaldehyde, osmium tetroxide), **sterilants** (e.g., peracetic acid, chlorine dioxide, peroxides, gluteraldehyde), and **anesthetics** (e.g., isoflurane, tribromoethanol, methane sulfonate, nitrous oxide, urethane, barbiturates). Each chemical product should be handled carefully using the label directions and recommended PPE in accordance with University guidelines and lab training. Safety Data Sheets (SDS) are available online. These provide additional information on the hazards and precautions related to a chemical’s use. Users must be certain that they understand the proper use of the chemical material before they use it.

4. **Animal Allergies**

Allergic reaction to animals is among the most common conditions that adversely affects worker health. The estimated prevalence of allergic symptoms among workers exposed to animals is from 10% to 40%. Workers who are continually exposed to animal allergens tend to have progressively more frequent and severe symptoms, and an estimated 10% develop
**asthma.** Hence, it is critical that all workers seek to minimize their exposure to animal allergens. Additionally, once animal allergy develops, the affected worker should minimize any additional allergen exposure to prevent progression of allergy symptoms.

Allergy is most often manifested by **nasal symptoms** (e.g., allergic rhinitis), **itchy eyes** (e.g., allergic conjunctivitis), and **rashes** (e.g., contact urticaria, atopy). Symptoms usually evolve over a period of 1-2 years and may lead to acute anaphylaxis in a small number of patients. In **rodents**, the allergen protein is of urinary origin and in **rabbits** it is contained in the fur, dander, and, to a lesser degree, the saliva and urine. In **Guinea pigs**, urine is the main allergen with dander, fur, and saliva contributing. Exposure to **birds** can cause rhinitis and asthma symptoms. Multiple bird proteins have been identified as allergens and can be found in serum and fecal droppings that contain serum. **Fish** proteins can be an inhalation allergen for those who are sensitized.

Prudent efforts to prevent allergen exposure and reduce the frequency of sensitization in animal workers require strict work practices and consistent use of PPE. Housing animals in filter-top cages, working in well-ventilated areas, and using ventilated hoods for soiled bedding disposal will minimize exposure to animal allergens.

The work area must be maintained clean to prevent inhalant and contact exposure. Procedures should be adopted that minimize release of airborne materials, including bedding dust and antibiotic aerosols, and the contamination of hands, arms, body, and face. Workers should adopt the use of PPE during each and every animal contact or allergen exposure. Wearing PPE “just some of the time” will not prevent exposure. Of particular importance is wearing a facemask to reduce inhalation and hand-to-face spread of allergens and covering all exposed skin (e.g., gloves, lab coat, sleeve protectors, and hair cover) to prevent allergen contact.

It is also important that once animal procedures are complete, all contaminated PPE and clothing are removed and properly disposed of to prevent repeated exposure while performing subsequent duties. Supervisors or RMS/BSO can provide further information and access to approved PPE devices.

5. **Latex Gloves and Related Allergies**

Allergic reactions to natural rubber latex have been increasing since 1987, when the Centers for Disease Control recommended the use of universal precautions to protect against potentially infectious materials, bloodborne pathogens, and HIV. Increased glove demand also resulted in higher levels of allergens due to changes in the manufacturing process. In additional to skin contact with the latex allergens, inhalation is another potential route of exposure. Latex proteins may be released into the air along with the powders used to lubricate the interior of the glove.

In June 1997, the National Institute of Occupational Safety and Health (NIOSH) issued an alert, “Preventing Allergic Reactions to Latex in the Workplace” (publication number DHHS (NIOSH) 97-135).

NIOSH studies indicate that 8-12% of healthcare workers regularly exposed to latex are sensitized, compared to 1-6% of the general population. Latex exposure symptoms include skin rash and inflammation, respiratory irritation, asthma, and shock. The amount of exposure
needed to sensitize an individual to natural rubber latex is not known, but when exposures are reduced, sensitization decreases.

NIOSH recommends the following actions to reduce exposure to latex:

- If latex gloves must be used, choose reduced-protein, powder-free latex gloves.
- Whenever possible, substitute another glove material.
- Wash hands with mild soap and water after removing latex gloves

When using antibiotic materials, procedures should be adopted that minimize release of airborne materials and skin contamination. Of particular concern are releases of penicillin-type (or other) antibiotics during syringe-loading from multi-dose vials. Persons who have had previous exposures and have developed sensitivity can quickly go into anaphylactic shock after inhaling a mist of antibiotic material. Be sure to handle these materials with caution and according to use directions. Use and caution inserts for each antibiotic are provided in the product packaging and should be read and understood prior to use. Investigators inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

6. **Zoonoses**

Zoonoses are diseases that can be transmitted between species (in some instances, by a vector) from animals to humans or from humans to animals (the latter is sometimes called reverse zoonosis or anthroponosis). They may be a significant exposure hazard in some laboratories where animals are used for research. Fortunately, many laboratory animal species today are bred to be free of zoonoses that were once more common in these animals. However, there remain zoonotic agents associated with laboratory animals, some which can be life-threatening. Field research with wild species also remains a clear source of zoonoses exposure. Prevention of exposure to these animal-related illnesses requires knowledge of the zoonoses related to the animals involved. In the sections that follow, the zoonotic agents listed for each animal species are those that may be present in the animals being used. If someone is exposed through bite, scratch, aerosol droplet, mucosal secretion, feces, or urine, there is the potential for infection, so medical consultation is highly recommended.

a) **Laboratory Mice**

Modern laboratory mice are bred to exclude all zoonotic agents. Therefore, unless the laboratory mice are infected as part of the research procedures or exposed to wild mice (those coming from the natural habitat outside the laboratory), there is limited concern for disease from these research mice. However, there is always concern about secondary infections that can occur with bites and scratches. Common skin, intestinal, and soil bacteria present on a person or an animal can infect the scratch or bite wound and cause these secondary infections. Therefore, users should handle all mice with care and always cleanse any wound immediately with soap and water or antiseptic and seek medical consultation for broken skin.
b) **Wild Rodents**

Wild rodents or laboratory rodents that have been exposed to wild rodents have the potential of carrying a variety of zoonotic bacteria and viruses that can be passed on to workers handling them. Tests should always be completed on wild rodents and those coming from foreign countries when they are received at UNT to screen for these zoonotic agents. Although this provides reasonable assurance that rodents will be free of zoonotic infections, the screening does not guarantee infection-free rodents. Therefore, because of the serious consequences of becoming infected, investigators must always follow good personal hygiene and animal handling procedures and use the provided PPE to protect from exposure. Rodents that have originated from the wild, have had contact with wild rodents, or are from foreign countries could be infected with one or more of the pathogens and should be considered ABSL-2.

(1) **Hantavirus**

Hantavirus is transmitted through inhalation of dried rodent feces and urine when such material is raised into the air from disturbed feces, bedding, or nesting material. Transmission can also occur through rodent bites and contamination of broken skin or mucous membranes. The infection progresses from flu-like symptoms to respiratory complications and has resulted in death in over 50% of clinical cases, particularly when medical care was not quickly obtained. It is possible to prevent exposure through the use of PPE, good personal hygiene, and properly ventilated handling of waste bedding material.

(2) **Lymphocytic Choriomeningitis (LCM) Virus**

LCM virus is transmitted to humans by inhalation, broken skin, or mucous membrane exposure to blood, urine, feces, and other body secretions from infected mice. The infection results in flu-like symptoms 1 to 3 weeks after exposure. More severe symptoms of meningitis and encephalitis can result. There is a special risk of exposure during pregnancy because the fetus can become infected. Because mice are well screened and provided from virus-free sources, the potential for exposure in UNT animal facilities is very limited. Again, use of proper PPE, such as disposable gloves and lab coat, along with careful hand washing will further reduce the likelihood of exposure.

c) **Laboratory Rats**

Modern laboratory rats are bred to exclude all zoonotic agents. Therefore, unless the laboratory rats are experimentally inoculated, cross-contaminated, or exposed to wild rodents (those coming from the natural habitat outside the laboratory), there is limited concern for disease from these research rats. However, there is always concern about secondary infections that can occur with bites and scratches. Common skin, intestinal, and soil bacteria present on you or the animal can infect the scratch or bite wound and cause these secondary infections. Therefore, personnel should handle all rats with care, always cleanse any wound immediately with soap and water or antiseptic, and seek
medical consultation for severe wounds.

Historically, rats have been known to carry the bacterium that causes Rat-Bite Fever. However, these bacteria have not been found in laboratory rats for decades due to the special efforts of commercial suppliers to eliminate these bacteria from breeding colonies.

d) Laboratory Rabbits

Modern laboratory rabbits contain few infectious pathogens. Of concern are scratches that can be inflicted with their strong hind legs and sharp claws or from bites. Secondary infection with common skin, intestinal, and soil bacteria present on personnel or the animal can result, so personnel should always cleanse wounds immediately with soap and water or antiseptic and seek medical consultation for severe wounds.

Historically, laboratory rabbits have been known to harbor the bacteria for human Tularemia (Rabbit Fever). Although this zoonotic agent remains present in wild rabbit populations, modern laboratory rabbits are free of this bacterium.

e) Birds

The birds used in research colonies are either caught in the wild or acquired from established flocks. In general, birds are not supplied disease-free and usually contain a number of microbial agents including *Mycobacterium avium*. Of zoonotic concern are the diarrheal bacteria such as *Salmonella* and the bacteria that cause psittacosis, which can cause a more severe type of infection.

(1) Salmonella

*Salmonella* bacteria is a common contaminant of fecal droppings and eggs. When ingested by humans, this bacterium has the potential for causing severe intestinal disease. Use of good personal hygiene measures including effective and thorough hand washing along with the proper PPE, such as disposable gloves and lab coat, will greatly reduce the likelihood of infection when handling birds and materials in their environment.

(2) Psittacosis

The bacterium *Chlamydia psittaci* is the cause of psittacosis, and it is found most widely in large, imported psittacine birds (e.g., parrots, parakeets, cockatoos, and macaws). Human infection is most often the result of exposure to these imported birds. The risk of exposure from domestic birds is very low.

However, because this bacterium is highly infectious, there is some potential that any bird or mammal may be infected. Acute infection in animals causes such symptoms as reddening of the eyes (conjunctivitis), difficulty breathing (pneumonia), swollen painful joints (arthritis), and reproductive problems. After
the acute infection, those animals that survive enter a period without symptoms during which stress can cause the animal to shed the bacterium. Stress can result from such things as the importation process or birds being handled in their new environment. Humans can be infected when coming in contact with the bird’s body secretions or feces. In humans, the symptoms include fever, headache, muscle pain, and chills, and may progress to pneumonia as well as liver, heart, and brain inflammation.

USDA regulations require that testing be performed on all psittacine birds imported from foreign countries during an initial 60-day quarantine period. There were no psittacine birds from foreign countries at UNT at the time this document was developed. However, in the event that UNT acquires psittacine birds from a foreign country, they would be need to be quarantined while testing is done and infected birds would be eliminated from the colony. The use of protective equipment and thorough hand washing would reduce the risk of any potential exposure.

f) *Fish and Amphibians*

Fish and amphibians used in research colonies are mostly wild-caught or raised on commercial farms. These animals often contain parasites and bacteria. Of zoonotic concern are gram-negative bacteria that cause secondary infection of contaminated wounds and breaks in the skin. These bacteria include *Aeromonas*, *Pseudomonas*, *Klebsiella*, and *Mycobacteria*. Use of proper PPE, such as disposable gloves, will help prevent contamination of skin surfaces. Likewise, thorough hand washing is very important to further reduce potential for infection.

**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, synthetic DNA
- Microbiological waste

The National Institutes of Health’s “Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules” (NIH Guidelines) requires UNT 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 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, unless an alternate disposal method has been pre-approved by RMS/BSO.

Soiled animal bedding from infected animals is placed by the animal care staff in sturdy plastic bags, sealed, and transferred to bins for movement from the facility. Bags of soiled bedding should be limited to 40 pounds to prevent back and shoulder injury during subsequent handling.

Animal carcasses are bagged, sealed, and stored in freezers located in each animal facility until pick up by the vendor for incineration.

All sharps utilized in the animal facility are disposed of in sharps containers, which, when full, are placed in the red barrels for biological waste when in an animal facility. All other biologically contaminated material is also placed in the red barrels. When the red barrel is full, it is the responsibility of the laboratory staff to contact RMS for pick-up.

B. Liquid Waste

Although the rules and definitions for liquid biohazard waste vary somewhat from solid waste procedures, 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 as defined under the UNT Bloodborne Pathogen Exposure Control Plan
- 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 UNT 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.

Medical Waste Rules do not allow chemical disinfection of regulated liquids followed by disposal to the sanitary sewer unless approval has been obtained from RMS.

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;
• Waste from animals intentionally infected with microbes, viral vectors, or toxins.

If you wish 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, and documented in the Laboratory-Specific Biosafety Plan.

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 etc. These bottles of chemicals cannot be poured down the sink or sanitary sewer. They must be discarded using the online hazardous waste pick up program 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 UNT 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:
  - closable;
  - constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping;
  - 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 to the eyes, irrigate with clean water, saline, or sterile irrigants;
4. Report the incident to your supervisor; and

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. If you are unable to speak with a person in RMS, during regular business hours, treatment should be obtained at the closest CareNow facility. 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 go to UNT Student Health & Wellness Center. If UNT Student Health & Wellness Center is closed, emergency care may be obtained at the nearest emergency room and reported to UNT Student Health Services and RMS/BSO the next business day.

Supervisors must report all accidents and injuries to RMS. Submit the Biohazard Incident Report Form to biosafety@unt.edu 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.

Disposal of Biohazardous Sharps

In UNT research laboratories, biohazardous sharps are collected directly into red, plastic containers available from FisherScientific (catalog #s 14-827-104, 14-827-63, 14-827-71, or 22-037-959 for contaminated; 14-830-107 for non-contaminated). These containers must bear the biohazard symbol marked with an “x” using autoclave indicator tape. To avoid accidents related to overfilling the containers, remove the containers for disposal when they are 2/3 full. When removing the sharps container from a biosafety cabinet, always decontaminate the exterior of the container. Containers of sharps contaminated with biohazardous materials should be autoclaved in a red autoclavable bag (FisherScientific polypropylene biohazard printed bags, catalog #s 01-828 A through E) marked with an “x” over the bag’s biohazard symbol. After autoclaving, the bags with the containers of sharps can be disposed of with the regular trash. Non-hazardous sharps should be placed in the white plastic sharps containers. The non-hazardous sharps containers should be disposed of in regular trash.
once they are 2/3 full. **Note:** If you use a biohazardous sharps box with non-biohazardous sharps, you must treat it as if it is a biohazard and it must be autoclaved.

While small shards of contaminated broken glass can be placed into the sharps containers identified above, large contaminated broken glass items must be autoclaved separately in a hard-walled container (such as a cardboard box) lined with a red biohazard bag bearing an autoclave tape indicator “x” over the bag’s biohazard symbol. Place the tape on the red bag before it is used to line the box to prevent contact with biohazardous materials and sharps. The universal biohazard symbol should also be found on the outside of the box. **After autoclaving (see Autoclave Waste Decontamination Procedures),** the glass waste can be disposed of in the regular trash.

**Additional Information**

It is intended that the Principal Investigator (PI) and supervisory personnel will supplement this information with instruction and guidance regarding specific practices and procedures unique to the work being done in their facilities.

**XIII. Disinfection and Decontamination**

Wastes associated with biological research materials must be disposed of in special ways.

**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 and disinfection are two ways to address microbial contamination.

**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.

Sterilization, disinfection, and antisepsis are all forms of decontamination.

**B. When to Decontaminate**

In most UNT 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**

<table>
<thead>
<tr>
<th>Blood and body fluids (Regulated medical waste)</th>
<th>Treated with bleach or autoclaved at 123°C for 70 minutes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiological Waste including Biosafety Level 1 and 2 organisms: (Regulated medical waste)</td>
<td>Autoclaved at 123°C for 70 minutes or chemically treated and sent to landfill.</td>
</tr>
<tr>
<td>Pathological waste (animal carcasses infected with human BSL1 and BSL2, this includes transgenic mice) (Regulated medical waste)</td>
<td>Animals are incinerated by vendor (TBD)</td>
</tr>
<tr>
<td>Uninfected Animal Carcasses</td>
<td>Animals are incinerated by vendor (TBD)</td>
</tr>
<tr>
<td>Non-hazardous Sharps</td>
<td>White plastic sharps containers sent to landfill</td>
</tr>
<tr>
<td>Biohazardous Sharps</td>
<td>Red plastic sharps containers are autoclaved at 123°C for 70 minutes then sent to landfill</td>
</tr>
</tbody>
</table>
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. See PI Autoclave Waste Decontamination Cycle Testing and Verification.

1. Autoclavable Waste Bags/Containers

Red polypropylene, preprinted, biohazard autoclave bags (FisherScientific 01-828 A through E) should be utilized in UNT 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, leakproof, have a lid and be of such a design so as not to be mistaken by Housekeeping as 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 FisherScientific 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 FisherScientific 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 as shown below. 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” (labels available appendix xxx). Biohazard bags placed in the white Brute containers and marked with the heat sensitive tape signal to Housekeeping 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 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. If you want to deviate from these parameters, a validated method must be submitted to the BSO.

<table>
<thead>
<tr>
<th>Material</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laundry</td>
<td>121°C</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Trash (biohazard bags, &lt;2/3 full, containing infectious waste)</td>
<td>123°C</td>
<td>70 minutes</td>
</tr>
<tr>
<td>Glassware</td>
<td>121°C</td>
<td>60 minutes</td>
</tr>
<tr>
<td>Liquids</td>
<td>121°C, each gallon</td>
<td>60 minutes</td>
</tr>
<tr>
<td>Animals</td>
<td>121°C</td>
<td>8 hours</td>
</tr>
</tbody>
</table>

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 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 at biosafety@unt.edu.

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 maintain 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.

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 E. 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 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 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 – used to collect liquid
B. Secondary flask (overfill flask) minimizes splash
C. In line filter between secondary flask and vacuum source (FisherSci 09-744-75)
D. Vacuum line that is occasionally serviced by lab workers or UNT 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 and respirators. 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 form the 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.

![Diagram of HEPA filter](image)

**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.

a) 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).
b) **Class II Biological Safety Cabinets**

Class II biological safety cabinets are the most commonly used biological safety cabinet at UNT 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 which 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.
c) **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. Don 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.

**NOTES:** 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.** Refer to the UNT rule on open flames in BSCs.

**d) 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. Most biological safety cabinets at UNT 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 maintains the certifications for the BSCs at UNT.** If the BSC in your lab is not currently certified, cease work in the BSC and notify the BSO at biosafety@unt.edu.

**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

UNT 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 UNT 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, please 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. UNT Laundry Facilities

Laundry facilities exist in a few departments at UNT. Follow departmental procedures for cleaning *mild to moderately* contaminated clothing. Generally, these facilities are for intra-department use only. Laboratory managers may launder mildly contaminated clothing using departmental laundry facilities where available. Contaminated clothing shall be washed, at a minimum in accordance with the manufacturer’s directions. However, departments are encouraged to launder contaminated clothing in hot water (160º F or greater). Where departmental facilities are not available, contaminated clothing must be laundered by a professional laundry service.

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. Where departmental facilities are not available, contaminated clothing must be laundered by a professional laundry service. 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 in which chemicals, biological, and radioactive materials are kept unless they have been labeled, “Not for Human Consumption.”

XIX. Nails and Jewelry

Principal Investigators (PIs) at UNT 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, 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 recommends nail length should be no longer than 0.25 inch beyond the end of fingertips. Artificial nails (e.g., nail extensions, nail wraps, nail jewelry) are not recommended when working in the laboratory.

XX. Transfers, Packaging, and Shipping of Biological Materials

A. Transfers

The transferring, packing, and shipping of select agents and toxins is HIGHLY regulated. Please contact the UNT Biological Safety Officer or the [Office of Research and Innovation](mailto:OfficeofResearchandInnovation) for more information.
For materials that are not 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. Please refer to UNT Export Controls.
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) are 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-carryed on common carriers, all applicable packaging, transport, and training regulations should be followed.
7. Develop and follow a protocol for intra-facility transfer (between laboratories on UNT campuses) of all biological and biohazards. Contact RMS/BSO 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 which 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 or Office of Research and Innovation.

C. Labeling

The outer shipping container of all materials containing etiologic/biohazards which are being shipped or transported must bear a special labels. Please contact RMS Biosafety and Biosecurity 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.

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.
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 UNT 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 Research Integrity & Compliance or find additional information regarding export controls [here](#).

1. **U.S. Public Health Service (USPHS)**
Importation permits are issued by the U.S. Public Health Service (USPHS) only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by U.S. Customs.

2. **CDC Application for Permit to Import Infectious Biological Agents into the United States**

Code of Federal Regulations Title 42 Chapter I Subchapter F Part 71 Subpart F §71.54 requires persons importing etiologic agents to obtain a permit through the CDC.

The permits offered by the CDC include Permit to Import Biological Agents or Vectors of Human Disease (A/BSL 2 and A/BSL 4; and ACL-2 and ACL-3) or Permit to Import or Transport Live Bats. Checklists for compliance with the requirements of the import permit regulations can be found on their [website](#).

Instead of an importation permit, a Letter of Authorization may be issued by the Centers for Disease Control and Prevention after review of an “Application to Import an Etiological Agent.” The letter is issued for materials that are judged to be noninfectious, but which U.S. Customs inspection personnel might construe to be infectious. Letters of Authorization may be issued for items such as formalin-fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine, cerebrospinal fluid, and other tissues or materials of human origin when there is no evidence or indication that such materials contain an infectious agent. Letters of Authorization are in effect for two years and do not require a shipping label to be issued by CDC.

Importation permits and Letters of Authorization are issued by the Biosafety Branch, Office of Health and Safety, CDC, 1600 Clifton Road, Atlanta, Georgia 30333, after review of a completed application form. Application forms may be obtained online or by calling CDC at their FAX Information System. Dial 1-888-CDC-FAXX and enter document number 101000. CDC can also be contacted on their [website](#). Completed forms may be returned to CDC by mail or FAX at 404-639-2294. Application to CDC for the importation permit should be made 15 working days in advance of the shipment date to allow time for processing, issuance, and delivery of the permit and shipping labels to the permittee.

Contact RMS/BSO ([biosafety@unt.edu](mailto:biosafety@unt.edu)) or [Research Integrity & Compliance](#) for additional guidance.

3. **Other Permits**

Imported shipments may require a United States Department of Agriculture (USDA) permit if your product contains ingredients derived from plants or animals or if there is domestic shipping of infectious agents of livestock, poultry, and other animal diseases, and any materials that might contain these agents. The majority of plant- or animal-derived ingredients do require a permit. It is better to apply for a permit and receive a Letter of No Jurisdiction than to have a shipment delayed or rejected for lack of a proper permit.

There are three main types of import permits offered through the USDA: Veterinary Service Permit and Plant Protection and Quarantine Permit; Animal and Plant Health Inspection Permit.
a) **USDA Veterinary Service Permit**

The Veterinary Service (VS) Permit, issued by the Veterinary Services branch of APHIS, specifies the conditions under which animals, animal products, or products with animal-origin ingredients may be imported into the United States. Often to the surprise of importers, a large number of products – some very unintuitive – require VS Permits. Learning this lesson the hard way, by a Customs Ag hold or Emergency Action Notification, will guarantee increased costs and delay and often USDA-refused products. The importation or domestic transfer of plant pests is also regulated by the USDA. Such a permit is required for plant pests, plant biological agents, or any material that might contain them. Information may be obtained by calling 301-734-3277 or through the web. USDA permits are required for certain live animals and all live bats. Call 800-358-2104 for further information.

b) **Plant Protection and Quarantine Permit**

The Plant Protection and Quarantine (PPQ) Permit, issued by the Plant Protection and Quarantine branch of APHIS, specifies the conditions under which plants, plant products, or products with plant-origin ingredients may be brought into the United States.

c) **USDA Animal and Plant Health Inspection Service (APHIS)**

USDA Animal and Plant Health Inspection Service (APHIS) issues permits for the import, transit and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms. Tissue culture materials, and suspensions of cell culture-grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are also controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U.S. Applications for USDA/APHIS permits may be obtained by calling the appropriate office at USDA APHIS using the numbers found at their website. However, they also have readily available electronic permit system through the website.

U.S. Fish and Wildlife Service (USFWS) permits are required for certain live animals, including bats. Please call 800-344-WILD for further information.

**Export** of infectious materials may require license from the Department of Commerce (DoC). Exporters of a wide variety of etiologic agents of human, plant, and animal diseases, including genetic material and products which might be used for culture of large amounts of agents will require an export license. A key in determining whether an export license is needed from the Department of Commerce is determining whether the item you intend to export has a specific Export Control Classification Number (ECCN). The ECCN is an alphanumeric code, e.g., 3A001 that describes the item and indicates licensing requirements. Information may be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the web.

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. UNT Risk Management Services (RMS/BSO) 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 UNT Biosafety inspection checklist, for more information about the biosafety audit form used by RMS/BSO.

RMS/BSO will make every attempt to schedule safety audits with faculty members. However, if the principal investigator is unavailable or is unresponsive, RMS/BSO will proceed with the safety audit. RMS/BSO 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 faculty member responsible for the laboratory. The faculty member is responsible for correcting the hazards. If the faculty member fails to correct the hazard, a second notice is sent to the department head with a copy to the faculty member. 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 UNT police if materials are damaged or missing from laboratories.
- Notify UNT 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


XXIII. Emergency Preparedness

Emergency guidelines and floorplans can be found online at the Office of Emergency Management & Safety Services. Emergency procedures for the following scenarios are provided at the links below:
- Active Shooter
- Acts of Threats of Violence
- Bomb Threat
- Civil Disturbance
- Crime Prevention
- Evacuation Procedures
- Fire
- Gas Leak
- Hazardous Materials
- Hostage Situation
- Inclement (Winter) Weather
- Medical Emergencies
- Power Outage
- Shelter-in-Place
- Stay Informed
- Suspicious Package of Object
- Tornado/Sever Weather
- Utility Failure

XXIV. Working Alone

All faculty, staff, students, and visitors working in an area (e.g., laboratory, animal holding room) 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\dagger 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.

‡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.

XXV. Recordkeeping

A. Inventory

Detailed inventory records must be maintained by the PI 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 (i.e., ATCC, Dr. Smith at UCLA), their storage location, and the assigned biosafety level. Additional information and templates for inventory management are available through RMS on the biosafety website. These must be submitted to biosafety@unt.edu annually.

B. Additional Records

The principal investigator 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 UNT Biosafety and Biosecurity Program.
Appendix A. Definitions

**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 which 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 an employee 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** – any UNT faculty member, staff employee, or student conducting research or other educational activities utilizing UNT facilities or due to his/her status as a UNT employee or student involving biohazardous agents, potentially hazardous human materials, or recombinant DNA molecules.

**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.
### Appendix B. Acronyms

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<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>AAALA</td>
<td>Association for Assessment and Accreditation of Laboratory Animal Care International</td>
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<tr>
<td>AC</td>
<td>Animal Care</td>
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<td>APHIS</td>
<td>Animal and Plant Health Inspection Service</td>
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<td>UNT</td>
<td>University of North Texas</td>
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<td>BMBL</td>
<td>Biosafety in Microbiological and Biomedical Laboratories</td>
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<td>BSC</td>
<td>Biological Safety Cabinet</td>
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<td>BSO</td>
<td>Biological Safety Officer</td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CFR</td>
<td>Code of Federal Regulations</td>
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<td>DEA</td>
<td>Drug Enforcement Administration</td>
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<td>RMS</td>
<td>Risk Management Services</td>
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<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
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<td>IBC</td>
<td>Institutional Biosafety Committee</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<td>PHS</td>
<td>Public Health Service</td>
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<td>PI</td>
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<td>PPE</td>
<td>Personal Protective Equipment</td>
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<td>SDS</td>
<td>Safety Data Sheet</td>
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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Appendix C. Biosafety Guidelines (BSL-1) (BMBL 5th ed.)

Guidelines for Good BSL-1 Practices

BSL-1 is suitable 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-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

1. Standard Microbiological Practices

   The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

   Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

   Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

   Mouth pipetting is prohibited; mechanical pipetting devices must be used.

   Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

   Precautions, including those listed below, must always be taken with sharp items. These include:

   Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

   Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

   Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

   Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic-ware should be substituted for glassware whenever possible.

   Perform all procedures to minimize the creation of splashes and/or aerosols.

   Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

   Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

An effective integrated pest management program is required.

The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

2. Special Practices

None required.

3. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Special containment devices or equipment, such as biological safety cabinets, are not generally required.

Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment.

Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

- Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
- Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
- Do not wash or reuse disposable gloves.
- Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

Laboratory Facilities (Secondary Barriers)

a) Laboratories should have doors for access control.

b) Laboratories must have a sink for hand washing.

c) The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

iii. Laboratories windows that open to the exterior should be fitted with screens.

Guidelines for Good BSL-2 Practices

BSL-2 builds upon BSL-1 practices. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

b) Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

c) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

d) Mouth pipetting is prohibited; mechanical pipetting devices must be used.

e) Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

iii. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic-ware should be substituted for glassware whenever
possible.

f) Perform all procedures to minimize the creation of splashes and/or aerosols.

g) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

h) Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

i) Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

j) Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

k) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory’s biosafety level, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory.

Agent information should be posted in accordance with the institutional policy.

j) An effective integrated pest management program is required.

k) The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

2. **Special Practices**

a) All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

b) Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

c) Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

d) A Laboratory-Specific Biosafety Manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

e) The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

f) Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing,
Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

h) Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

i) Animals and plants not associated with the work being performed must not be permitted in the laboratory.

j) All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a biological safety cabinet or other physical containment devices.

3. Safety Equipment (Primary Barriers and Personal Protective Equipment)

a) Properly maintained biological safety cabinets (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

i. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

i. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

b) Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

c) Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the biological safety cabinet or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

d) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

e) Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

a) Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

b) Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.[

c) The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

j) Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

e) Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

f) Biological safety cabinets must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Biological safety cabinets should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

g) Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

h) An eyewash station must be readily available.

i) There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

j) HEPA filtered exhaust air from a Class II biological safety cabinet can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure
proper safety cabinet performance and air system operation must be verified.

k) A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Guidelines for Good BSL-3 Practices

NO WORK AT BSL-3 IS APPROVED AT UNT.

Biological Safety Level 3 (BSL-3) is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets, other physical containment devices, or by personnel wearing appropriate personal protective equipment. A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

**Standard Microbiological Practices**

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

b) Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

c) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

d) Mouth pipetting is prohibited; mechanical pipetting devices must be used.

e) Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

iii. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
f) Perform all procedures to minimize the creation of splashes and/or aerosols.

g) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

h) Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

2. 

ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

i) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory’s biosafety level, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

j) An effective integrated pest management program is required.

k) The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

Special Practices

a) All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

b) Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

c) Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

d) A Laboratory-Specific Biosafety Manual must be prepared and adopted as policy. The biosafety manual must be available and
accessible.

e) The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.

f) Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

g) Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

h) Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

i) Animals and plants not associated with the work being performed must not be permitted in the laboratory.

j) All procedures involving the manipulation of infectious materials must be conducted within a biological safety cabinet, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a biological safety cabinet, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

a) All procedures involving the manipulation of infectious materials must be conducted within a biological safety cabinet (preferably Class II or Class III), or other physical containment devices.

b) Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls is worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.

c) Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

d) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:

i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the
laboratory.

iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

e) Eye, face, and respiratory protection must be used in rooms containing infected animals.

4. Laboratory Facilities (Secondary Barriers)

a) Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

b) Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.

c) The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

i. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

ii. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

iii. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

e) All windows in the laboratory must be sealed.

f) Biological safety cabinets must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Biological safety cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

g) Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
h) An eyewash station must be readily available in the laboratory.

i) A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

   i. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

   ii. The laboratory exhaust air must not recirculate to any other area of the building.

   iii. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

j) HEPA filtered exhaust air from a Class II biological safety cabinet can be safely recirculated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. Biological safety cabinets should be certified at least annually to assure correct performance. Class III biological safety cabinets must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

k) A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

l) Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

m) Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

n) Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

o) The BSL-3 facility design, operational parameters, and procedures must be
verified and documented prior to operation. Facilities must be re-verified and documented at least annually.
Appendix D. Animal Biosafety Guidelines (ABSL-1)

Guidelines for Good ABSL-1 Practices

Animal Biosafety Level 1 (ABSL-1) is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans and present minimal potential hazard to personnel and the environment.

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment.

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following standard practices, safety equipment, and facility requirements apply to ABSL-1.

1. Standard Microbiological Practices

   a) The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

   Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

   Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the IBC.

   b) A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

   c) The animal facility supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

   d) An appropriate medical surveillance program is in place as determined by risk assessment. The need for an animal allergy prevention program should be considered.

   Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, and animal care and manipulations. This is accomplished at UNT by having the Occupational Health and Safety Program physician and/or nurse regularly attend IACUC meetings.

   Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations, or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and
guidance.

Personnel using respirators must be enrolled in the UNT Health Services respiratory protection program.

e) A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the animal facility supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. However, at UNT all research with biohazard agents are conducted at ABSL-2 or higher.

Security-sensitive agent information should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans as a contingency for man-made or natural disasters.

f) Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.

All persons including facility personnel, service workers, and visitors, are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

g) Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious, and hazardous materials, and when handling animals.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye, face, and respiratory protection should be used in rooms containing infected animals as dictated by the risk assessment.

h) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designed and used for this purpose.

i) All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

j) Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

k) Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

i) Use of needles and syringes or other sharp instruments in the animal facility is limited
to situations where there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

i. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

ii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

v. Equipment containing sharp edges and corners should be avoided.

l) Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent and after any spills, splashes, or other overt contamination.

m) Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or manipulated.

n) An effective integrated pest management program is required.

o) All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local, and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

Special Practices

None required.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

a) A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

b) Special containment devices or equipment may not be required as determined by appropriate risk assessment.

c) Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing.

Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.

d) Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with non-human primates must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed.

e) Gloves are worn to protect hands from
exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task, and alternatives to latex gloves should be available.

Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

f) Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated. Hand washing should occur after the removal of gloves.

4. Laboratory Facilities (Secondary Barriers)

a) The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

b) The animal facility must have a sink for hand washing.

Sink traps are filled with water and/or appropriate liquid to prevent the migration of vermin and gases.

c) The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

It is recommended that penetrations in floors, walls and ceiling surfaces, including openings around ducts, doors, and doorframes, be sealed to facilitate pest control and proper cleaning.

d) Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

e) External windows are not recommended; if present, windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

f) Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. No recirculation of exhaust air may occur.

It is recommended that animal rooms have inward directional airflow.

Ventilation system design should consider the
heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

g) Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

h) If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

i) Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F (82°C). If manual cage washing is utilized, ensure that appropriate disinfectants are selected.

j) Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

k) Emergency eyewash and shower are readily available; location is determined by risk assessment.

Guidelines for Good ABSL-2 Practices

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and 4) a biological safety cabinet (BSC) or other physical containment equipment is used when procedures involve the manipulation of infectious materials or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs is required.

The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

Standard Microbiological Practices

a) The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC and the IBC.

b) A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

Consideration should be given to specific biohazards unique to the animal species and protocol in use.

c) The animal facility supervisor must ensure that animal care, laboratory, and support personnel
receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions, and staff attendance.

d) An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, and animal care and manipulations.

Personal health status may impact an individual’s susceptibility to infection and ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in the UNT Health Services respiratory protection program.

e) A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the animal facility supervisor’s name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Contact RMS/BSO for more information.

Advance consideration should be given to emergency and disaster recovery plans as a contingency for man-made or natural disasters.

f) Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

g) Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious, and hazardous materials and when handling animals.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or
animals are housed or manipulated.

Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or manipulated.

Eye, face and respiratory protection should be used in rooms containing infected animals as dictated by the risk assessment.

h) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.

i) All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

j) Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

k) Policies for the safe handling of sharps such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:

i. The use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

ii. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

iii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

v. Use of equipment with sharp edges and corners should be avoided.

l) Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent and after any spills, splashes, or other overt contamination.

m) Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

n) An effective integrated pest management program is required.

o) All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local, and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

2. Special Practices
a) Animal care, laboratory, and routine support personnel are provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present before entry into animal rooms.

When appropriate, a baseline serum sample is stored.

b) Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.

c) Decontamination by an appropriate method (e.g., autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.

A method for decontaminating routine husbandry equipment as well as sensitive electronic and medical equipment should be identified and implemented.

Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or manipulated must be placed in a durable, leak-proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local, and state requirements. Autoclaving of content prior to incineration is recommended.

d) Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or manipulated.

e) Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

f) Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Contact UNT Health Services for more information. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

3. Safety Equipment (Primary Barriers and Personal Protective Equipment)

a) Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential
exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.

b) A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

Gowns, uniforms, laboratory coats, and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

c) Eye and face protection (mask, goggles, and face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with non-human primates should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.

d) Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task, and alternatives to latex gloves should be available.

Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated. Hand washing should occur after the removal of gloves.

Laboratory Facilities (Secondary Barriers)

a) The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Doors to areas where infectious materials
and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

b) A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.

If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.

c) The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.

Penetrations in floors, walls, and ceiling surfaces, including openings around ducts, doors, and doorframes, are sealed to facilitate pest control and proper cleaning.

Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.

d) Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

e) External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

f) Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

g) Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

h) Floor drains must be maintained and filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.

i) Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F (82°C). The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.

j) Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

k) If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper
operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.

All BSCs should be used according to manufacturer’s specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.

l) If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.

m) An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.

n) Emergency eyewash and shower are readily available; location is determined by risk assessment.

Guidelines for Good ABSL-3 Practices

NO WORK AT ABSL-3 IS APPROVED AT UNT.

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

The ABSL-3 laboratory has special engineering and design features. ABSL-3 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3.

1. Standard Microbiological Practices

   a) The animal facility animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

   UNT must assure that worker safety and health concerns are addressed as part of the animal protocol review.

   Prior to beginning a study, animal protocols
must be reviewed and approved by the IACUC and the IBC.

b) A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential and special hazards and are required to read and follow instructions on practices and procedures.

Consideration must be given to specific biohazards unique to the animal species and protocol in use.

c) The facility supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions, and staff attendance.

d) An appropriate medical surveillance program is in place as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility to include those associated with the research, animal husbandry duties, animal care, and manipulations.

Personal health status may impact an individual’s susceptibility to infection as well as their ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in the UNT Health Services respiratory protection program.

e) A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the facility supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Contact RMS/BSO for more information.

Advance consideration should be given to emergency and disaster recovery plans as a contingency for man-made or natural disasters.

f) Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where
infectious materials and/or animals are housed or manipulated.

All persons, including facility personnel, service workers, and visitors, are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

g) Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious/hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.

Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or manipulated.

Eye, face, and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

h) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

i) All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

j) Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

k) Policies for the safe handling of sharps such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:

i. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

ii. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

iii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

v. Use of equipment with sharp edges and corners should be avoided.
l) Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent and after any spills, splashes, or other overt contamination.

m) Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or manipulated.

n) An effective integrated pest management program is required.

o) All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local, and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

2. Special Practices

a) Animal care, laboratory, and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

When appropriate, a baseline serum sample should be stored.

b) All procedures involving the manipulation of infectious materials, handling of infected animals, or the generation of aerosols must be conducted within BSCs or other physical containment devices when practical.

When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

Restraint devices and practices are used to reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications).

c) The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems.

d) Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate operational malfunctions.

e) A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials and/or animals are housed or manipulated (e.g., autoclave, chemical disinfection, or other approved decontamination methods).

Consideration must be given to means for decontaminating routine husbandry equipment as well as sensitive electronic and medical equipment.

Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) by an appropriate method.
before removal from the areas where infectious materials and/or animals are housed or manipulated.

It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or manipulated, preferably within the caging system.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local, and state requirements.

f) Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.
   Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

   Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

   g) Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Contact UNT Health Services for more information. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

3. Safety Equipment (Primary Barriers and Personal Protective Equipment)

   a) Properly maintained BSCs and other physical containment devices or equipment should be used for all manipulations for infectious materials and, when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

   The risk of infectious aerosols from infected animals or bedding can be reduced by primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets, ventilated cage rack systems, or, for larger species, cages placed in inward flow ventilated enclosures or other equivalent systems or devices.

   b) A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

   Personnel within the animal facility wear protective clothing such as uniforms or scrub suits. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits or wrap-around or solid-front gowns should be worn over this clothing before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.

   Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or manipulated. Scrub suits and uniforms are removed before leaving the animal facility. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

   c) All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate eye, face, and respiratory protection. To
prevent cross contamination, boots, shoe covers, or other protective footwear are used where indicated.

Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

d) Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task, and alternatives to latex gloves should be available.

Procedures may require the use of wearing two pairs of gloves (double-glove).

Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated. Hand washing should occur after the removal of gloves.

Laboratory Facilities (Secondary Barriers) 4.

a) The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open.

Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.

b) A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.

If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water and/or appropriate liquid to prevent the migration of vermin and gases.

c) The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.

Penetrations in floors, walls, and ceiling surfaces, including openings around ducts
and, doorframes, are sealed to facilitate pest control, proper cleaning, and decontamination. Walls, floors, and ceilings should form a sealed and sanitizable surface.

Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.

Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

d) Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Equipment and furnishings with sharp edges and corners should be avoided.

e) External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

f) Ventilation of the facility should be provided in accordance with the Guide for Care and Use of Laboratory Animals. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. HEPA filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations, and use conditions. The exhaust must be dispersed away from occupied areas and air intakes.

Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. Alarms should be considered to notify personnel of ventilation and HVAC system failure.

g) Internal facility appurtenances such as light fixtures, air ducts, and utility pipes are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

h) Floor drains must be maintained and filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.

i) Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F (82°C). Cages should be autoclaved or otherwise decontaminated prior to removal.
from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage cleaning process.

j) Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

k) BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or exhausted directly to the outside through a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance.

Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.

All BSCs should be used according to manufacturers’ specifications.

When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

l) An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials to designated alternate location/s within the facility.

m) Emergency eyewash and shower are readily available; location is determined by risk assessment.

n) The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.

o) Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions or other applicable federal, state, or local regulations.
### Appendix E. Disinfection Tables

<table>
<thead>
<tr>
<th>DISINFECTANT ACTIVITY</th>
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<tbody>
<tr>
<td>Disinfectants</td>
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<tr>
<td>Type</td>
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<td>Liquid</td>
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<td>Gas</td>
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</table>

NE=not effective  B=Variable results dependent on virus  *=Available halogen (1:100)
<table>
<thead>
<tr>
<th>DISINFECTANT</th>
<th>Important</th>
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<tbody>
<tr>
<td>Type</td>
<td>Category</td>
</tr>
<tr>
<td>Liquid</td>
<td>Quaternary Ammonia Compound</td>
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<tr>
<td>Phenolic Compounds</td>
<td>+</td>
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<tr>
<td>Chlorine Compounds</td>
<td>+</td>
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<tr>
<td>Iodophor</td>
<td>+</td>
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<tr>
<td>Alcohol, Ethyl</td>
<td>+</td>
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<tr>
<td>Alcohol, Isopropyl</td>
<td>+</td>
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<tr>
<td>Formaldehyde</td>
<td>+</td>
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<tr>
<td>Glutaraldehyde</td>
<td>+</td>
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<tr>
<td>Gas</td>
<td>Ethylene Oxide</td>
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<tr>
<td>Paraformaldehyde</td>
<td>N/A</td>
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</tbody>
</table>

N/A=not applicable (A)=Protected from light and air (B)=Neither flammable nor explosive in 90% CO2 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.
<table>
<thead>
<tr>
<th>Type</th>
<th>Disinfectants</th>
<th>Important Characteristics</th>
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<tbody>
<tr>
<td>Liquid</td>
<td>Quaternary Ammonia Compound</td>
<td>+</td>
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<td>Phenolic Compounds</td>
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<tr>
<td></td>
<td>Paraformaldehyde</td>
<td>+</td>
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</table>
Appendix F. 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”

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 of 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, nonintact skin, or bites; direct contact with mucous membranes; and inhalation of aerosols) (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 LD50 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. For example human tissues or cell lines make harbor pathogens (Table 2);
- Availability of vaccines and/or prophylactic interventions; and
- 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
o Pouring, splitting, or decanting liquid specimens
o Removing caps or swabs from culture containers, opening lyophilized cultures, opening cryotubes
o Spilling infectious material
o Filtering specimens under vacuum
  o Preparing smears, performing heat fixing, staining slides
  o Performing serology, rapid antigen tests, wet preps, and slide agglutinations
  o Throwing contaminated items into biohazardous waste
  o 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;

Examples of possible hazards are 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 immnosuppressive drugs, chronic respiratory conditions, pregnancy, nonintact 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; and
• The most common routes of exposure or entry into the body (i.e., skin, mucous membranes, lungs, and mouth) (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 nonintact 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>
<thead>
<tr>
<th>Routes of exposure/transmission</th>
<th>Activities/practices</th>
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</thead>
<tbody>
<tr>
<td><strong>Ingestion/oral</strong></td>
<td>• Pipetting by mouth</td>
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<tr>
<td></td>
<td>• Splashing infectious material</td>
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<tr>
<td></td>
<td>• Placing contaminated material or fingers in mouth</td>
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<td></td>
<td>• Eating, drinking, using lipstick or lip balm</td>
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<tr>
<td><strong>Percutaneous inoculation/nonintact skin</strong></td>
<td>• Manipulating needles and syringes</td>
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<td>• Handling broken glass and other sharp objects</td>
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<td></td>
<td>• Using scalpels to cut tissue for specimen processing</td>
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<td>• Waste disposal (containers with improperly disposed sharps)</td>
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<tr>
<td><strong>Direct contact with mucous membranes</strong></td>
<td>• Splashing or spilling infectious material into eye, mouth, nose</td>
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<tr>
<td></td>
<td>• Splashing or spilling infectious material onto intact and nonintact skin</td>
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<tr>
<td></td>
<td>• Working on contaminated surfaces</td>
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<td></td>
<td>• Handling contaminated equipment (i.e., instrument maintenance)</td>
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<td></td>
<td>• Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material</td>
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<tr>
<td></td>
<td>• Bites and scratches from animals and insects</td>
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<td></td>
<td>• Waste disposal</td>
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<td></td>
<td>• Manipulation of contact lenses</td>
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<tr>
<td><strong>Inhalation of aerosols</strong></td>
<td>• Manipulating needles, syringes, and sharps</td>
</tr>
<tr>
<td></td>
<td>• Manipulating inoculation needles, loops, and pipettes</td>
</tr>
<tr>
<td></td>
<td>• Manipulating specimens and cultures</td>
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<td></td>
<td>• Spill cleanup</td>
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<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Source</th>
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<tbody>
<tr>
<td>Adenovirus</td>
<td>Human kidney, pancreas, some adenovirus transformed cell lines, rhesus monkey kidney cells</td>
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<tr>
<td>Bovine viruses:</td>
<td>Bovine serum, fetal bovine serum (substantially lower risk today due to ultrafiltration of bovine serum)</td>
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<tr>
<td>Bovine rhinotracheitis virus</td>
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<tr>
<td>Bovine diarrhea virus</td>
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<tr>
<td>Parainfluenza type 3</td>
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<tr>
<td>Bovine enterovirus</td>
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<td>Bovine herpesvirus</td>
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<tr>
<td>Bovine syncytial virus</td>
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<tr>
<td>Cytomegalovirus</td>
<td>Kidney, human foreskin, monkey kidney cells</td>
</tr>
<tr>
<td>Epstein-Barr virus (EBV)</td>
<td>Some lymphoid cell lines and EBV-transformed cell lines, human kidney</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Human blood, liver</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Human kidney</td>
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<tr>
<td>Herpesvirus group</td>
<td>Monkey kidney cells</td>
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<tr>
<td>Human or simian immunodeficiency virus</td>
<td>Blood cells, serum, plasma, solid organs from infected humans or monkeys</td>
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<tr>
<td>Human papilloma virus (HPV)</td>
<td>HeLa cell lines</td>
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<tr>
<td>HTLV-1</td>
<td>Human kidney, liver</td>
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<tr>
<td>Lymphocytic choriomeningitis virus</td>
<td>Multiple cell lines, mouse tissue</td>
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<tr>
<td>Mycoplasmas</td>
<td>Many cell cultures</td>
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<tr>
<td>Myxovirus (SV5)</td>
<td>Monkey kidney cells</td>
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<tr>
<td>Porcine parvovirus</td>
<td>Fetal porcine kidney cells, trypsin preparations</td>
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<tr>
<td>Rabies virus</td>
<td>Human cornea, kidney, liver, iliac vessel conduit</td>
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<tr>
<td>Simian adenoviruses</td>
<td>Rhesus, cynomologous, and African green monkey kidney cells</td>
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<tr>
<td>Simian foamy virus</td>
<td>Rhesus, cynomologous, and African green monkey kidney cells</td>
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<tr>
<td>Simian virus 40 (SV40)</td>
<td>Rhesus monkey kidney cells</td>
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<tr>
<td>Simian viruses 1–49</td>
<td>Rhesus monkey kidney cells</td>
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<tr>
<td>Swine torque teno virus</td>
<td>Trypsin, swine-origin biological components</td>
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<tr>
<td>Squirrel monkey retrovirus</td>
<td>Multiple cell lines, commercial interferon preparations</td>
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<tr>
<td>West Nile virus</td>
<td>Human blood, heart, kidney, liver, lung, pancreas</td>
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</table>
**REVISION HISTORY**

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

<table>
<thead>
<tr>
<th>Date of Review</th>
<th>Changes Made</th>
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