UNT BIOSAFETY MANUAL

Aug 2025

This latest version of the Biosafety Manual has been updated by the Office of Research Integrity and Compliance, the Biosafety Officer, and the Institutional Biosafety Committee (IBC) at the University of North Texas (UNT). This Biosafety Manual provides university-wide safety guidelines, rules, and procedures for the use, possession, manipulation, and transport of biological materials

Revision	Date	Name	Description
1	2021	Megan Shoff	Annual Updates and Editorial Revision
2	Jan 2022	Veena Naik	Updates to information and transfer from EH&S to RIC
3	July 2023	Veena Naik	Annual Updates
4	July 2024	Veena Naik	Annual Updates
5	Aug 2025	Veena Naik	Annual Updates

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1 INTRODUCTION

FORWARD

This Biosafety Manual has been updated by the Office of Research Integrity and Compliance, Biosafety Officer and the Institutional Biosafety Committee (IBC) at the University of North Texas (UNT). This manual is part of the UNT Biosafety Policy and Program instituted to accomplish the following goals:

- To protect against exposures of personnel (UNT employees and students, community members and visitors) to biological agents
- To prevent environmental contamination
- To provide an environment for high-quality research while maintaining a safe workplace
- To comply with applicable federal, state, and local regulations and guidelines
- To comply with Guidelines implemented by federal funding agencies and accepted by UNT as a condition of funding eligibility
- To create a secure laboratory environment to prevent unauthorized utilization of a biological agent.

This Biosafety Manual provides university-wide safety guidelines, rules, and procedures for the use, possession, manipulation, and transport of biological materials. Although the implementation of these procedures is primarily the responsibility of the Principal Investigator (PI), its success depends largely on the cooperative efforts of laboratory supervisors, employees, and students. Please read the section on responsibilities for additional information. Planning for and implementation of biological safety must be part of every laboratory activity in which potentially biohazardous material is used. Recommendations in this Biosafety Manual define a "standard of practice" that laboratories should follow.

In general, the possession, handling, manipulation and transport, and disposal of biological materials, including (but not limited to):

- Recombinant and synthetic DNA molecules;
- Infectious or potentially infectious agents, including human or non-human primate-derived material, cultures, and genetically modified cells;
- Microbial agents (e.g. viruses, bacteria, mycobacteria, rickettsia, yeast, fungi, prions, parasites) or specimens that may be exposed to microbial agents;
- Toxins of biological origin; and
- Animals, including animal-derived material, cultures, and genetically modified cells; both vertebrate and invertebrate
- Plants including plant-derived materials

require the use of various precautionary measures depending on the material(s), facilities, personnel and their experience, and procedures involved. This manual will assist in the evaluation, containment, and control of these biohazards. It is required that all parties involved and/or working with these materials be familiar with the contents of this manual, complete the required training, and that they seek additional advice when necessary. The Biosafety Officer, IBC Chairperson, as well as the IBC members, are available to assist in this endeavor.

This manual focuses on Biosafety Levels 1 and 2, as all UNT laboratories fall within these designations as of Jan 2025. No BSL-3 or BSL-4 containment-level facilities are available at UNT.

We urge you to use the manual as a road map to compliance within your laboratory. Consult the sections relevant to your research and apply the appropriate safety procedures. The Biosafety Officer is available for consultation if you have any questions or concerns with any aspect of the Biosafety Program at UNT. The credo, "Think before you act," and "If you do not know, ask," are relevant to the use of this manual. If you are unsure of a requirement or biosafety practice, please contact the Biosafety Office for assistance at IBCprogram@unt.edu. We also would appreciate any feedback or comments that you may have with the use of this manual and will incorporate any suggestions in future versions.

EMERGENCY PHONE NUMBERS AND SAFETY, COMPLIANCE AND OPERATIONS CONTACTS

Emergency Telephone Numbers	
Ambulance/Fire/Police	911
Risk Management Services	940.565.2109 askRMS@unt.edu
Emergency Operations Center	940.369.6155
Student Health Center	940.565.2333
Research Integrity and Compliance	
Autumn Pinckard, Sr. Director, Research Integrity and Compliance	940.369.8374 autumn.pinckard@unt.edu
Institutional Biosafety Committee (IBC)	IBCprogram@unt.edu
Dr. Veena Naik, Biosafety Officer	940-369-7260 veena.naik@unt.edu
Environmental Health and Safety (EHS) Contacts	
Chris Erickson, Director, Environmental Health & Safety	940-565-2167 Chris.Erickson@unt.edu
Todd Germain, Chemical Hygiene Officer	940-565-4196 Todd.Germain@unt.edu
Jordan Murray, Biological Safety Specialist	Jordan.Murray@unt.edu
Karla Henson, Environmental Program Manager	940.369.8055 karla.henson@unt.edu
Tony Roman, Laboratory Safety Officer	940.565.2123 anthony.roman@unt.edu
Institutional Animal Care and Use Committee (IACUC)	untiacuc@unt.edu
Human Subjects IRB Review Board	940 565-4643, untirb@unt.edu.

ROLES AND RESPONSIBILITIES

Please refer to the Institutional Biosafety Committee Standard Operating Procedures for full accounting of responsibilities related to the IBC and biosafety program.

1.1.1 Department Chairperson and/or Center/Institute Director

The Department Chairperson and/or Center/Institute Director bears overall responsibility for the implementation and maintenance of safe practices and procedures in the department.

Department/Center/Institute Heads have the following responsibilities:

- To ensure that prior to initiation of work, each Principal Investigator (PI) of a Research laboratory, Clinical Director or each clinical laboratory, and/or Instructional Course Director within their department which may expose UNT personnel, students, animals, the environment or the public to biological material files a Biosafety Protocol Registration for review by the IBC through the Biological Safety Officer and that approval has been granted prior to the initiation of the research.
- To ensure that students, staff, and faculty within their department/center/institute have had instruction in safety procedures in research and teaching laboratories or field situations where biological agents are used or collected.
- To ensure that resources are made available to researchers, health care providers, laboratory and/or clinic staff, instructors, and/or students who require health screening and/or vaccination due to the potential risk of exposure to particular biological materials.
- To assume responsibility for maintaining the appropriate Biosafety standards and documentation of shared departmental facilities or delegate that responsibility to an appropriate faculty member within the department.
- To provide leadership in laboratory or clinic safety at the management level in the department or institute.

1.1.2 Principal Investigators, Clinical Directors, and/or Instructional Course Directors

The PI, Clinical Director, and/or Instructional Course Director in which biological material is used bears the ultimate responsibility and authority for assessing risks, establishing policies and procedures, training personnel, and maintaining the facility and equipment.

The PI, clinical director, and/or instructional course director are responsible for:

- Performing an appropriate risk assessment of research projects. The level of detail should be dependent on the hazard associated with the organism under study (e.g., an assessment of risk associated with research on Risk Group 1 agents might reasonably be less detailed than a risk assessment of a Risk Group 2 or unknown agents). Each evaluation should be completed before work is undertaken and the project should be reassessed periodically as new data is obtained and before the investigation of any specific research, clinical, or teaching methods that may affect that risk (e.g., procedures requiring highly concentrated amounts of microorganisms or inoculation of laboratory animals). No human or animal pathogen should be studied without prior written approval of the UNT IBC. The procedures for handling unclassified agents must be reviewed by the UNT IBC as well as Institutional Animal Care and Use Committee (IACUC) if work with animals is anticipated.
- Registering research work involving biological materials, particularly recombinant DNA and potential animal, human, or plant pathogens, with the IBC. This Biosafety Protocol (BSP) application must detail the nature of the proposed experiments and an assessment of the levels of physical and biological containment required for them as established by the NIH and CDC/BMBL guidelines. The research described in IBC protocol applications must be accurate and complete descriptions of the research projects in the laboratory and reflect the agents, locations, operations, experiments, manipulations, personnel, and safety measures that will be employed in the laboratory. A Risk Assessment Form and a Standard Operating Procedure document along with completed training certificates must be included with each IBC submission.
- Changes in the protocols must be submitted as amendments to the IBC protocols to the IBC prior to its initiation. PIs, Clinical Directors, and/or Course Directors are responsible for ensuring that no research is initiated by any laboratory personnel prior to review and approval by the IBC.
- Developing, establishing, and implementing appropriate safety practices and procedures within their laboratories prior to bringing new biological agents to campus and/or before initiating any new research project (independent of funding status) to ensure safe operation and instructing students and staff of potential hazards. This involves:
    - Being knowledgeable in good laboratory practices and maintaining current knowledge of new safety practices and/or equipment which may improve safety within the laboratory.
    - Demonstrating a positive safety attitude.
    - Making available to the laboratory staff copies of the written procedures that describe potential biohazards, precautions, and actions to be taken in response to spills and accidents to include decontamination procedures and emergency procedures. These procedures and other information addressing biological safety-related issues will be produced in the form of a standard operating procedure (SOP) for the work. The SOP will reside within the laboratory and be easily accessible for reference and provided to the IBC for review as part of the Biosafety Protocol application.
    - Maintaining up-to-date knowledge to changes in international federal, state, and local regulations and guidelines pertaining to biological materials, in consultation with the Biosafety Office, and modifying the laboratory procedures to comply with these.

    - Ensuring 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;
    - Ensuring proper decontamination of the laboratory or animal facility and the equipment as necessary to ensure safety during any required inspection, calibration, and recertification activity
- Approving research personnel to work in the laboratory and documenting that personnel are competent to conduct the work. PIs, Clinical Directors, and/or Course Directors are responsible for the safety of personnel on their Biosafety Protocols (IBC protocols) and their actions. This includes:
    - Providing laboratory staff with *documented* formal and informal instruction and training in the practices and techniques required to ensure safety and in the procedures for dealing with accidental spills, personnel contamination, and other laboratory accidents or emergencies.
    - Informing the laboratory staff of the risks involved with the biological agents in the laboratory and the reasons and provisions for any precautionary medical practices (e.g. physical examinations, serum collection, and vaccinations).
    - Making provisions for any precautionary medical practices, including occupational health physical examinations, vaccinations, and/or other medical surveillance of personnel when required by the agents and the nature of the experiments.
    - Supervising and monitoring the performance of the staff to ensure that required safety practices and techniques are employed.
    - Ensuring the authorized staff completes the appropriate IBC-required training modules and keeping these training records up-to-date.
- Maintaining a liaison with the Biosafety Office. This includes:
    - Amending and modifying Biosafety Protocols to reflect changes in agents, personnel, locations, experimental application, operations, and/or safety equipment.
    - Reporting, in writing, any accident, potential environmental exposure or exposure of personnel, suspected illness, and/or release from containment of any biohazardous agents. Any significant problems pertaining to the operation and implementation of containment practices, procedures, or facilities should also be reported to the Biosafety Office.
    - Providing accurate information for compliance verification processes when requested.
    - If required maintaining an internal inventory of all vectors, microbial strains, viruses, and other potentially biohazardous materials (including animals and animal tissues) used or stored in their laboratory and make it available for inspection and submit annually to RMS/BSO.
- Maintaining compliance with all Federal, State, and/or local regulations related to the possession, use, transfer, and/or disposal of biohazardous materials. This would include the following regulations:
    - Federal Select Agent regulations (42 CFR §73.3, 7 CFR §331.3, and 9 CFR §121.4) for the use, possession, and transfer of Select Agents and Toxins
    - U.S. Department of Transportation Regulations (49 CFR), U.S. Public Health Service (42 CFR §72) and IATA guidelines for shipping and/or transport of hazardous or etiological materials
    - U.S. Departments of State, Commerce, and Treasury Regulations related to Export control laws
    - U.S. Department of Agriculture Animal and Plant Health Inspection Service Regulations related to transportation, importation, or exportation of animal or animal products, genetically engineered organisms, plants or plant products, and/or soil samples.
    - U.S. CDC importation requirements for Etiologic Agents, any arthropod and/or other animal host or vector of human disease, including unsterilized specimens of human and animal tissues (such as blood, body discharges, fluids, excretions, or similar material) containing an infectious or etiologic agent

    - Texas (25 TAC §96) and OSHA (29 CFR §1910.1030) standards for blood-borne pathogen handling, medical surveillance, training and record-keeping
    - Texas Medical Waste Management (30 TAC 326 and 25 TAC 1.136)
- Ensuring that the terms and conditions of NIH Grants Policy Statement are maintained within the laboratory for all research projects (independent of funding status or sponsor). This includes compliance with the NIH *Guidelines for Research with Recombinant DNA Molecules (NIH Guidelines)*, the Occupational Health and Safety Administration (OSHA) standards included in 29 CFR Part 1910, and other applicable safety guidelines, including those in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories (*BMBL*)*.
    - Obtaining IBC approval before initiating recombinant or synthetic nucleic acid molecule research or potentially biohazardous research*;*
    - Initiating or modifying no recombinant or synthetic nucleic acid molecule research or potentially biohazardous until that research or the proposed modification thereof has been approved by the IBC and has met all other requirements of the *NIH Guidelines* and BMBL;
    - Making the initial risk assessment and determination of biological containment levels in accordance with the *NIH Guidelines* and BMBL when registering research with the IBC ;
    - Developing specific biosafety standard operating procedures for recombinant or synthetic nucleic acid molecules and biohazards used in the laboratory;
    - Making an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines* and BMBL;
    - Selecting appropriate microbiological practices and laboratory techniques to be used for the research;
    - Submitting the initial research protocol and any subsequent changes as amendments (e.g., changes in the source of DNA, host-vector system, or personnel), to the IBC for review and approval or disapproval;
    - Remaining in communication with the IBC throughout the conduct of the project and submitting annual registration updates;
    - Adhering to IBC-approved emergency plans for handling accidental spills and personnel contamination; and
    - Immediately reporting any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents, illnesses, or releases to the BSO, Greenhouse/Animal Facility Director, IBC, NIH Office of Biotechnology Activities, and other authorities, as appropriate.

1.1.3 Research, Laboratory, Clinical Personnel, and UNT Students

Research, Laboratory, Clinical Personnel, and UNT students are responsible for:

- Completing requirements for approval to work in the laboratory and ensuring that all work is conducted in compliance with UNT, NIH, CDC, OSHA, state labor and waste management laws, Department of Transportation (DOT), and other applicable guidelines and regulations and UNT IBC policies prior to initiation of any work with biological materials at UNT (See Section 2, Biosafety Requirements). Follow the UNT Biological Safety Manual.
- Participating in appropriate training and instruction to ensure that they are adequately trained and fully understand the instructions. This includes taking refresher courses as applicable.

- Learning the standard operating procedures (SOPs) for the laboratory, the potential hazards of the infectious agents in use, and emergency procedures. Personnel are responsible for helping to maintain the facility in good working condition and maintaining compliance with the laboratory Biosafety Protocol and SOPs.
- Maintaining their work areas neat and clean. All containers in which biological materials are placed should be appropriately labeled with biohazard stickers. Biohazardous waste must be disposed of according to EH&S requirements.
- Completing any medical surveillance requirements as required by the PI's Biosafety Protocol Agreement with the IBC prior to initiation of work with biological materials at UNT and obtain necessary and recommended vaccinations, or submit declination forms as permitted.
- Reporting to the PI, Clinical Directors, and/or Course Directors any medical restrictions, reportable illnesses, and any event that may be an exposure or result in the creation of a potential hazard.
- If inexperienced in handling human pathogens, tissue culture, recombinant DNA, and/or microorganisms, receiving additional training from the PI and demonstrate proficiency in these practices to the PI prior to initiation.
- Reporting thefts, security incidents, accidents, spills, contamination incidents, and near misses to supervisor and/or EH&S/BSO.
- Performing responsibilities assigned to them by the PI, Clinical Director, or Instructional Course Director. The operation of the facility is the responsibility of the users; therefore a number of tasks must be assigned. These tasks may include the following:
    - Training of other staff members
    - Autoclave maintenance and waste management
    - Freezer, refrigerator, equipment maintenance
    - Cleaning
    - Vacuum trap and filter maintenance
    - Maintenance of supplies, including personnel protective equipment
    - Security of infectious agents; i.e. store infectious agents in a locked freezer in a locked laboratory

1.1.4 Environmental Health and Safety (EHS),

- Department of Environmental Health and Safety (EH&S) This department along with the Institutional Biosafety Committee is responsible for evaluating existing and potential biohazardous conditions at the UNT, implementing safety standards, and providing support to IBC and the IBC Office .
- The EH&S Biosafety should have expertise in developing and supporting the Biosafety Program (including BSL-1, BSL-2).
- Assist with biohazardous waste management aspect of all labs working with all types of biological materials.
- Help implement policies, procedures and processes required for an effective, compliant and efficient biosafety program. Play a role in providing technical support to the UNT IBC Office.
- They along with the IBC committee review research proposals, laboratory operations and laboratory facilities for all aspects of biosafety to assure appropriate safety controls, containment and compliance with federal, state and local regulatory agencies as well as seeing that UNT requirements are met.
- They work closely with research staff, faculty, students, university units and institutional committees, Biosafety Officer to promote safe laboratory practices, procedures, and proper use of containment equipment and facilities.
- They conduct laboratory safety and compliance audits, safety equipment audits (biosafety cabinets,

fume hood, eyewash, safety showers etc.) identify corrective actions, and prepare written status and compliance reports.

- They long with the Biosafety Officer to develop and provide educational materials and training.
- They also respond to, investigate and follow up with biological safety incidents.

1.1.5 Biological Safety Office and Biosafety Officer (BSO)

The Biosafety Office serves as a resource to researchers, administration, compliance, and maintenance departments, as resources allow. The Biosafety Office along with EHS shares the lab safety responsibility. Some responsibilities include:

- Providing information and consultation on the operation of the use of biological materials (registered with IBC) to ensure compliance with CDC, NIH, USDA, OSHA, Environmental Protection Agency (EPA), state and local requirements to researchers, administrators, and other institutional compliance and maintenance offices;
- Voting member of the IBC.
- Assist in evaluation and inspection of laboratory facilities that have registered Biosafety Protocols with IBC for work with infectious agents, recombinant DNA, and other potentially hazardous biological agents. This includes advising on safety measures and equipment for new procedures that may be utilized to mitigate risks associated with working with potentially hazardous materials.
- Review and vote on the protocol that seeks IBC permission for work with rDNA experiments documented in the Biosafety Protocols to ensure compliance with the NIH Guidelines;
- Providing biosafety training programs for responsible conduction of research related to proper handling of biological materials and maintenance of training records for compliance with federal, state, and University requirements (laboratory-, agent- and operation-specific training is the responsibility of the PI (see Section 1.2.2);
- Providing guidance, advice and assistance in the event of large, high-hazard, or public biological material spills;
- Along with EH&S assist in investigation of laboratory incidents, accidents, exposures, potential exposures, and illnesses that may have resulted from potential exposures to biological material in the laboratory, releases, or possible releases from containment of biological materials to ensure appropriate emergency follow-up procedures have been followed. If alternate incident mitigation and/or management procedures are required to circumvent future similar incidents, the Division of Environmental Health and Safety will make recommendations to the appropriate safety committees to address these;
- Reporting to the IBC 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 unless the Biological Safety Officer determines that a report has already been filed by the PI;
- Along with EH&S develop emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;
- Providing advice on laboratory security; and
- Providing technical advice to PIs and the IBC on research safety procedures.

1.1.6 UNT Institutional Biological Safety Committee (IBC)

The UNT IBC serves to maintain institutional compliance with the *NIH Guidelines*, the BMBL, and International, Federal, state, and local regulations pertaining to the handling of biological materials. The IBC shall also advise the President, Provost, Vice President of Research and Innovation, and the Director of the Division of Environmental

Health and Safety (EHS) and/or other pertinent offices on policy matters concerned with the protection of personnel from biohazardous agents including both infectious organisms and allergens that may be present in either laboratory materials or the environment. The IBC shall also recommend guidelines relating to procedures and facilities used at the University, including such matters as safety training and health surveillance. As per NIH guidelines the committee should consist of Biosafety Officer who is a voting member of the committee, one voting individual with expertise in plant, plant pathogen, or plant pest containment principles , one voting individual with expertise in animal containment principles At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community. Ensure that when the institution participates in or sponsors recombinant or synthetic nucleic acid molecule research involving human participants: (i) the Institutional Biosafety Committee has adequate expertise and training (using ad hoc consultants as deemed necessary) and (ii) no human gene transfer experiment shall be initiated until Institutional Biosafety Committee approval has been obtained and all other applicable institutional and regulatory authorization(s) and approvals have been obtained. Institutional Biosafety Committee approval must be obtained from the clinical trial site.

The IBC shall offer its counsel to all University personnel regarding matters of biological safety. The President, Provost, and/or Vice President of Research and Innovation may ask the Committee to inform the community about developments in the general area of biological safety.

The IBC's composition, roles, and responsibilities adhere to those dictated in NIH Guidelines and as outlined in the IBC SOP. As such, the Committee is required to review applications for research involving biological materials and recombinant DNA to determine whether the facilities, procedures, and practices meet the standards required by the University and the NIH. Meetings called for the purpose of such review and certification may be open to the public. Minutes of these meetings shall be kept and made available for public inspection. Some of the IBC's responsibilities include:

- Review applications and perform comprehensive risk assessments to determine the appropriateness and adequacy of containment levels and safety measures proposed and/or used in research, clinical duties, and teaching.
- Assess the adequacy of containment facilities for biological agents and rDNA molecules as required by NIH or other funding or regulatory agencies. The IBC may down-grade or up-grade containment levels as appropriate to address the risks associated with the proposed activities.
- Assess the adequacy of facilities, procedures, practices, training, and expertise of personnel involved in the research, clinical duties, and/or instructional activities.
- Periodically review biohazardous research, clinical duties, and/or instructional activities being conducted at UNT to ensure that the requirements of the University, funding sources, and regulatory agencies are being fulfilled.
- Recommend to UNT Administration appropriate sanctions for noncompliance with biological safety standards, guidelines, or regulations.
- Reporting any significant problems, violations, or significant research-related accidents or illnesses to the NIH Office of Biotechnology Activities within 24 hours or 30 days, as required.

1.1.7 The University of North Texas (UNT)

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.

The University of North Texas and its administrative officers are ultimately responsible for the following:

- Developing and maintaining appropriate policies regarding the conduct of potentially biohazardous research, education, and service activities.
- Developing mechanisms for ensuring adherence to Biological Safety policies.
- Establishing an IBC with adequate expertise and training.
- Providing the resources necessary for the construction of safe research, clinical, and teaching facilities and for the implementation of the Biological Safety Program.
- Providing adequate resources for IBC member training on biohazards and biological safety procedures, including training programs and workshops.
- Providing resources for appropriate medical surveillance measures to protect the health and safety of employees.
- Providing appropriate and sufficient legal protection for faculty and staff members who conduct activities in compliance with appropriate regulations and guidelines.
- Coordinate efforts between institutional safety and compliance offices to ensure compliance with International, Federal, State, and Local regulations and guidelines for research and clinical care.
- Reporting any significant problems, violations, or significant research-related accidents or illnesses to the NIH Office of Biotechnology Activities within 24 h or 30 days, as required.

1.1.7.1 *Other Offices and Committees at the University of North Texas (UNT)*

1.1.7.1.1 *Facilities Operations & Maintenance*

The UNT Facility Operations and Maintenance is responsible for:

- Ensuring that the design and construction of all laboratory and clinical facilities meet the standards of containment to maintain compliance with Federal, State, and Local Regulations, *NIH Guidelines*, and BMBL, among other standards and their intended purposes.
- Making sure the mechanics and integrity of the facilities are maintained to ensure proper protection of workers within the facilities and proper protection of the environment from hazardous materials within the buildings.
- Notifying the EH&S, laboratory director, Biosafety Office, and building manager of any planned maintenance of the facilities which would require even short suspension of utility operations required to maintain safety in the laboratory. This may include tests or repairs of HVAC, electrical, plumbing, or vacuum systems.
- Immediately reporting any possible failures in facility containment that may have resulted in the environmental release of biological materials or potential exposures of any personnel to biohazardous materials to the EH&S and the Biosafety Office (IBCprogram@unt.edu),well as the building directors, departmental chairs, and researchers.
- Reporting any issues of non-compliance that are noticed while within laboratories, particularly if these may potentially place facilities personnel at higher risk for exposure (*e.g.* inappropriate waste disposal for sharps or biological material).

Because proper design and function of laboratory facilities is one of the key components involved in the management of risk within a laboratory, Facility Operations, and Maintenance is encouraged to consult and coordinate with the IBC/Biosafety Office during the design of new or renovated laboratory facilities and/or during construction or maintenance operations which may require untrained personnel to enter areas containing biohazardous materials.

1.1.7.1.2 *The Institutional Animal Care and Use Committee (IACUC)*

To comply with federal laws and institutional policies governing the humane care and treatment of laboratory animals, UNT requires that all use of vertebrate animals for research, education, or for any other purpose be documented in an Animal Use Protocol (AUP) which must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to initiation of the work. The IACUC administrative office specifically supports the IACUC compliance mission to address laboratory animal care and welfare issues. Duties regarding the IACUC are outlined in the Animal Care and Use Policy.

Although the IBC's oversight of the health and safety of the (human) researchers, the community, and the environment (including control of infection within the animal care areas) may overlap with the IACUC's concerns for animal welfare and caretaker safety, each committee reviews research protocols from different perspectives. Therefore, PIs may need to submit both AUP and IBC applications to the IACUC and IBC, respectively, in order to receive both committees' approvals to maintain compliance. To address common concerns, the IACUC and IBC work in close collaboration with each other. IBC approval may be required by IACUC before initiation of projects involving biohazardous materials or recombinant DNA in conjunction with live animals or prior to completion of processing IACUC approvals.

1.1.7.1.3 *The Institutional Review Boards (IRBs)*

The purpose of the UNT-approved Institutional Review Boards (IRBs) is to ensure the principles outlined in the Department of Health and Human Services (DHHS) policies, the Belmont Report, the Nuremberg Code, and the Declaration of Helsinki are maintained in all UNT investigations involving human subjects. These principles were established to safeguard the rights and welfare of human subjects of research investigations and to fulfill the moral and legal obligations and commitments of the institution.

The IRBs evaluate Human Use/Clinical Protocols based on several criteria, most of which focus on the **ethical and legal obligations of UNT toward human research subjects** including:

- The rights and welfare of the human research subjects involved
- The appropriateness of the methods used to obtain informed consent from research subjects
- The risks to the human subjects and the potential benefits of the investigations to the patients and mankind.

With the exception of protocols that may involve the delivery of recombinant DNA or other biological materials (*e.g.,* cells, tissues, toxins of biological origin), IBC reviews focus more on the health and safety of the researchers, the community, and the environment; each differs. Therefore, PIs may need to complete two separate applications and receive both committees' approvals to maintain compliance with both the human use and biosafety requirements. Although the IRB and IBC reviews are independent of one another, some of the issues (e.g. delivery/collection of biohazardous material or recombinant DNA into/from human research subjects) overlap; therefore, the IRB and IBC work in close collaboration. IBC approval may be required before the completion of IRB approvals for projects involving biohazardous materials or recombinant DNA in conjunction with human research subjects.

1.1.7.1.4 *Other Organizations*

Other committees, such as the Radiation Safety Committee, must consult and coordinate with the IBC and EH&S/BSO on any proposals under their purview which involve the use of biohazards.

1.1.8 Visitors, Vendors, and Contractors

Contractors must ensure that appropriate personal protective equipment is available for their own workers. Laboratory supervisors are responsible for visitors to their laboratories. All visitors, vendors, and contractors are responsible for:

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

2 BIOSAFETY REQUIREMENTS

The following information describes the requirements for UNT researchers as defined by the UNT IBC and the UNT Biosafety Office. It is the responsibility of each PI, Clinical Director, and/or Instructional Course Director to ensure their workplaces are in compliance.

REGISTRATION FOR THE USE OF BIOLOGICAL MATERIAL

All PIs working with biological materials are required to complete and submit a IBC Protocol Form prior to bringing new biological materials to campus or the initiation of the research independent of its funding status. Incoming PIs establishing a new laboratory at UNT must submit the registration prior to bringing biological materials to campus (see Section 2.3.1, Incoming New Faculty to UNT). The BSP must be kept current to accurately reflect the biological materials and their manipulation, the personnel handling the material, and the locations in which the material may be handled or stored.

The Biosafety Protocol Registration form can be accessed online through the IBC website. The risk assessment and mitigation methods associated with the PI's research project(s) must be performed and documented in this application (See Section 3, Risk Assessment, and Section 4, Risk Mitigation for further information) to enable the IBC to evaluate and perform a mandated independent comprehensive risk assessment.

2.1.1 Annual Affirmation of Biosafety Protocol Information

EHS and IBC must maintain accurate information regarding the use of biological materials (e.g. microorganisms, cell lines, human materials, animals, and toxins) by UNT personnel. Approvals for IBC protocols typically issued by the IBC are for a three-year term, as long as the research, personnel, practices, and locations have not changed. Therefore, the IBC requires all PIs, Clinical Directors, and/or Instructional Course Directors annually review their IBC protocols to ensure that the information provided continues to accurately reflect the current situation in their laboratories and submit a written affirmation to the IBC that their BSP(s) are up-to-date and accurate. IBC Protocol Renewal forms may be found online on the Biosafety and IBC websites.

2.1.2 Amendments to Biosafety Protocols

Changes or modifications to approved protocols (i.e. change in or additional of research personnel, room changes, new procedures, or agents) must be reviewed and approved by the IBC prior to initiation.

Amendments must comprehensively describe the new proposed changes and document any new risk assessment(s) and proposed risk management methods, as appropriate. All questions can be addressed to the IBC (IBCprogram@UNT.EDU).

2.1.2.1 *Major Changes*

Major changes are those that change the scope of the review or that are inconsistent with the focus of the approved protocol. **For major changes, the PI must submit a new IBC Protocol Form.** Major changes are typically not eligible for the amendment process.

Significant changes are changes to approved protocols that are extensive and include, but are not limited to, the following:

- Change in PI
- Change of an infectious agent or toxin biosafety level
- Change of protocol components that are not exempt from the *NIH Guidelines*
- Changes that affect the risk assessment of the protocol

Significant changes will initially be reviewed by a designated reviewer and will require approval at a convened IBC meeting prior to initiation of work or obtaining materials.

2.1.2.2 *Minor-risk changes*

Minor-risk changes are changes to approved protocols (including recombinant) that are minor and do not affect the risk assessment or applicable *NIH Guidelines* such as:

- Addition of human biological materials (fluids, cells, or tissue) from sources not known to be infectious (if already working with human materials)
- BSL-1 viral vectors from approved sources
- Genes and host changes that do not significantly change the focus of the project
- Additional cell lines
- Changes in BSL-1 agents, vectors (except viral), hosts, and genes

Minor-risk changes can be reviewed through designated review by a designated qualified member(s) and may be approved outside a convened IBC meeting. Proposed minor-risk changes falling under the *NIH Guidelines* require designated review and IBC approval during a convened meeting prior to initiation.

2.1.2.2.1 *Amendments for New or Modified Biological Agents or Operations to a Biosafety Protocol*

Any changes in the Biological Agents to be utilized in the laboratory *that are not covered in the previously-approved protocol* must be documented to enable comprehensive risk assessment by the IBC and evaluation of whether the documented mitigation methods are appropriate for the new materials or operations.

In particular, any changes in non-exempt recombinant DNA materials or applications must be documented and submitted to the full IBC for review to maintain strict compliance with *NIH Guidelines*. This includes changes in inserts, vectors, target cells/tissues/organisms, or operations. This is a condition of NIH funding to the entire institution and applies to all research at the institution, independent of its funding source.

Any change which might alter the risk of a BSP will require IBC review and approval. For instance, if the infectious potential status of biological material handled differs from that previously documented and approved in a BSP, either a new biosafety protocol registration (for major changes) or an amendment (minor-risk changes) must be submitted. For instance, if a previous BSP included approval for handling blood samples obtained from non-infectious patients

and the PI wishes to initiate a study involving taking blood specimens from infectious patients (*e.g.,* HIV+ patients), this change must be submitted and approved by the IBC prior to initiation. Similarly, if a new operation will be employed with the biological agent(s) which may involve additional risk, this must also be documented. For instance, if a previous BSP included approval for handling the biological agents *in vitro*, and the PI wishes to initiate a study involving *in vivo* administration of the biological agent into animals or humans, this change would be a major change and must be submitted and approved by the IBC prior to initiation via a new Biosafety Protocol Registration. Another example of operational changes that may alter the risk of a BSP includes utilizing new procedures with the biological agent which may involve additional splash, spray, or aerosol exposure risks (*e.g.,* sonication, homogenization, FACS sorting, etc.). See Section 3, Risk Assessment for further details.

BSP Amendments involving changes in agents or operations are reviewed and approved by the IBC via the same procedures as the original BSP.

2.1.2.3 *Non-significant changes*

Non-significant changes are changes to approved protocols (including recombinant) that are non-significant, do not affect the risk assessment, or apply to *NIH Guidelines* such as changes to:

- Research personnel other than PI
- IACUC and IRB protocol numbers or grant numbers
- Lab Biosafety Manual
- Research locations

Non-significant changes may be approved by administrative approval by an IBC member or BSO staff outside a convened IBC meeting. Proposed non-significant changes require administrative review and approval prior to initiation.

2.1.2.3.1 *Adding New Personnel to or Removal of Personnel from a Biosafety Protocol*

Before a new employee, student, volunteer, or visitor may handle the biological material documented on a Biosafety Protocol, their name must be added to the list of authorized personnel on the BSP. PIs should first submit a Biosafety Amendment Protocol. In doing so, the PI takes responsibility for ensuring that all training and laboratory entrance requirements listed in the laboratory SOPs and BSP document have been completed by this new person before authorizing them to work in the laboratory.

The Biosafety Office will verify that the new person has completed the IBC-required training modules (as described in Section 2.2, Training), in addition to any special training requirements as required by the IBC for the BSP before administratively adding their names to the list of authorized personnel of the Biosafety Protocol.

As with all personnel listed in the BSP, the PI, Clinical Director, and/or Instructional Course Director is responsible for communicating the risks associated with the biological material and procedures in the laboratory, as well as the laboratory-specific mitigation methods. Therefore, the PI should review the laboratory standard operating procedures (SOPs), biosafety protocol(s), and UNT Biosafety Manual with any new personnel, including emergency response procedures and entrance requirements before allowing them to start work within the laboratory. The PI is also responsible for ensuring proper supervision and training are provided to any new personnel until proficiency in handling the materials and mastery of the techniques can be demonstrated.

New personnel should also be offered and provided with employee health counseling and/or vaccinations as documented in the Biosafety Protocol prior to initiation of work within the laboratory.

A Biosafety Protocol Amendment should also be completed by the PI, Clinical Director, or Instructional Course Director if someone should be removed from their list of authorized personnel upon their departure from the laboratory.

2.1.2.3.2 *Modifying the Locations or Equipment on a Biosafety Protocol*

If a PI's biological agents are handled or stored in locations other than those documented on their IBC-approved IBC protocols, the new location(s) must be documented and approved via a Biosafety Protocol Amendment by the IBC in order to ensure that the containment is appropriate to handle the risks presented by the agents. A laboratory assessment by the Biosafety Office will likely be required prior to approval of the amendment.

Some common locations (such as core laboratories or common storage rooms) may have previously been approved by the IBC for other IBC protocols involving similar agents/operations; Biosafety Office. However, any area proposed for handling or storage of biological materials which has not been previously reviewed and approved by the IBC will need to be authorized by the IBC before its use to ensure appropriate containment.

Because major equipment used to contain or handle biological agents, such as Biosafety Cabinets or centrifuges, may not only impact the risk of the protocol but may also require certification documentation prior to use, such amendments must also be submitted with assistance from EH&S (Biosafety Cabinet Certification) to the Biosafety Office.

TRAINING REQUIREMENTS

Successful completion of a range of biosafety training programs may be required prior to the initiation of your work at UNT.

Please review the following table for information on the IBC-required training modules. The Biosafety Office must be able to document the completion of the required training modules listed in this table prior to completion of any IBC- approval paperwork:

Table 2.A.: Training Requirements		
Intended for all personnel who work on UNT projects or areas, who:	**You must satisfactorily complete the following training:**	**Training Requirements**
Work with biological materials in a basic laboratory setting. Includes Recombinant DNA, microbial agents ≤ RG2, cell culture, biological materials, and with animals	Biosafety	*An Initial Training session is required prior to the initiation of work.* *Annual Refresher is required thereafter.*
Work with human or primate blood, tissues, fluids other potentially infectious materials, and bloodborne pathogens	Bloodborne pathogens training	*Required once prior to initiation of work and annually thereafter*
May be in contact with Biological materials	Laboratory-specific Training (includes a review of SOPs, IBC protocols, and hands-on training);	*Required once prior to initiation of work, ongoing with changes, and annually thereafter.* The PI, Clinical director, or Instructional Course Director is responsible for educating

	Documentation required	all personnel in the laboratory related to the laboratory-specific risk issues and mitigation methods as well as laboratory-specific operations.
Animals	Animal Biosafety Training	*Required once prior to initiation of work and annually thereafter*
Recombinant Materials	NIH Guidelines Training for PIs	*Required for PIs who work with recombinant materials prior to initiation of work and every three years thereafter*
Hazard Communication	Hazard Communication module	*Required for all new personnel*

LABORATORY ESTABLISHMENT, CLOSE-OUTS, AND MOVES

To ensure appropriate containment is maintained, safety measures are implemented, and compliance with all Federal, State, local regulations and guidelines for all biological materials at UNT have been met, documentation must be provided, reviewed, and approved by the IBC prior to moving any biological agents into any new facilities (which includes any materials which may be brought to campus by incoming new faculty or materials which may be moved to new locations not previously authorized as part of the PI's, Clinical Director's, or Instructional Course Director's IBC-approved Biosafety Protocol). In addition, to ensure that the biological materials are properly removed and facilities are decontaminated prior to the closing of a laboratory, PIs, Clinical Directors, or Instructional Course Directors must document compliance with the appropriate close-out procedures listed below.

2.1.3 Incoming New Faculty to UNT

New UNT researchers must receive authorization from EH&S and IBC **prior to transfer of any biological material to campus and prior to shipment of this material**. Initial applications to transfer biological agents to UNT campus must, at minimum, document:

- The biological agents to be transferred to UNT
- The method of transfer (which must be in compliance with IATA/DOT standards, and any required USDA or CDC permits for infectious materials must be obtained by the PI prior to shipment.
- The location(s) at which these materials will be stored prior to the new faculty member's laboratory establishment
- The UNT personnel responsible for the materials prior to arrival of the new faculty member (if applicable).
- Initiate Materials Transfer Agreements and Permits

The IBC will review the above to ensure containment issues have been addressed and compliance with regulations, guidelines, and policies are met, and will typically issue authorization only for the transfer of the material. Prior to initiation of research in UNT facilities, the incoming faculty member is expected to:

- Complete and submit a complete Biosafety Protocol Registration for IBC review and approval (including documentation of all of the locations in which their biological materials may be handled or stored and submission of the laboratory-specific Standard Operating Procedures)
- Ensure all laboratory personnel listed on the BSP have completed the IBC training requirements (see Section

2.2, Training, for further details)

- Have all laboratory facilities (for those working in BSL1 or BSL2) assessed by the Biosafety Office and UNT EH&S. Once the facilities have been equipped for research, the PI should contact the Biosafety Office to make an appointment for a full laboratory assessment (IBCprogram@unt.edu). All other non IBC registered research will work with EH&S for safety assessment.

Incoming faculty members are encouraged to contact the Biosafety Office (IBCprogram@unt.edu) for assistance with these IBC protocol submission processes as early as possible. The Biosafety Office will help "walk" new faculty members through the process and assist them in obtaining any permits or authorizations required to transfer materials to campus. All research materials transferred must be transferred to UNT via an MTA agreement from the institutes the faculty is transferring from. All unauthorized transfers will be asked to be destroyed by the IBC.

New faculty members are advised that review and approval of IBC protocols involving non-exempt recombinant DNA or any materials which may pose a higher risk than those previously approved at UNT may take several (3-7) weeks due to requirements for full IBC review (the IBC meets once per month, and the deadline for submission for Biosafety Protocols for review is three weeks prior to each meeting). Because of these time constraints, the IBC recommends that Department chairs, Institute Directors, and/or Departmental Managers notify the Biosafety Office of any incoming faculty members in their programs as soon as possible after recruitment to initiate these application processes to enable the faculty member to begin research as promptly as possible after arrival at UNT.

2.1.4 Laboratory Close-outs

If work will continue at UNT under the same PI in a new laboratory, PI must submit a Biosafety Protocol Close out form indicating the closure of the old laboratory and indicating the new space. If work is will continue at UNT under a new researcher, the new PI must submit a new Biosafety Protocol to transfer the research to the new UNT PI. The new PI must assume responsibility for all materials, research, and associated personnel on the disclosure.

- Decontamination/deactivation and disposal of the biological material (as per the PI's SOPs) and with the assistance of EH&S team.
- Disposal in the authorized Biohazard waste containers (as per the PI's SOPs) as per EH&S guidelines and IBC protocol.
- Transfer of the biological materials to another authorized user (or to another authorized location).
    - If the material is to be transferred to another UNT laboratory, written documentation of this transfer should be provided to the Biosafety Office (an email to IBCprogram@unt.edu is sufficient). If the materials are being transferred to a different PI, the receiving PI should have authorization to possess this material in the new locations prior to transfer. The receiving PI must also agree to take responsibility for the new materials.
    - If the material is to be transferred outside of UNT, this must be done in accordance with IATA/DOT standards by personnel with documented training (since many biological materials and dry ice are considered hazardous materials by the federal government). Any transport, import, and/ or export permits must also be obtained by the PI prior to transfer for shipping. Contact Risk Management Services (RMS) for assistance.
- Follow the new institution's procedures to gain approval to receive the biohazards
- Contact EH&S for assistance with proper containment and shipping procedures
- Initiate Materials Transfer Agreements and Permits

After removal of the biological materials from the laboratory, all surfaces and equipment (interior and exterior) must be decontaminated using the appropriate disinfection procedures **as documented** in the laboratory standard operating

procedures prior to departure from the laboratory. Some equipment, such as biosafety cabinets, may require additional decontamination methods, such as gaseous fumigation with paraformaldehyde or vapor hydrogen peroxide (VHP), prior to removal from the laboratory. After the removal of all biological materials and decontamination of the laboratory and equipment, the Biosafety Office must be notified to complete the "clearance" process.

Please utilize the Laboratory Closeout Form (Appendix E) and submit the closeout form to RMS prior to departure. Follow RMS guidelines to dispose of remaining biohazards or chemicals used in the research.

- Complete the UNT Lab closeout form found in Appendix E
- Schedule a laboratory close-out inspection with RMS, Biosafety, the laboratory's building owner, and your department's safety officers, as applicable.

Once all hazardous material has been disposed of, email IBCprogram@unt.edu to close your biosafety disclosure.

2.1.5 Laboratory Moves

If biological materials or equipment are to be transferred from one UNT laboratory to a new UNT laboratory, the PI, Clinical Director, and/or Instructional Course Director must:

- Submit a BSP Amendment request to the Biosafety office for IBC authorization of the new locations for the biological materials prior to moving the materials (see Section 2.1.2, Amendments to Biosafety Protocols or the IBC SOP for more details). Work with EH&S to make sure that the new facility is appropriate.
- Transport the biological materials in a method that complies with their laboratory standard operating procedures. Contact the Biosafety Office or Biosafety Specialist for assistance and see below for special notes about transport considerations.
- Arrange for a full laboratory assessment with the EH&S and Biosafety Office to ensure appropriate containment measures are in place in the new laboratory prior to initiation of work in the new laboratory (this is usually a stipulation in the IBC approval of any new location)
- Complete the laboratory close-out procedures (as described above in 2.3.2, Laboratory Close-outs) for the former laboratory

Standard Operating Procedures for biological materials typically require the biological material to be transported outside of the laboratory:

- By authorized personnel (i.e., those personnel listed on the IBC protocol who are familiar with the risks associated with the biological material, the emergency spill, and exposure/release procedures)
- Contained in a sealed, leakproof primary container inside a well-labeled, sealed, leakproof, durable secondary container.
- Add appropriate biohazard label to the container with the PI's name, the research materials contained and the emergency contact information.
- Decontaminate the outer container and use cart while transporting samples to avoid accidental spills and ease of movement.

Transport of large amounts of biological materials in laboratory moves poses unique challenges. First, laboratory moves are often accomplished with the help of non-authorized personnel (*e.g.,* professional movers or personnel from the UNT's Facilities). PIs are reminded that these personnel are not authorized to handle the biological materials because they have not been fully educated in the risks, received appropriate medical evaluation/vaccinations, and are not prepared for any emergency procedures should spills or releases occur *en route*.

Therefore, whenever possible, equipment containing biological materials should be transported separately by authorized personnel. However, if this is not feasible (due to the size/weight constraints of the equipment), the equipment containing the biological materials should be securely sealed to prevent moving personnel from exposure (*e.g.,* refrigerators and freezers should be locked and/or securely taped shut), the exterior of the equipment should be decontaminated prior to the movers handling them, and during the move, the movers and equipment should be accompanied by at least one member of the authorized laboratory staff equipped with the appropriate spill clean-up materials to ensure proper emergency procedures are followed should a spill/release occur *en route*. Request assistance from EH&S team or the biosafety officer if required.

In preparation for moving, any glass, breakable items, or other hazardous materials (*e.g.,* chemicals or radiological materials) should be removed from any refrigerators or freezers. Biological materials should be contained in one or more forms for sealed, durable containment within the refrigerator or freezer and secured to prevent spillage or scattering of biological materials or samples within the freezer. Keep in mind: freezers and refrigerators require tipping to transport moving dollies; a good deal of tipping is often required to get large equipment into tight spaces, such as elevators. For instance, sealed microcentrifuge tubes should be contained within boxes with lids (not in open racks); plastic tubes should have tightly secured lids and should be in fitted containers that would prevent movement during transport (or contained within ziplock bags). Filling any open spaces within the freezers/refrigerators with packing materials will also prevent the shifting of materials during transport (foam rubber works well for this purpose). The refrigerator or freezer should be sealed shut (locked or securely taped shut) and the exterior decontaminated prior to moving.

Because cryo tanks cannot be completely sealed during transport to prevent leakage of liquid nitrogen if tipped, and any vehicle transporting these materials on public roads may require special placarding for DOT regulatory compliance, these items should not be transported via moving truck. Alternate methods of moving this equipment should be discussed with EHS and the Biosafety Office prior to moving.

The need for gaseous decontamination of a Biosafety Cabinet (BSC) must be evaluated by the EH&S before moving. Should a BSC require decontamination, arrangements must be made in advance to perform this service before the BSC can be moved.

LABORATORY RECORD-KEEPING

At least one copy of the Laboratory-Specific Biosafety Manual must be maintained within each laboratory for training and reference of all laboratory personnel. This manual should include the following material, as appropriate:

- The Laboratory Biosafety Protocol(s) , amendments and renewal documentation
- The Laboratory-specific Standard Operating Procedures (SOPs)
    - Laboratory procedures
    - Safety procedures
- The Risk Assessment signed off on by all participants
- Hazard communication (e.g. agent summaries)
- Lab-specific exposure control plan(s), if applicable
- Post-exposure plan(s), as appropriate
- Copies of documentation of training of all laboratory staff. This should include:
    - Copies of certificates of completion of any IBC-required training modules
    - Documentation that all authorized personnel having access to the lab have received laboratory-specific education and training from the PI or his/her designated representative.
    - Signed/dated statements should affirm that training included:
        - Laboratory-specific risk communication and training. This includes providing references

and a discussion of the risks associated with the materials within the laboratory, any possible signs/symptoms which may suggest occupational exposure has occurred, and any incident/emergency procedures that may be required.
    - Each staff member should verify that they have reviewed and understand the materials documented within the Biosafety Manual, Laboratory Biosafety Protocols, and the Laboratory SOPs.
- If appropriate, copies of signed documentation that each person authorized to work within the laboratory have been offered Employee Health screening/counseling and vaccinations by the PI for the agents within the laboratory and as described in the laboratory SOPs and IBC Approval documents, if applicable.
- Records of any incident, exposure, possible exposure, spill, release from primary containment, or environmental exposure of any Risk Group 2 biological agent (see Section 3, Risk Assessment for further information on Risk Groups). This should include the date of the incident, the agents, locations, and personnel involved, a narrative description of the circumstances and outcome, the dates when the Biosafety Office was informed, and any follow-up measures taken (*e.g.,* was health care sought for those potentially exposed? are subsequent tests/health care follow-up measures required and performed and when? were any SOPs altered to prevent similar incidents in the future?)
- Reference sheets for biosafety guidelines/policies (e.g. NIH Guidelines, BMBL)

PAPERWORK FLOW

There are two critical routine administrative processes that occur within the Biosafety Office:

a. Receipt and administration of new Biosafety Protocols (IBC protocols) or amendments, presentation of these to the IBC for review and processing of IBC approvals.
b. Verification that IBC approval has been received *for a particular project*, IRB protocol, or IACUC protocol by a PI to other campus offices (OGCA, IRB, or IACUC).

2.1.6 IBC Review/Approval Process for Biosafety Protocols and Amendments

Biosafety Protocols and amendments are first reviewed by the Biosafety Office to determine the most appropriate review/approval track for the protocol.

1. All IBC protocols or amendments involving recombinant DNA agents or those involving higher risk organisms (RG-2 and above) are required to be reviewed by the full IBC during a convened meeting in which minutes are recorded as per NIH and CDC Guidelines.
    - The IBC meets monthly; typically on the second Wednesday of the month
    - All IBC protocols and amendments received four weeks prior to an IBC meeting will be reviewed at that meeting if all documents are in order before the meeting.
    - Decisions regarding protocols or amendments are expected approximately 3-7 weeks from the date of submission for completed IBC protocols applications and training documentation to allow for IBC review.
2. IBC protocols or amendments which do not involve recombinant DNA or involve only exempt recombinant DNA may be eligible for an expedited review and conditional approval by an IBC subcommittee. Responses are requested from subcommittee members within 5 working days of receipt of the completed application or amendment.
3. Simple amendments of previously-IBC approved Biosafety protocols (IBC protocols) which do not involve additional risk (e.g. an addition of a grant title/clinical protocol title to the protocol; most personnel changes) are handled administratively within the Biosafety Office.

During the IBC meeting, the committee may vote to: approve the BSP/amendment without contingencies, approve

the BSP/amendment *with* contingencies, disapprove or table the proposed BSP/ amendment. The PI will be notified of the IBC decision subsequent to the meeting.

If the BSP/amendment is approved with contingencies, the PI will be notified and will be given a deadline to address these contingencies. The BSP/amendment is not considered finally approved until all contingencies have been met. If the contingencies are not met within the window after receipt of the contingencies, the IBC PROTOCOL/amendment application will be considered withdrawn and must be re-submitted for further IBC reconsideration.

BIOLOGICAL MATERIALS AND APPLICATIONS REQUIRED FOR APPROVED BY IBC

Each PI is responsible for the preparation of the Biosafety Protocol Registration Form and supporting documents for all research involving potential biohazards, including the assignment of the required Biological Safety Level (BSL) in the given laboratory, as determined by a risk assessment, to the proposed biological research. The IBC, in conjunction with the Biological Safety Officer, will review all submitted registration documents; inspect laboratory space if not done already; 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. All biological research must be registered, even if exempt from NIH oversight. Registration information can be found on the IBC website.

All proposed research or other educational activities involving the use of the following materials must be approved by the IBC prior to the acquisition of the materials or initiation of the activity.

2.1.7 Recombinant DNA Experiments

As a condition for receipt of NIH funding, UNT must ensure that all such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*. The *NIH Guidelines* are available via the NIH Office of Biotechnology Activities (NIH OBA) web page.

On behalf of the institution, the UNT IBC is required by NIH to review all recombinant DNA research conducted at or sponsored by the institution and perform a comprehensive risk assessment of containment levels, facilities, procedures, practices, training, and expertise of personnel for the proposed research to ensure compliance with the *NIH Guidelines*.

In addition, the IBC is expected to periodically review recombinant DNA research conducted at the institution and report any significant problems with, violations of the *NIH Guidelines*, or any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 calendar days, unless the IBC determines that a report has already been filed by the PI, with some incidents requiring 24h notifications. **The UNT IBC therefore requires registration and/or approval prior to the initiation of any recombinant or synthetic DNA (r/sDNA) experiments.**

2.1.7.1 *Definition of Recombinant DNA*

The *NIH Guidelines* (Section I-B) defines recombinant DNA molecules as either:

i. *molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or*
ii. *molecules that result from the replication of those described in (i) above. NIH Guidelines also specifies that synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a*

toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.

Therefore, according to *NIH Guidelines*, the following are technically **not** considered "recombinant DNA":

- Isolation of genomic DNA or RNA from natural sources.
- PCR amplification of genomic DNA or cDNA from non-cloned templates (e.g. for genotyping purposes), as long as the resultant amplicons are *not* subsequently cloned.
- Chemically synthesized oligonuleotides such as those used in some siRNA/shRNA or DNA sequencing applications. Please note, methods of producing siRNA or shRNA other than chemical synthesis of oligonucleotides typically involve recombinant DNA templates and/or cloning techniques and are therefore **not exempt** from *NIH Guidelines* (e.g. production via *in vitro* transcription, expression of siRNA from an expression plasmid or viral vector, or expression of a PCR-derived siRNA expression cassette).

2.1.7.2 *Categories of rDNA Work That Require Registration*

NIH categorizes recombinant DNA experiments into six categories, as described in Sections IIIA through F of the *NIH Guidelines*. Five of these categories (described in Sections IIIA through E of *NIH Guidelines*) refer to research that the NIH considers "non-exempt" from the requirements for notification and/or approval of internal institutional review committee(s) (i.e. the IBC and/or IRB) and/or external agencies (NIH Office of Biotechnology Activities (OBA), the Recombinant DNA Advisory Committee (RAC) and/or the NIH Director). Although the NIH does not explicitly require notification or approval for recombinant DNA experiments which fall into a sixth ("exempt rDNA") category (as described in Section III-F of *NIH Guidelines*) prior to initiation of the experiment, the UNT IBC requires researchers register **all** r/sDNA experiments with the IBC in an effort to avoid accidental misclassification of rDNA experiments.

2.1.7.2.1 *Cloning a therapeutic antibiotic resistance gene into a human, animal or plant pathogen, if the transfer could compromise the ability to treat or control the disease. (Section III-A-1)*

Note: Registration with the UNT IBC is still required even if:

- this drug resistance is acquired naturally;
- the transferred resistance gene is related to a drug that is an end of the line alternative treatment (2nd, 3rd, 4th, or 5th line drug);
- the drug was used years ago, but is not the preferred treatment today (it may be the only treatment in developing countries);
- the drug is only used to treatment a very small portion of the population (i.e. those with specific contraindications to front line drugs); or
- working with antibiotic resistance strains of pathogens also require registration (even if you did not create them).

Examples:

- Cloning a gene for Erythromycin resistance into *Borrelia burgdorferi*
- Cloning a gene for Chloramphenicol resistance into *Rickettsia typhi*
- Cloning a gene for Pyrimethamine resistance into *Toxoplasma gondii*
- Cloning a gene for Rifampin resistance into *Mycobacterium tuberculosis*

Caution:

- Be careful when using old plasmids for cloning experiments involving pathogens. Many of the old plasmids carry genes for antibiotics that have been used therapeutically or are related to front line drugs.

- Avoid using these plasmids when working with related pathogens;
- Verify that the antibiotic resistance gene is not in a location on the plasmid that can be transferred to the pathogen via a double cross over event.

Website: NIH OSP FAQ – Major Actions

https://osp.od.nih.gov/biotechnology/faqs-about-major-actions-under-section-iii-a-of-the-nih-guidelines-for-research-involving-recombinant-or-synthetic-nucleic-acid/

2.1.7.2.2 *Cloning DNA encoding for a low LD50 toxin or work with vectors that express toxins with a low LD50 (< 100 ug/kg body weight). (Section III-B-1)*

Examples of toxins with low LD50's are:

- Botulinum toxin
- Staphylococcal enterotoxin B
- Tetrodotoxin
- Clostridium tetanus toxin

Websites: Table of Toxins: http://www.selectagents.gov/PermissibleToxinAmounts.html
Univ. of Florida – Toxin Lists: http://www.ehs.ufl.edu/Bio/toxin.htm
Biological Toxins List: https://riskmanagement.unt.edu/sites/default/files/05_table_of_biological_toxins.pdf

2.1.7.2.3 *Human Gene Transfer Experiments (Section III-C-1)*

The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants are subject to the *NIH Guidelines*. This includes the transfer of DNA with defective viral vectors, such as retroviral, adenoviral and lentiviral vectors, along with the use of liposomes and other methods of delivery.

Human gene transfer experiments with synthetic nucleic acid molecules also require registration if any of the following criteria are met: The synthetic nucleic acid molecules:

- Contain more than 100 nucleotides; or
- Possess biological properties that enable integration into the genome (e.g. cis elements involved integration); or
- Have the potential to replicate in a cell; or
- Can be translated or transcribed

These experiments require approval from the UNT IRB and possibly U.S. Food and Drug Administration.

Website:
NIH OSP Frequently Asked Questions on Human Gene Transfer Experiments: https://osp.od.nih.gov/biotechnology/faqs-on-the-nih-guidelines-research-synthetic-nucleic-acid-molecules/ and https://osp.od.nih.gov/wp-content/uploads/PTC_from_AppendixM_042319_508.pdf

2.1.7.2.4 *rDNA Experiments involving the use of a human, animal, or plant pathogen (whether the recombinant or synthetic nucleic acid molecules originated from your lab or another). (Section III-D-1, III-D-2, III-D-3)*

Examples:

- Cloning a gene into a pathogen (i.e. expressing a gene into VSV, Vaccinia Virus, Tobacco Mosaic Virus, Mouse Cytomegalovirus)
- Cloning a pathogen into a lower eukaryotic or prokaryotic cell;
- Using a defective pathogen vector with or without helper virus in cell culture or animal experiments, examples include:
    - Poxviruses (Vaccinia)
    - Herpesvirus vectors (HSV)
    - Lentivirus vectors (HIV, FIV based)
    - Retroviruses (murine retroviruses)
    - Adenoviruses
    - Adeno-Associated Virus vectors
    - Vesicular Stomatitis Virus vectors
    - Sindbis Virus vectors

Helpful guidance documents developed by Stanford University for experiments involving viral vectors can be accessed at the following websites :
https://ehs.stanford.edu/reference/recombinant-viral-vector-biosafety-levels
https://ehs.stanford.edu/reference/lentivirus-fact-sheet
https://ehs.stanford.edu/reference/adenovirus-fact-sheet.

Note that rDNA experiments involving ≥ 50 % of genetic material from Risk Group 2 organisms must also be registered with the IBC.

2.1.7.2.5 *Cloning DNA or RNA from Risk Group 3 or Risk Group 4 human pathogens, restricted animal or plant pathogens, or Select Agents. (Section III-D-2)*

Any rDNA experiments with these materials must be registered with and approved by the UNT IBC, **even if you are working with only one base pair of DNA or RNA from these agents.**

Websites: NIH Appendix B (Risk Groups)
https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html#_Toc3114384

American Biological Safety Association Risk Group Classifications of Etiologic Agents: https://my.absa.org/Riskgroups

List of restricted animal pathogens, BMBL appendix D
https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF

Select Agent List:
https://www.selectagents.gov/SelectAgentsandToxinsList.html

2.1.7.2.6 *rDNA Experiments involving whole animals, plants, and arthropods (and insects)… (Section III-D-4, III-D-5, III-E-3)*

Experiments in this category include:

- Experiments involving toxins, pathogens, defective vectors, and other genetically modified materials used in animal, plants or insects.

- Creation of transgenic animals
  - Mice, rats
  - Zebrafish
  - Drosophila, butterflies
  - Other

Note: For rodents only, the purchase or transfer of transgenic rodents is exempt from the NIH rDNA Guidelines and does not require approval (if the transgene used does not code for a toxic, virulent, or oncogenic sequence), but does require IBC protocol registration for use and breeding at UNT. Purchase is defined as buying a transgenic rodent that has been created by another entity outside of your laboratory. The transfer of a transgenic rodent to your laboratory is also exempt (provided the transgene doesn't code for toxic, oncogenic or potentially harmful gene). Transfer is defined as the acquisition into your research lab of a transgenic animal created (made) by another entity.

Note: In each case above, *for rodents only*, you may have designed or created the gene that has been inserted into the developing embryo of the transgenic rodent, but if you are not the group that has performed the actual procedure (i.e. the lab that inserted the gene into the embryo), you are exempt from the r/sDNA Guidelines. However, the use of these animals in your lab is not exempt from IBC registration and you must provide the gene inserted in these animals if you have designed it. If your lab will insert the gene into the embryo, you must get approval for this work. Regardless, you will still be required to register your project with the IBC.

2.1.7.2.6.1 Knock-out Animals

Knock-out (gene silencing, gene ablation, etc.) *rodents* are exempt from the *NIH Guidelines* as long as the method to generate the knock-out animal does not leave any "new" genetic material behind in the genome after the procedure. If DNA from the molecule used to create the knock-out is permanently inserted into the genome, the experiment will require approval by the UNT IBC.

2.1.7.2.6.2 Exemption for Breeding Transgenic Rodents

Note: Generation of transgenic rodents by breeding to create a new strain shall be EXEMPT from the *NIH Guidelines* if the following criteria are met. Exempt experiments must still register with the IBC.

- Both parental rodents can be housed under BSL1 containment; AND
- Neither parental transgenic rodent contains the following genetic modifications:
  - Incorporation of more than 50% of the genome of an exogenous eukaryotic virus from a single family of viruses; OR
  - Incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); AND
- The transgenic rodent that results from this breeding is not expected to contain more than 50% of an exogenous viral genome from a single family of viruses. This exemption DOES NOT pertain to other transgenic animals such as zebrafish, drosophila, rabbits, pigs, etc. It also DOES NOT pertain to transgenic experiments involving plants.

2.1.7.2.7 Large Scale rDNA Experiments (Section III-D-6)

Any r/sDNA experiments at any level or Risk Group, including exempt and non-exempt experiments that generate a volume of culture that is in excess of 10 liters, requires approval by the UNT IBC. Note: Work with 10 L may be in a single fermentation vessel (10 L or larger) or a series of flasks whose aggregate volume would exceed 10 L. Examples include: Growing up five 2 L flasks of *E. coli* K-12 cultures expressing your gene of interest. Growing 10

L of *Saccharomyces cerevisiae* in a fermentation apparatus to get a sufficient yield of the desired protein.
2.6.1.2.8. The following guideline is intended to further assist researchers in properly classifying their recombinant DNA experiments. Please contact the Biosafety Office IBCprogram@unt.edu for any additional assistance:

Table 2.B. Simplified Guideline for Classifying Recombinant DNA Experiments according to *NIH Guidelines*. UNT IBC requests that all recombinant DNA experiments be registered with the Biosafety Office prior to initiation to avoid potential misclassification errors. *(Table based on that from Yale University's Biosafety Manual)*

"Non-exempt" Recombinant DNA		"Exempt"
Experiments which must be registered with the IBC and approved prior to initiation	Experiments that require registration with the IBC simultaneous with initiation	Recombinant DNA experiments that do not require IBC approval but do require IBC registration

1. Deliberate transfer of a drug trait to a microorganism not known to acquire it naturally (if it could compromise the use of the drug to control disease agents in humans, animals or agriculture)[2]. *(Note: this would likely exclude most subcloning procedures using antibiotic selectable markers in E. coli K12 derivatives)* 2. Cloning of DNA encoding toxic molecules lethal to vertebrates at an LD50 of <100 µg/ kg body weight[2,3]. 3. Human gene transfer/therapy experiments[2]. 4. Cloning using human or animal pathogens as host-vector systems. 5. Cloning of DNA from all Risk Group[4] 2, 3, 4 or restricted human or animal pathogens (including HIV and related viruses and viruses capable of infecting human cells). 6. Experiments using more than 2/3 of the genome of infectious animal or plant viruses or defective viruses grown in the presence of helper virus or complementing helper virus components. 7. Recombinant DNA experiments involving whole animals, plants, or arthropods (and insects), including transgenic or knockout rodent experiments requiring BSL-2[4] containment; or transplantation of genetically engineered cells into organisms. 8. Large scale DNA projects (≥10 liter cultures at any moment in time).	1. Experiments using as vectors < 2/3 of the genome of a eukaryotic virus, demonstrated to be free of helper virus or complementing helper virus components. 2. Transgenic or knockout rodent experiments for which BSL-1[4] containment is appropriate (NOTE: the purchase of transgenic rodents for BSL-1 experiments falls into the "exempt" category; however, breeding of these animals with different strains of mice is considered non-exempt rDNA experiments).	1. rDNA containing less than 1/2 of an eukaryotic viral genome propagated in cell culture (with the exception of expression of DNA from Risk Group[4] 2, 3, 4 or restricted agents); 2. rDNA work involving E. coli K12 derivatives, *S. cerevisiae*, and *B. subtilis* host-vector systems (with the exception of expression of DNA from Risk Group 2, 3, 4[4] or restricted agents).

[1]For further explanations/details, please see *NIH Guidelines*

[2]Please note that these experiments may require approval of NIH Director, NIH's Office of Biotechnology Activities (OBA) approvals or registration and/or the Recombinant DNA Advisory Committee (RAC) review. Please contact the Biosafety Office as soon as possible if your experiments fall into these categories for assistance.

[3]LD50 for many toxins can be viewed on commercially available Safety Data Sheets (SDSs), or contact the Biosafety Office if you need assistance.

[4]See the following links for assistance in Risk Group classification and recommended Biosafety Level usage:

- American Biosafety Association Risk Group Guide: https://my.absa.org/Riskgroups
- Public Health Agency of Canada MSDSs: http://www.phac-aspc.gc.ca/msds-ftss/
- *NIH Guidelines* for Recombinant DNA Research: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf
- CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL): https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF

2.1.7.3 *Viral Vectors in Animal Use*

All work involving recombinant nucleic acids must be approved by the UNT IBC (in addition to the IACUC approval) as required by the *NIH Guidelines*. The IBC requires that all viral vectors used for transgene expression must:

- Be free of detectable replication competent virus
- Minimize probability of homologous and end joining recombination which might reestablish wild type virus
- Be produced in the absence of helper virus
- Utilize a homologous packaging system
- Utilize self-inactivating derivatives

The biosafety level of a viral vector defaults to the Risk Group (BSL-1, ABSL-1 or BSL-2 or ABSL-2; See Section 4 for information on risk groups and biosafety levels) of the wild type viral strain from which the vector is derived. This biosafety level is applied during preparation, during use in cell culture systems, and for the first 72 hours after inoculation into animals while the vector is considered infectious (though non-replicating). In addition, the transgene being inserted and the source of the viral vector should be considered. The following checklist will assist a researcher in determining the proper biosafety level:

- Is the viral vector derived from a wild-type virus pathogenic to humans or primates (HIV, SIV, Human Adenovirus, etc.)?
- Does the transgene encode a product that is potentially hazardous (oncogene, toxin, etc.)?
- Does the vector or transgene encode more than 2/3 of the viral genome?
- Is the viral vector obtained from a non-commercial source?

If all answers are "NO", then the viral vector can be handled at BSL-1 or ABSL-1 pending IBC approval. Examples of these types of vectors include adeno-associated virus (AAV), murine retrovirus, feline immunodeficiency virus (FIV) and vesicular stomatitis virus (VSV). Exceptions must be requested via IBC protocol review process.

2.1.7.4 *Recombinant DNA Registration Requirements*

PIs and/or Lab Supervisors must submit or amend Biosafety Protocols:

- before bringing new recombinant DNA materials to campus which had not already been approved by the IBC
- before initiating any new recombinant DNA work.

New submissions of Biosafety Protocol describing new work must be submitted by the PI using the Biosafety Protocol Registration form, which is available online on the UNT Biosafety Office and IBC web sites.

Amendments must be submitted by the PI fully describing the new work if the scope of the work has changed from that approved by the IBC utilizing the Biosafety Protocol Amendment form. Changing the scope of the work would include changes in: vectors or inserts, administration or exposures of new target materials or animals, creation of a new transgenic strain or species, or embarking upon large-scale culture (≥10 liters), changes in locations, personnel or major equipment. All questions can be addressed to the Biosafety Office (IBCprogram@unt.edu).

2.1.8 Hazardous or Potentially Hazardous Biological Agents (or Materials Potentially Contaminated with these Pathogens or Toxins)

All proposed research or other educational activities involving risk group 2 (RG-2) or BSL-2 labs must be approved by the IBC; see Section 3 and 4 for information on risk groups and biosafety levels. Special considerations are required prior to possession and/or transfer of any known human, animal, and/or agricultural pathogens or toxins or materials which may be potentially contaminated with these pathogens or toxins to UNT campus. Inactivated biological samples derived from BSL-2 and above agents or attenuated pathogens derived from BSL-2 and above agents must also be approved. This may include laboratory inspections for compliance with Biosafety standards and permits for transfer of these agents. The Biosafety Office can assist researchers in determining whether special inspections and/or permits may be required.

The UNT IBC and the IACUC must approve all experiments involving the introduction of infectious agents or potentially hazardous biological materials into animals prior to initiation.

All researchers working with etiologic agents (Risk Group 2) must receive training in both biosafety and the microbiological procedures that will be utilized for the experiment. Biosafety training sessions for new staff and faculty are provided through Environmental Health and Safety online or contact IBCprogram@unt.edu to schedule in-person training. The PI is responsible for ensuring that all researchers are trained in the appropriate procedures and techniques used in the laboratory.

Consultation in Risk Group classification of biological agents can be sought through the Biosafety Office (IBCprogram@unt.edu). Information can also be found at the following URLs:

- CDC BMBL: https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF
- *NIH Guidelines*: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf
- ABSA Risk Group Classification Database: https://my.absa.org/Riskgroups
- Public Health of Canada Biological MSDSs: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

Work with Risk Group 3 and 4 agents (or those requiring Biosafety Level 3 or 4 containment) is not currently permitted at UNT.

Work with Select Agents or Toxins will require additional registration and training requirements by the PI and laboratory staff as well as additional registration and approval requirements from federal agencies. Contact the Biosafety Office (IBCprogram@unt.edu) for additional information before you start or purchase these agents/toxins. Updated list can be found at http://www.selectagents.gov/sat/list.htm

2.1.9 Toxins of Biological Origin

Because of the risks posed by the acute toxicity of some biological toxins (i.e., toxins derived from biological sources), the IBC will require documentation of Standard Operating Procedures (SOPs) to ensure tracking, safety handling, and disposal procedures for any toxin of biological origin with an LD50 ≤ 100 μg/kg body weight as part of the PI's Biosafety Protocol (BSP). IBC approval must be obtained prior to acquisition of the toxins. Safety and handling procedures for biological toxins with LD50 ≤ 1000 μg/kg body weight must also be documented in the laboratory biosafety protocols. These should incorporate the risk assessment and management considerations as described in Appendix I of the BMBL. See the biosafety website for representative biological toxins and their LD_{50}'s and information on deactivation methods for many biological toxins.

These SOP considerations should include:

- Special training should be provided to all personnel related to the unique risk characteristics of toxins and particular emergency spill and/or exposure procedures should be provided
- Special procedures and protective equipment required to prevent exposure to any dry/freeze-dried toxins, since the potential for electrostatic dispersal of concentrated forms of toxin pose uniquely high risks. Respiratory protective measures must be considered.
- The containment practices and equipment required to reduce the risks of accidental exposure by direct contamination of mouth, eyes, or other mucous membranes by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin while working with solutions of biotoxins. When working with toxins that pose direct percutaneous hazards, special care must be taken to select gloves that are impervious to the toxin and the diluents or solvents employed.
- Restricted access to designated isolated rooms while toxins are in use. The room should be clearly posted: "Toxins in Use—Authorized Personnel Only." while toxin work is ongoing and unrelated and nonessential work should be restricted from areas where stock solutions of toxin or organisms producing toxin are used.
- Special deactivation/decontamination procedures may be required for surfaces and liquids contaminated with the toxins. Toxins may not be deactivated by typical disinfectants used against pathogens, and therefore, special procedures should be documented and followed for deactivation and disposal (see Section 7.4 for further information and guidance on deactivation of biological toxins).
- Additional security measures which may be required to limit access to the stored biological toxins.

2.1.10 Select Agents and Toxins (BSATs)

A subset among human, animal, and/or agricultural pathogens and toxins are the Select Agents and Toxins (BSATs). Select agents/toxins are agents that the U.S. Department of Health and Human Services (HHS) and/or the Department of Agriculture/Animal & Plant Health Inspection Service (USDA/APHIS) considers to have the potential to pose a severe threat to human health, animal or plant health, or to animal or plant products. Only small quantities of these toxins may be handled in BSL2 level labs. Please contact the BSO and EH&S before considering to work with these toxins.

The BSAT regulations pertain not only to the intact agents or toxins, but also several genetic elements or recombinant nucleic acids from these agents and recombinant Select Agent organisms. The National Select Agent Registry has established a URL that serves as an informational center, as well as a source of all registration materials and forms. http://www.selectagent.gov/

Among these requirements of these regulations is that any entity (defined by HHS/CDC and USDA/APHIS as "any government agency (Federal, State, or local), university, corporation, company, partnership, society, association, firm, sole proprietorship, or other legal entity") wishing to use, possess, or transfer SATs must register with either the Centers of Disease Control (CDC) or USDA/APHIS. This registration process requires the following:

- Designation of a Responsible Official (RO) for the entity, which is the person "designated by an entity to act on its behalf…" and that "…this individual must have the authority and control to ensure compliance with the regulations in this part". Currently, UNT is not registered with the Federal Select Agent Program.
- Approval of the HHS or USDA Secretary or Administrator based on a Security Risk Assessment (SRA) by the U.S. Attorney General for any individual or entity who may potentially have access or control over any Select Agent or Toxin. Note: an application for an individual may be denied or a certificate of registration revoked or suspended if an individual is reasonably suspected by any Federal law enforcement or intelligence agency of:
    - Committing a crime specified in 18 U.S.C. 2332b(g)(5),
    - Knowing involvement with an organization that engages in domestic or international terrorism (as defined in 18 U.S.C. 2331) or with any other organization that engages in intentional crimes of violence, or
    - Being an agent of a foreign power (as defined in 50 U.S.C. 1801)
- Identification of the particular physical location in which these BSATs may be present, and the associated comprehensive documentation must be provided and approved by the CDC and/or USDA for the specific locations, agents and experiments proposed for use in these facilities:
    - Safety plans
    - Security plans
    - Incident/Emergency response plans
    - Precise Inventories and all records of transfer and use.
- Agent- and Use-specific training of all who may have access must be documented and approved, and must include drills and exercises.
- Notifications of any theft, loss, or unaccounted samples, releases from containment and possible exposures must be made to the CDC and/or USDA, and may require further Federal investigation and cooperation.

Any individual who intends to possess, use, or transfer any Select Agent or Toxin to UNT campus must contact the Biosafety Office (IBCprogram@unt.edu) immediately to discuss registration procedures and the adequacy of the facilities to secure the agents or toxins in question, and the measures that will need to be taken to qualify for registration.

2.1.10.1 *Exclusions from Select Agents and Toxins Regulations*

HHS/USDA select agents or toxins that meet any of the following specific criteria are excluded from the Select Agent of Toxin Regulations as described in 42 CFR §73.3 (d) & (e), 7 CFR §331.3 (d) & (e) and 9 CFR §121.4 (d) & (e):

- Any select agent or toxin that is in its naturally occurring environment provided the select agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source is

excluded.

- Non-viable select agents or nontoxic select toxins are excluded.
- Except as required in § 73.16(l), the aggregate amount of the toxin under the control of a PI, treating physician or veterinarian, or commercial manufacturer or distributor is excluded as long as it does not, at any time, exceed the following amounts:

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

Provided that,

  - (i) The toxin is transferred only after the transferor uses due diligence and documents the identification of the recipient and the legitimate need (e.g., prophylactic, protective, bona fide research, or other peaceful purpose) claimed by the recipient to use such toxin.
  - Documentation should include, but is not limited to, the recipient identity information (name, institution, address, telephone number and email address); name of the toxin and the total amount transferred; and the legitimate need claimed by the recipient.
- An animal inoculated with or exposed to an HHS select toxin is excluded.
- HHS select toxins identified in an original food sample or clinical sample are excluded.
- For those laboratories that are not exempt under § 73.5 (a) and § 73.6 (a), Botulinum neurotoxin that is produced as a byproduct in the study of Botulinum neurotoxin producing species of Clostridium is excluded so long as the toxin has not been intentionally cultivated, collected, purified, or otherwise extracted, and the material containing the toxin is rendered non-toxic and disposed of within 30 days of the initiation of the culture.
- Waste generated during the delivery of patient care by health care professionals from a patient diagnosed with an illness or condition associated with a select agent is excluded as long as the waste is decontaminated or transferred for destruction by complying with state and federal regulations within seven calendar days of the conclusion of patient care.
  - Waste including specimens associated with patient care must be secured against theft, loss, or release during the period between identification and transfer or destruction, and any theft, loss, or release of the waste including specimens must be reported to FSAP. All patient-generated waste including specimens kept more than seven days after the conclusion of acute patient care are subject to the select agent regulations. There is no requirement to document the transfer or destruction of waste, including specimens generated from the patient, provided that the waste is decontaminated or transferred for destruction by complying with state and federal regulations within seven calendar days of the conclusion of patient care.

While these exclusions apply, the IBC requires registration and approval of these materials prior to acquisition and use of any of these agents, even if excluded by regulation.

The PI must contact the Biosafety Officer immediately when any material containing a Select Agent or Toxin is identified or is likely to be transferred to campus (even if it may qualify for an exemption or exclusion) to ensure that the strict compliance standards with these Federal laws are maintained.

2.1.10.2 *Criteria for Handling Select Agents or Toxins Contaminated Specimens in Clinical/ Diagnostic Laboratories under the Exemption Clause*

Please note, if a BSAT is contained in a specimen presented for diagnosis or verification, please be aware that the following conditions must be met:

1. Contact the Biosafety Office immediately (IBCprogram@unt.edu).
2. The BSAT needs to be transferred or destroyed on-site within 7 calendar days in accordance with specific guidance in the Federal laws.
3. The BSAT must be secured against theft, loss or release prior to transfer or destruction.
4. Clinical or diagnostic laboratories and other entities that have identified select agents or toxins (select agents & toxins list) contained in a specimen presented for diagnosis or verification are required by regulation (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73) to report the identification within 7 calendar days to the Federal Select Agent Program. In addition, these laboratories or entities are required to report the identification of select agents and toxins from samples received for proficiency testing within 90 days of receipt of the sample.
5. In addition to reporting the identification of a select agent or toxin contained in a specimen presented for
6. diagnosis or verification using the APHIS/CDC Form 4, the following select agents and toxins are required to be immediately (i.e. within 24 hours) reported to Federal Select Agent Program (e.g., via telephone, fax, or email):
    - *Bacillus anthracis*
    - *Bacillus cereus* Biovar *anthracis*
    - Botulinum neurotoxins
    - Botulinum neurotoxin producing species of Clostridium
    - *Burkholderia mallei*
    - *Burkholderia pseudomallei*
    - Ebola virus
    - Foot-and-mouth disease virus
    - *Francisella tularensis*
    - Marburg virus
    - Rinderpest virus
    - Variola major virus (Smallpox virus)
    - Variola minor virus (Alastrim)
    - *Yersinia pestis*

2.1.11 Human Blood, Body Fluids, Cells, Tissues and Other Potentially Infectious Materials

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens (BBP) Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard) to minimize or eliminate exposure to infectious agents that may be present in any human blood, tissues or certain body fluids (bloodborne pathogens). Further, Texas Bloodborne Pathogen Control (25 TAC §96) governs the University of North Texas also references the OSHA standard. For these reasons, it is UNT's IBC policy to adhere to the OSHA standards for Bloodborne Pathogens in addition to the Texas Bloodborne Pathogen Control Standard.

The Bloodborne Pathogens Standard applies to all employers having employees that are "occupationally exposed" to human blood, materials which may have been exposed to human blood or other potentially infectious materials. An employee is considered occupationally exposed if there is "reasonably anticipated skin, eye, mucous membrane, or parenteral [via injection, infusion, cut exposure, or transdermal methods] contact with human blood or other potentially infectious materials in the performance of an employee's duties." An individual is also considered occupationally exposed even if they do not have direct contact with blood or other potentially infectious material, if the employee uses equipment that is used to process or store blood, other potentially infectious materials, or bloodborne pathogens.

Potentially infectious materials other than human blood may include:

- Human or non-human primate cell, tissue, or organ cultures, this includes
  - All cell lines (primary and established) of human/primate origin
  - All cell lines derived from lymphoid or tumor tissue
  - All cell lines exposed to or transformed by any oncogenic virus
  - All cell lines exposed to or transformed by amphotropic packaging systems
- All human clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy)
  - All mycoplasma-containing cell lines
- Any unfixed human tissue or organ, other than intact skin, from a human being (living or dead)
- Human body fluids, potentially contaminated with blood. This includes:
  - semen
  - vaginal secretions
  - cerebrospinal fluid
  - synovial fluid
  - pleural fluid
  - pericardial fluid
  - peritoneal fluid
  - amniotic fluid
  - saliva in dental procedures
- All body fluids in situations where it is difficult or impossible to differentiate between body fluids or is contaminated with blood.
- Blood, organs, or other tissues from experimental animals infected with HIV or HBV or other bloodborne pathogens. OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens such as (not an exhaustive list):

  - Hepatitis B virus (HBV)
  - Hepatitis C virus
  - Hepatitis D virus
  - Human Immunodeficiency Virus (HIV)
  - *Plasmodium* species
  - *Treponema* species
  - *Babesia* species
  - *Borrelia* species
  - *Brucella* species
  - *Leptospira* species

- *Francisella* species
- *Streptobacillus moniliformis*
- Colorado Tick Fever viruses
- Fever viruses
- Arboviruses
- *Spirillum minus*
- Creutzfeldt-Jakob virus
- Human T-lymphotropic Virus Type I
- Hemorrhagic

It is not acceptable to handle any of the above materials on a clean bench or horizontal laminar flow hood. 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. When human blood or tissue donors are involved, the PI must also determine whether a human subject Institutional Review Board application is required. Work done with NHP blood or tissue must also be approved by the IBC.

Persons working with human blood, body fluids, cells, tissues and other potentially infectious materials should refer to the Bloodborne Pathogen Exposure Control Plan for information on the applicable standards, exposure control plan, training requirements, work practices, housekeeping, engineering controls, personal protective equipment, signs/label requirements, Hepatitis B vaccination, emergency actions, exposure incident procedures, post-exposure evaluation and follow-up, and recordkeeping.

2.1.11.1 *Cultures of Cell lines of Human and/or Non-Human Primate Origin*

Human cell lines are commonly used in biomedical research, yet appropriate biosafety requirements for handling human cell lines are often subject to debate within the scientific community. While human blood, most body fluids, unfixed human tissues and organs were clearly included within the scope and application of the OSHA Bloodborne pathogen standard (29 CFR Part 1910.1030), the inclusion of human cell lines was ambiguous. In 1994, OSHA issued a letter of interpretation related to the applicability of the BBP Standard towards human cell lines:

According to the interpretation, human cell lines are considered to be potentially infectious and within the scope of the BBP Standard unless the specific cell line has been characterized to be free of hepatitis viruses, HIV, Epstein-Barr virus, papilloma viruses and other recognized bloodborne pathogens. In alignment with this interpretation, the American Type Culture Collection (ATCC) recommends that all human cell lines be accorded the same level of biosafety consideration as a line known to carry HIV, and recommends handling all cultures under BSL-2 conditions. Moreover, Appendix H of the 5th Edition of the BMBL recommends that human and other primate cells should be handled using Biosafety Level 2 (BSL-2) practices and containment.

In consideration of the aforementioned regulatory interpretation and consensus guidelines and other factors, the UNT IBC has adopted the following guidance regarding the use of Human and Non-Human Primate cell lines: **in addition to human and non-human primate blood, most body fluids, unfixed tissues and organs, all cell and organ cultures of human and non-human primate origin including established cell lines shall be handled in accordance with the OSHA Bloodborne Pathogens Standard and under Biosafety Level 2 (BSL2) containment. Existing and future university laboratory safety policies should reflect this guidance.** See the Human and NHP Cell Lines Guidance for additional information.

2.1.11.2 *Human Embryonic Stem (hES) Cell and Embryonic Germ Cell Lines*

Human embryonic stem cell (hESC) research requires additional regulatory approval by an Embryonic Stem Cell Research Oversight (ESCRO) Committee. Prior to obtaining human embryonic stem cells, researcher must verify that an ESCRO is available to meet and review protocols. UNT does not have a standing ESCRO committee, and no hESCs may be brought on to campus without this prior approval. Contact IBCprogram@unt.edu with questions.

On March 9, 2009, President Barack H. Obama issued Executive Order 13505: Removing Barriers to Responsible Scientific Research Involving Human Stem Cells. The Executive Order states that the Secretary of Health and Human Services, through the Director of NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell (hESC) research, to the extent permitted by law.

These Guidelines implement Executive Order 13505, as it pertains to extramural NIH-funded stem cell research, establish policy and procedures under which the NIH will fund such research, and helps ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law. Internal NIH policies and procedures, consistent with Executive Order 13505 and these Guidelines, will govern the conduct of intramural NIH stem cell research.

Website: https://stemcells.nih.gov/policy/2009-guidelines.htm

NIH Human Embryonic Stem Cell Registry

The Registry lists human embryonic stem cell lines that are eligible for use in NIH-funded research.

Website: https://grants.nih.gov/stem_cells/registry/current.htm

2.1.12 Animals and the use of Biological Materials in Animals

All research experiments involving animals must be conducted in accordance with the associated UNT IACUC approved animal use protocol (AUP). If the animals are infected, infectious, or pose a higher risk for human infection such that the animals or their tissues require Biosafety Level-2 containment, research with these animals will require IBC approval as well. This may include working with animals in the field. All animal work should be registered through the Biosafety Protocol Submission process, though not all work requires full IBC approval.

In any laboratory or field work which involves the use of and/or exposure to live animals (including invertebrates/insects/arachnids), 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. All laboratory personnel and animal care workers must be fully informed of the biosafety practices necessary to prevent an accidental exposure from an infected animal. It is the PI's responsibility to inform animal care workers associated with the research of the potential risks and appropriate biosafety practices. This includes requiring all workers to complete annual animal biosafety training.

Animal research that involves the introduction of biological hazards (*e.g.,* agents which require BSL-2 containment or recombinant DNA), poisonous, or venomous animals must have an *approved* Biosafety Protocol (BSP) to fully describe and disclose the use of these biological agents/recombinant DNA and risk mitigations in their BSP applications. **The IBC must approve the work prior to initiation.** Biosafety Protocol Registration forms can be found

on the Biosafety website or please contact the Biosafety Office (IBCprogram@unt.edu) for assistance. Once approved by the IBC, the UNT IACUC will be contacted prior to initiation to ensure that a safety protocol have been established and appropriate facilities are obtained for these experiments.

Animal containment, biosafety, and biosecurity must be considered with every animal protocol.

2.1.12.1 *Insects/Arthropods*

Insects present unique challenges to containment which are not encountered with either microbial pathogens or biological toxins. In addition, because some arthropods are venomous or may serve as vectors for disease transmission, special containment considerations, including facilities (insectaries) and practices specifically designed to prevent accidental escape of arthropods are crucial for health and environmental safety. With this in mind, the American Committee of Medical Entomology (ACME), a subcommittee of the American Society of Tropical Medicine and Hygiene (ASTMH) has developed Arthropod Containment Guidelines (ACG) and related materials which must be considered during the risk assessment process. Contact IBCprogram@unt.edu for a copy of these guidelines.

Special care and consideration must be given regarding research with live insects concerning containment procedures. Research projects involving live insects should be limited to only those involving insects which do generally not serve as disease vectors (*e.g., Drosophila melanogaster* (*i.e.,* fruit flies)), which may or may not carry exempt (RG-1) recombinant DNA, unless the facility has been modified to properly house and contain the disease vector. Even with experiments involving insects/ arthropods which are not potential disease vectors, special precautions should be taken to prevent accidental escape/ release of laboratory insects, particularly those which have been genetically engineered or are venomous, to prevent injuries or environmental contamination.

Animal research that involves the use of insect vectors, the introduction of biological hazards, or poisonous or venomous insects must have an *approved* Biosafety Protocol to fully describe and disclose the use of these biological agents/recombinant DNA and risk mitigations in their applications. **The IBC must approve the work prior to initiation.** Biosafety Protocol Registration forms can be found on the Biosafety website or please contact the Biosafety Office (IBCprogram@unt.edu) for assistance.

2.1.13 Dual Use Research of Concern (DURC)

All proposed research or other educational activities involving Dual Use Research of Concern (DURC) must be approved by the IBC and outside Federal Agencies prior to acquisition of the materials or initiation of the activity. 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. Refer to the DURC training and information on the DURC website for additional information.

3 RISK ASSESSMENT

Responsibility for biosafety exists at all levels and is shared throughout the University. The President, Provost, and Vice President of Research acknowledge the institution's role in providing a safe workplace and have given the IBC and Biosafety Office the authority to administer the campus biosafety program. The IBC establishes policies for the safe use of biohazards and for compliance with all applicable regulations and guidelines to secure the safety of the university, personnel, research assets, the environment and the surrounding community. The UNT EH&S helps with the implementation of those guidelines in the lab spaces and UNT facilities. The Biosafety Office acts as the agent of the IBC in ensuring compliance with these policies, disseminating pertinent information, consulting with faculty, staff, students and visitors, and monitoring non-compliance.

The researchers, clinicians, students, and technicians who perform work with biohazards are perhaps the most important component of the biosafety program, as they must incorporate the biosafety requirements and safety precautions into all facets of their work.

As per the NIH guidelines the PI is ultimately responsible for safety within the laboratory and in the field. An integral part of this responsibility is to conduct a review of proposed work to identify potential hazards (risk assessment) and to adopt appropriate safety procedures before initiation of the experiments (risk management). The PI must also monitor the work to ensure that the safety procedures are being utilized by the staff and assess whether any improvements should be made based on logistics of the experiments or additional safety concerns.

The information identified by risk assessment will provide a guide for the selection of appropriate biosafety level, microbiological practices, safety equipment, PPE, and facility safeguards that can prevent LAIs, protect persons that are not directly associated with the laboratory, and reduce environmental contamination risk.

Certain experiments require advanced registration and Biological Safety Committee approval prior to initiation (See Section 2.6).

A risk assessment/risk management matrix has been prepared to illustrate key elements of the process (see below). Relevant sections providing additional details are indicated within the matrix. Information on the routes of exposure is included at the end of this section. A general risk assessment worksheet is available on the biosafety/IBC website, and one is also provided when completing the IBC form. The information in sections 3 and 4 of this manual can help you to adequately determine the risk and mitigation to complete the risk assessment form.

The five P's of risk assessment and risk management are:

- Pathogen – hazardous biological agent.
- Procedures – proposed experimental manipulations and safe work practices.
- Personnel – appropriate training and skills.
- Protective equipment – protective clothing and safety equipment.
- Place – laboratory design.

Consider the five P's in each facet of laboratory work. Properly conducted, risk assessment can help prevent exposure to biohazards and minimize the potential for laboratory acquired infection. Remember that prior planning prevents poor performance from both biosafety and research integrity standpoints.

The primary factors to consider in risk assessment and selection of precautions are agent hazards and laboratory procedure hazards. Careful judgment is crucial to guarantee that the risks are neither underestimated nor the laboratory burdened unnecessarily with too rigorous safeguards.

After reading this section and relevant sections of the Biological Safety Manual contact the Biosafety Office IBCprogram@unt.edu or Biosafety Specialist (biosafety@unt.edu) for help applying the principles of risk assessment and risk management to experimental procedures.

Table 3.A.: Risk Assessment and Mitigation Table		
	Risk Assessment	**Risk Management**
Pathogen	• Agent classification • Routes of infection/exposure • Infectious disease process • Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors	• Registration – See Section 2.6 o Biosafety Office o Biological Safety Committee o USDA – restricted agents or Select Agents/Toxins o CDC – Select Agents/Toxins o FDA/NIH - human gene therapy • Ensure strain identity, attenuation, replication competency (safety tests & documentation)
Procedures	• Aerosol risk: sonicating, centrifuging, homogenizing, blending, shaking, etc. • Percutaneous risk: needles, syringes, glass Pasteur pipettes, scalpels, cryostat blade/knife, etc. • Splash/splatter risk: pipetting, microbial loop, etc. • Transport/shipping of agents outside of laboratory	• Written set of standard operating procedures (SOPs) with safety practices incorporated • Adherence to basic biosafety principles • Label labs, areas, and equipment housing BSL-2 agents • Conduct lab inspections to review practices and containment equipment • Use trial experiments with non-infectious material to test new procedures/equipment
Personnel	• Host immunity o Neoplastic disease . o Infection . o Immunosuppressive therapy o Age, race, sex, pregnancy o Surgery (splenectomy, gastrectomy) o Diabetes, Lupus • Immunization • Post-exposure prophylaxis • Serum banking • Attitude toward safety • Comfort • Open wounds, non-intact skin, eczema, dermatitis	• Safety training • Prior work experience with biohazards • Demonstrated proficiency with techniques • Prompt reporting of all exposure incidents, near misses, as well as signs and symptoms of related disease to PI and Biosafety Office • Investigation/review of incidents/spills, etc. to prevent future occurrence
Protective Equipment	Protection (containment) for: • Aerosols – respirable size particles <5µm • Droplets/splatter • Sharps	• Personal protective equipment (PPE): • Respirators – HEPA, N-99, N-95, etc. • Surgical masks (for droplets) • Face (eye, nose, mouth) protection – mask and safety glasses, or chin length face shield. • Solid front gown or lab coat • Gloves • Biological safety cabinets • Centrifuge safety buckets/rotors • Plexiglas shielding, glove box
Place – Laboratory facility	• Risk group/biosafety level requirements • Aerosol risk • Restricted access	• Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open • Labels • Containment laboratory with directional airflow

(From: Yale Biosafety Manual http://ehs.yale.edu/sites/default/files/files/biosafety-manual.pdf)

CONSIDERATIONS IN A RISK ASSESSMENT

In general, a risk assessment includes considerations of the following three items for all of the following factors listed below:

a. Identification of the potential hazards in an experiment.
b. Characterization of the relative potential for the hazards to become an issue.
c. Characterization of the potential severity of the consequences if the hazard exposure did occur (to personnel, community, environment, institutional image)

3.1.1 Risk Groups: Hazardous Characteristics of a Biological Agent

The principal hazardous characteristics of an agent are:

- Its capability to infect and cause disease or pernicious response in a susceptible human or animal host, which includes the following considerations about the agent:
    - Infective dose
    - Attenuation
    - Allergenicity
    - Physiological activity
    - Oncogenicity
- Its virulence as measured by the severity of disease that results from infection, which includes the following considerations:
    - Pathogenicity
    - Attenuation
    - Genetic modifications (toxic effects, oncogenecity, allergenicity, physiological activity)
- The availability of preventive measures and effective treatments for the disease, which includes the following considerations about the agents:
    - Are there vaccinations or treatment modalities available against the agent?
    - Is this a drug resistant strain?

The World Health Organization (WHO), *NIH Guidelines*, and CDC/NIH BMBL established a risk-group (RG) classification system based on the risks that the agent, alone, presents to the health of **healthy human adults** based on an assessment of the characteristics described above. See Table 3.B. for a general description of these risk groups. The risk group of an agent should be only one factor to be considered in association with mode of transmission, procedural protocols, amount of material present, experience of staff, and other factors in determining the appropriate biosafety containment level (BSLs) which the work will be conducted.

Keep in mind that these guidelines are based on the effects on healthy human adults, and do not account for individual health considerations, such as allergies, pregnancy, breast feeding, medication effects, a compromised immune system (due to illnesses or medical treatments such as steroids or chemotherapy) or other illnesses which may make individuals more susceptible to agents. In addition, the potential for differential effects of these agents in the immature systems of minors are also not considered in these guidelines. Therefore, the guidelines represent only a starting point for a biological agent's risk assessments. Other known health considerations should also be factored in when performing a comprehensive risk assessment. Also, for this reason, for their own safety, any individual with special health concerns is strongly encouraged to discuss these with the PI, Clinical Director, or Instructional Course Director prior to initiation of work within the laboratory.

Additional guidance in determining the appropriate Risk Group for microbial agents can be found at:

- NIH Guidelines, Appendix B
- American Biological Safety Association Risk Group Database:
- CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL), particularly Section VIII:
- Public Health of Canada Pathogen Safety Data Sheets

Table 3.B. *NIH Guidelines* (2019) Definitions of Risk Groups	
Risk Group Classification	**Description of Risk of Agents**
Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

3.1.2 Routes of Exposure

In order for microbiological agents to cause disease, they must:

1. Enter or invade the body in sufficient numbers. Routes of entry include oral, respiratory, and parenteral, via mucous membrane and/or via animal contacts (bites, scratches). See the image below for additional information on routes of exposure or contact the Biosafety Office (IBCprogram@unt.edu).
   Any risk assessment process should consider the possible unique routes of entry presented during the course of the proposed experiment in addition to the risks associated with potential spread of the infectious organism outside of the laboratory should a researcher be inadvertently infected. Please note: these infectious routes of entry may differ. For instance, although a parasitic infection may normally require a specific arthropod vector to transmit the infectious agents, and the risk of having the arthropod vector within the laboratory may be extremely low, the researcher must also consider that a parasitic infection may arise in the laboratory after accidental parenteral introduction of the organism (via a needlestick, for instance).
   It is difficult to determine a **minimum infectious dose** when discussing biohazards. The same dose of a pathogen may produce no disease symptoms in one individual but may cause serious or even fatal disease in another. There are microorganisms for which it is thought one organism entering the body is sufficient to invade and promote the disease process; *Mycobacterium tuberculosis*, the bacteria that causes tuberculosis or *Coxiella burnetii*, the causative agent of Q fever are examples. For many pathogens, 10 to 100 or more organisms must enter the body to cause infection leading to disease.

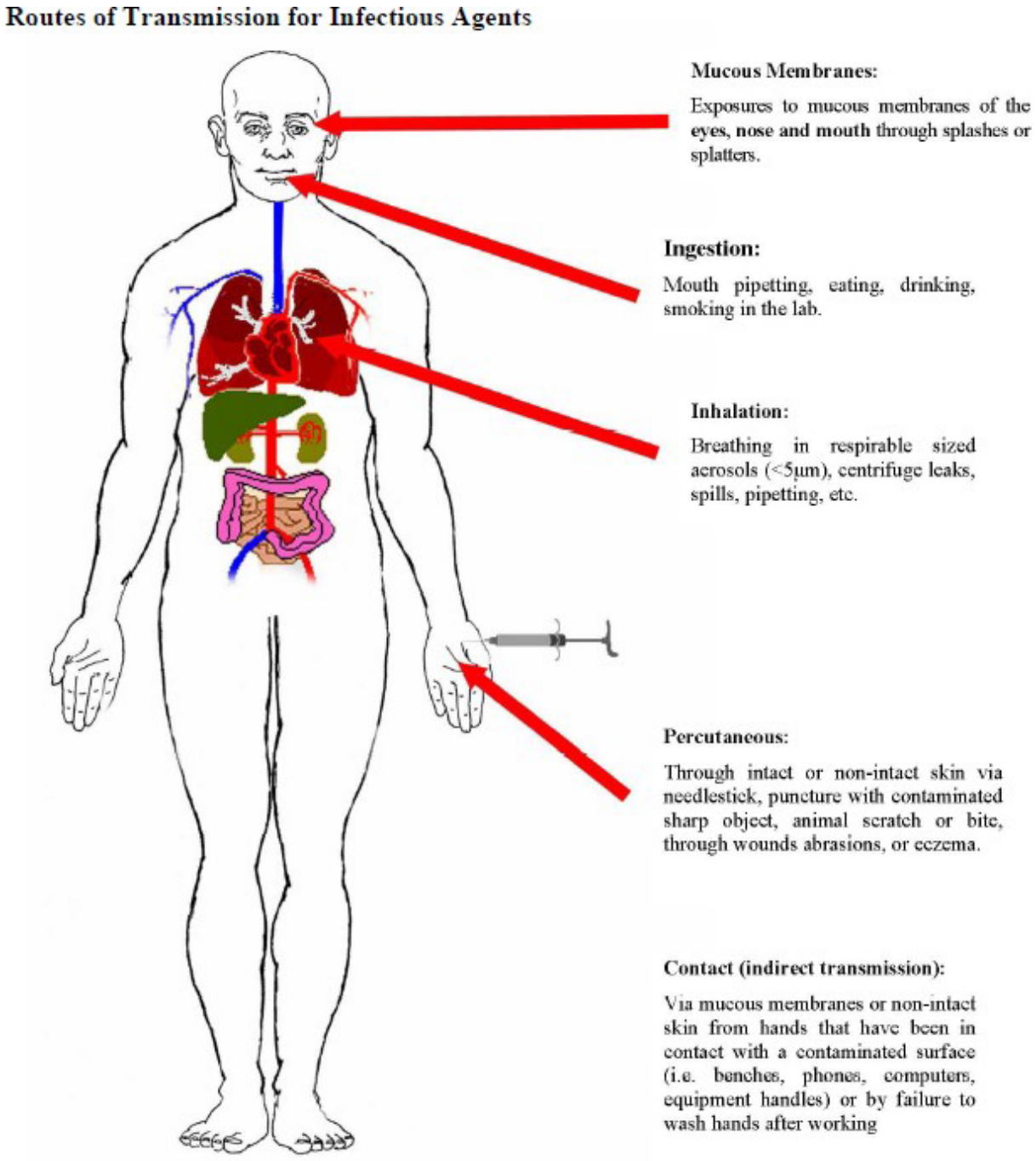
2. Once inside the body, microbial agents must colonize in a hospitable area and establish an infection in the host body cells, tissues, and/or organs

3. Lastly, the microorganisms must overcome the host body's natural defense mechanisms and/or mutate or adapt to body changes to persevere within the host organism.

Other factors contribute to an individual's susceptibility to the disease process. These include age, immunological state, occupation, physical and geographic environment, and predisposing conditions (such as alcoholism and other drug abuse, pregnancy, and diseases such as diabetes).

Therefore, all factors must be considered in a comprehensive risk assessment process. When performing risk assessments using recombinant DNA materials, all of the above factors must also be considered in addition to issues more specific to recombinant DNA, including the recombinant agents' ability to replicate once established inside the host, its ability to spread horizontally (to others or to unintended regions of the host's body), and/or the ability to transfer recombinant DNA vertically via germ cells into future generations.

Once the risks are assessed, appropriate mitigation/management measures can be developed which specifically address the risks posed by the agents and how these will be used (See Section 4, Risk Mitigation, for further information).



(From: Yale Biological Safety Manual (http://ehs.yale.edu/sites/default/files/files/biosafety-manual.pdf))

Figure 3.A. Routes of Transmission for Infectious Agents

3.1.3 Operations which may be associated with additional risk of exposure

The following laboratory operations are associated with additional risk for aerosol, splash and/or spray production. Therefore, additional risk considerations must be factored in during the risk assessment, and these activities must be documented on all Biosafety Protocols for the IBC to review.

• Blowing out pipettes
• Cell sorters (FACS)
• Shaking , vortexing or stirring tubes
• Opening lyophilized cultures
• Opening snap top tubes
• Breakage of culture containers
• Flaming loops or slides
• Pulling needles out of septums
• Filling a syringe
• Placing liquids under pressure
• Pouring liquids
• Centrifugation
• Sonication
• Homogenizing
• Blending
• Grinding
• Cell disruption with French press
• Intranasal inoculation of animals
• Cage cleaning, changing animal bedding
• Harvesting infected material from animals, eggs, and other virology procedures
• Necropsies of infected animal

3.1.4 Animal Research Risks

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.**

3.1.4.1 *Bites and Scratches*

The risk of animal bites and scratches are associated with handling of animals and is best avoided by proper handling techniques and wearing appropriate 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 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 or scratch (or puncture from sharps exposure) is to immediately and thoroughly wash the injury with soap and water. Inform a supervisor and EH&S and BSO and record the injury in the bite and scratch log located in the animal facility. Medical consultation and treatment should be obtained.

3.1.4.2 *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.

Additional risks associated with working with animals may include those related simply with the logistics for working in animal facilities. Heavy and large equipment is often used in these facilities, such as cage racks or changing stations, and the metal caging can often become bent in the day-to-day activities in the animal laboratory. These additional risks, including risks for crush injuries or lifting hazards must also be considered by the Facility Director.

3.1.4.3 *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.

3.1.4.4 *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.

3.1.4.5 *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.

3.1.4.6 *Zoonoses*

Use of animals and animal tissues may pose additional inherent risks due to the somewhat uncontrollable nature of animals as well as the potential risk of exposure to zoonotic diseases, which are diseases which are communicable from animals to humans and in many cases, *visa versa,* under natural conditions. Some animals are natural carriers of infections which may be associated with mild symptoms in animals, and therefore difficult to diagnose; however, some of these diseases can be quite serious or lethal in humans. Several of these illnesses may pose a particular hazard to pregnant women or their developing fetuses (Q-fever, toxoplasmosis); therefore pregnant workers should consider discussing their special risks and protective measures with a physician. Familiarization of the potential symptoms of the zoonotic diseases which may be carried by the research animals used in the laboratory should be part of the laboratory-specific training/education measures provided to all personnel working with the materials and should be communicated to any health care provider, should any exposures or symptoms present. Protective measures should always be addressed in each laboratory's standard operating procedures (SOPs) to mitigate the risk of these diseases. A recommended initial reference for information on zoonotic diseases and their associated pathogens is the document "Animal-Related Health Risks" at http://www.netcegroups.com/1048/Course_94921.pdf.

Please note, gloves are required PPE when working with any animals at UNT.

3.1.4.7 *Birds*

Birds carry diseases such as psittacosis and an avian form of tuberculosis. Only inspected, properly quarantined birds should be used in research studies or teaching demonstrations. Mycological fecal contamination is also frequent. The causative agents for some common avian transmitted diseases are described below and the following links describe some of the potential illnesses associated with birds. Contact the Biosafety Office for further information. A good resource is Stanford University's EHS "Working Safely with Birds".

Chlamydia psittaci: 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). However, a study has shown that more than 41% of U.S. raptors may be infected with this bacterium (Fowler et al 1990).

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.

Transmission to humans occurs by exposure via the inhalation route for the fungal infections (*Histoplasma*, *Cryptococcus*) due to inhaling spores. Contact with tissues through cuts or scratches may also pose a risk. Another route of exposure may be surface contact while handling avian fecal specimens.

Those at risk include investigators, animal caretakers, laboratory personnel, or others who routinely handle birds, their tissues, and feces. Scratches or cuts involving birds or injuries from objects contaminated with body fluids or feces from birds require immediate first aid and medical attention.

The following links describe some of the potential illnesses associated with dogs and may be found online:

- *Chlamydia psittaci* Canadian PSDS
- *Newcastle Disease* USDA/APHIS SOP
- *Histoplasma capsulatum*: Canadian PSDS
- Influenza (Canadian PSDS A, Type A, Type B and C and http://www.cdc.gov/flu/about/disease.htm)
- *Cryptococcus neoformans*: Canadian PSDS
- *Mycobacterium avium*: Canadian PSDS
- *Campylobacter jejuni:* Canadian PSDS
- *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. Canadian PSDS

Gloves, masks, and a laboratory coat (or other dedicated protective clothing such as a scrub suit) should be worn when working with birds, including field work. In some cases, protective eye wear is also indicated. Do not eat, drink, or apply cosmetics while working in an aviary, and always wash your hands after handling birds. Remember that unfixed tissues, body fluids, and other materials derived from birds may also pose a risk. Guano (feces), hair, and feathers may also exacerbate allergies. **Gloves must always be worn when handling laboratory animals or animals in the field.**

3.1.4.8 *Rodents (mice, rats, hamsters, gerbils, guinea pigs)*

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. Note: those working with rodents should also be aware of possible allergic reactions. These allergic reactions are often associated with cage cleaning due to the dust hazards of bedding and surface contact with rodent urine proteins.

Wild rodents or laboratory rodents that have been exposed to wild rodents pose additional concerns. Wild-caught animals may act as carriers for such viruses as hantavirus and lymphocytic choriomeningitis (LCMV) depending on where they were captured. Additionally, each rodent species may harbor their own range of bacterial diseases, such as tularemia and plague. These animals may also have biting insect vectors which can act as a potential carrier of disease (mouse to human transmission). 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.

The following links describe some of the potential zoonotic illnesses associated with wild rodents and may be found on-line:

Lymphocytic Choriomeningitis Virus (LCMV) : 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. Canadian MSDS

Hantaviruses (Hemorrhagic fever with renal syndrome or Korean hemorrhagic fever): 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. Canadian MSDS

Tularemia *(Francisella tularensis):* Canadian MSDS

Plague (*Yersinia pestis*): https://phc.amedd.army.mil/PHC_Resource_Library/Plague_FS-18-056-0919.pdf and the Canadian MSDS

Bites or scratches involving these rodents or injuries from objects contaminated with body fluids from rodents require immediate first aid and medical attention.

Gloves, masks and a laboratory coat (or other dedicated protective clothing such as a scrub suit) should be worn when working with rodents, as determined by risk assessment. In some cases protective eye wear is also indicated. Do not eat, drink, or apply cosmetics while working in an animal use area, and always wash your hands after handling rodents. Remember that unfixed tissues, blood, serum, urine, and other materials derived from rodents may also pose a risk. Bedding, hair, and fur may also exacerbate allergies. **Gloves must always be worn when handling laboratory animals or animals in the field.**

3.1.4.9 *Ferrets*

Commercially-raised laboratory ferrets are typically free of infections that could pose a risk to humans. Disease development from typical exposure to laboratory ferrets is not recognized as a significant public health risk. Risk of rabies is minor due to pre-arrival and on-site conditioning of ferrets. Zoonotic agents that can be transmitted by ferrets include:

- *Salmonella* spp. (Canadian PSDS)
- *Campylobacter* spp. (Canadian PSDS)
- *Cryptosporidia* (Canadian PSDS)
- *Giardia* spp. (Canadian PSDS)
- *Leptospira* (Canadian PSDS)
- Influenza (Canadian PSDS A, Type A, Type B and C and http://www.cdc.gov/flu/about/disease.htm)

Ferrets are very susceptible to influenza viruses and have served for years as an animal model in the laboratory. In ferrets, flu is characterized by sneezing, fever, lethargy, mucoserous nasal discharge, conjunctivitis, and photophobia. The course of the influenza infection usually lasts less than a week. The disease can be severe in young ferrets.
Human cases of influenza have occurred from contamination by aerosols from infected ferrets. Similarly, ferrets can be infected by humans shedding the virus.

Ferrets should not be allowed to roam freely, and their feces should be discarded in a hygienic manner. They also share parasites with dogs and cats (*Toxocara, Dipylidium*) as well as dermatophytosis (*Microsporum canis, T. mentagrophytes*).

Risk of exposure of workers to zoonotic materials may result from the following activities: changing animals from dirty (exposed to animals or their wastes) to clean cages; handling animals for injections, surgery, etc.; handling dirty

(exposed to animals or their wastes) animal room supplies; interacting with people who have entered animal areas and have not changed clothes or showered; and eating and touching the face with contaminated hands (exposed to animals or their wastes). There is a moderate risk of injury from ferrets bites or scratches. Bites or scratches or other potential exposures or injuries involving ferrets or objects contaminated with body fluids from ferrets require immediate first aid and medical attention

Gloves, masks and a laboratory coat (or other dedicated protective clothing such as a scrub suit) should be worn when working with ferrets, as determined by risk assessment. In some cases protective eye wear is also indicated. Do not eat, drink, or apply cosmetics while working in an animal use area, and always wash your hands after handling ferrets. Remember that unfixed tissues, blood, serum, urine, and other materials derived from ferrets may also pose a risk. Bedding, hair, and fur may also exacerbate allergies. **Gloves must always be worn when handling laboratory animals or animals in the field.**

3.1.4.10 *Fish, Amphibians, and Reptiles*

Fish, amphibians, and reptiles 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. **Gloves must always be worn when handling laboratory animals or animals in the field.**

Zoonotic agents that can be transmitted by amphibians and reptiles also include:

- *Salmonella* spp. (Canadian PSDS)
- *Aeromonas*
- *Pseudomonas*
- *Klebsiella*
- *Mycobacteria*

3.1.4.11 *Pigs/Swine*

Swine harbor a range of parasites and diseases that can be transmitted to humans. When working with pigs/swine or performing field work where pigs/swine are common, it is important to note the parasites and diseases they may harbor, including:

- Trichinosis (Canadian PSDS)
- Cysticercosis (https://www.cdc.gov/parasites/cysticercosis/index.html)
- Brucellosis (Canadian PSDS)
- *Salmonella* spp. (Canadian PSDS)
- Pathogenic *E. coli* (Canadian PSDS *enterohemorrhagic*, *enteropathogenic*, *enterotoxigenic*).
- Influenza (Canadian PSDS A, Type A, Type B and C and http://www.cdc.gov/flu/about/disease.htm)

Pigs are also known to host large concentrations of parasitic ascarid worms (Canadian PSDS) in their digestive tract.

Pigs can be susceptible to pneumonia, usually caused by weather. Pigs have small lungs in relation to body size; for this reason, bronchitis or pneumonia can kill a pig quickly.

Pigs can be aggressive and pig-induced injuries are relatively common in areas where pigs are reared or where they form part of the wild or feral fauna. Their relatively large size and weight also pose a physical hazard for animal care givers and researchers.

Gloves, masks and a laboratory coat (or other dedicated protective clothing such as a scrub suit) should be worn when working with swine. In some cases, protective eye wear is also indicated. Do not eat, drink, or apply cosmetics while working in an animal use area, and always wash your hands after working with animals. Remember that unfixed tissues, blood, serum, urine and other materials derived from swine may also pose a risk. Bedding, hay, dust and hair may also exacerbate allergies. Bites or scratches involving swine or injuries from objects contaminated with body fluids from swine require immediate first aid and medical attention. **Gloves must always be worn when handling laboratory animals or animals in the field.**

3.1.4.12 *Large Hooved Mammals (Cows, Horses, Sheep, Goats)*

The size of hooved mammals pose additional concerns for researchers, due to the physical hazards of weight and strength of the animal. Hooved mammals may resist handling and may require multiple workers to administer medication or other functions.

With regard to pathogens, sheep are known to shed a rickettsia, *Coxiella burnetii*, that is the causative agent for Q-Fever. Ruminants and pigs may harbor their own range of bacterial pathogens and parasites, such as *Salmonella*, *Campylobacter* and *Cryptosporidium*. Skin conditions, such as Erysipelas and Orf may result after contact with pigs and sheep and goats, respectively. In addition, these animals may carry biting insect vectors who can act as a potential carrier of disease.

The following links describe some of the potential illnesses associated with hooved mammals, farm animals, and may be found on-line:

- *Coxiella burnetii*/ Q-fever (Canadian PSDS and US APHC Fact Sheet)
- Salmonellosis (Canadian PSDS)
- Orf ("Sore Mouth" infection) https://www.cdc.gov/poxvirus/orf-virus/
- *Pasteurella* Pneumonia (Canadian PSDS)
- *Campylobacter jejuni* (Canadian PSDS)
- *Brucella spp.*/Brucelosis (Canadian PSDS)
- Anthrax (*Bacillus anthracis*) (TX Dept of Health)

Bites or scratches involving these species or injuries from objects contaminated with body fluids from hooved mammals require immediate first aid and medical attention.

Gloves, masks and a laboratory coat (or other dedicated protective clothing such as a scrub suit) should be worn when working with hooved mammals. In some cases, protective eye wear is also indicated. Do not eat, drink, or apply cosmetics while working in an animal use area, and always wash your hands after working with hooved mammals. Remember that serum, urine, and other materials derived from hooved mammals may also pose a risk. Bedding, hay, dust, and hair may also exacerbate allergies. Gloves must always be worn when handling laboratory animals or animals in the field.

High quantities of infectious material pose a greater risk of spread than small quantities. Large volumes of cultures

require additional considerations of the risk of spills, splashes, and laboratory logistics. These considerations should be factored into a comprehensive risk assessment. Cultures of recombinant materials in volumes exceeding 10 liters have specific biosafety standards which should be followed, as described in the *NIH Guidelines*.

3.1.4.13 *Arthropod Vectors*

Natural cycles of infection involve may involve transmission from mosquitoes, ticks, midges, or sandflies. In the laboratory setting, transmission can occur via parenteral inoculation, aerosol exposure, contamination of unprotected broken skin, and possibly animal bites. Exposure can occur through research directly with the arthropod vector or through the animal host.

The arboviruses (arthropod-borne viruses) are taxonomically diverse, each involving its own web of mammalian or avian hosts (or both) and specific arthropod vectors (Benenson 1995b; Tsai 1991). The presence of arboviral infection among laboratory animals generally would be restricted to situations where these agents are the focus of experimental study, wild-caught animals are brought into the laboratory for study, or nontraditional laboratory animals are housed outdoors, permitting the perpetuation of the natural cycle of arboviral infection.

Q fever is caused by the rickettsial agent *Coxiella burnetii. C. burnetii* has a worldwide distribution perpetuated in two intersecting cycles of infection—in domestic animals and in wildlife animals and their associated ticks. Infection is widespread within the domestic-animal cycle, which includes sheep, goats, and cattle. Cats, dogs, and domestic fowl also can be infected (Fox et al 1984). An outbreak of Q fever with one death in a human cohort exposed to a parturient cat and her litter and cases of the disease associated with exposure to rabbits indicate that other species should not be overlooked as possible sources of the infection in the laboratory environment (Langley et al 1988; Marrie et al 1990).

Dogs, rodents, and their ticks and fleas are the reservoirs for *Rickettsia rickettsia. R. akari, R. prowazekii*, and *R. typhi* are found in wild rodents and their associated fleas and mites (Fox et al 1984). *Ehrlichia canis* produces natural infection only in dogs; human infections result from the bites of infected ticks. These rickettsial infections are considered rare in the United States.

- Arboviruses (CDC Division of Vector-Borne Diseases, Arbovirus Catalog
- Q Fever (*Coxiella burnetii*) (Canadian PSDS)
- *Rickettsia* spp. (Canadian PSDS *R. akari*, *R. prowazekii*, and *R. rickettsii*)
- Zika (TX HHS, CDC)
- Dengue (Canadian PSDS)

Experience of Staff

UNT personnel receive basic biosafety training during the initial training class and annual refresher training modules via CITI program. However, specialized training in laboratory-specific operations is the responsibility of the PI.

If a laboratory is utilizing technology which no one, including the PI, has previous experience in (*e.g.* recombinant viral vector systems or use of new equipment, such as a French Press), additional training should be sought by the PI before embarking on these experiments. Often the Biosafety Office can be of assistance in providing additional training, or identifying appropriate trainers for the laboratory. Contact the Biosafety Office (IBCprogram@unt.edu) or Biosafety Specialist for assistance.

Mode of Transmission

Infectious diseases may spread between people via different modes. Some, like the diseases associated with the most common bloodborne pathogens (HIV, Hepatitis B & C) require contact with blood via open wounds, needlestick, or sexual transmission. This makes these diseases inherently more difficult to spread than those transmitted via an airborne route of transmission, such as influenza. The extent of risk to the public health and environment may factor in to the natural modes of transmission of diseases which may result from inadvertent infection of individuals.

Environmental Stability

Because of their resistance to common disinfectant measures and their heat stability, biological agents such as endospore-forming species of bacteria (*e.g.,* strains of *Bacillus*, *Clostridium*), and cyst-forming protozoan parasites pose an additional risk than agents which are more easily decontaminated. See Section 7 for further information on decontamination.

Institutional Public Image

Some biological agents may present additional considerations based on the perceived risk that others may assess who are outside of the laboratory or the university. A good example of this might be a laboratory who wishes to work with the avirulent Sterne strain of *Bacillus anthracis*. Although this strain is commonly used as a vaccination in animals, and may be available through veterinary suppliers, and poses very little risk of infection in healthy adults, if someone became aware that a laboratory works with anthrax, a higher level of risk may be perceived than actually exists. Anticipation and addressing these sorts of concerns prior to use of these agents may be necessary in order to maintain the institution's public image.

Section 3 References

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4 RISK MITIGATION: BIOSAFETY LEVELS

Management of the risks associated with research involving biological materials is accomplished via a combination of Practices, Safety Equipment, and Facilities. Biosafety Levels (BSLs) refer to the level of containment which is required and appropriate to contain the risk as assessed as described in Section 3, Risk Assessment. The CDC, WHO, and NIH have established standards for four biosafety containment levels for work with all biohazardous materials in the BMBL. These standards are reflected (although not always identical) to the four BSLs described for work with recombinant DNA materials in the *NIH Guidelines*. Both publications provide general descriptions of the combination of microbiological practices, laboratory facilities, and safety equipment as well as their recommended uses in order to contain biological agents which may be infectious to humans or impact the environment.

Because working with hazardous materials in animals imposes additional risks to the laboratory worker or the environment and laboratory logistics, there are also standards for four biocontainment levels for research with biohazardous materials and animals or ABSLs (Section V of BMBL for all biohazardous material). Similarly, the American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene has also developed arthropod containment levels (ACL1-4) for research with arthropods. *NIH Guidelines* also has developed specific biosafety containment levels for large scale cultures (>10 liters) and for work with recombinant plant materials.

These Biosafety containment level standards are intended as a guideline for management of the risks as determined during the risk assessment. However, this is not a prescriptive, formulaic process. Mitigation and management methods should always provide prudent measures to contain the materials and protect exposure of the users to the materials based on the risks assessed. Therefore, since the risks in each laboratory are unique to the laboratory, the Standard Operating Procedures (SOPs) should be specific for the laboratory, agents, operations, and practices within the laboratory. Protective measures to block the routes of transmission, based on the operations involved and locations must be reviewed (See Figure 4 below for suggestions of appropriate mitigation methods to prevent exposure via the common routes of transmission within the laboratory). Keep in mind that on occasion, a risk may be present which may require an additional or alternative protective measure (*e.g.,* development of a special practice or procedure, use of special equipment, or restriction to a facility) to protect personnel and the environment from the biological agents proposed for use. These may go beyond those explicitly stated in the BMBL or *NIH Guideline* standards; however, it is the IBC's responsibility (as per *NIH Guidelines*) to ensure the assessed risks involved in a proposed experiment have been reduced to an acceptable level based on the mandated comprehensive risk assessment. Therefore, the IBC may stipulate special containment measures as a condition of receiving approval.

Below is a summary of practices, equipment and facility requirements for agents assigned to basic biosafety levels BSL-1–4 (BL 1–4) (Table 4.A.). Additional information on biosafety levels may be found in the BMBL or *NIH Guidelines*.

Only work at biosafety levels 1-2 is currently permitted at UNT. Most laboratories at the University of North Texas

work under BSL-1 containment levels. Currently, no Biosafety level 3 or 4 work is allowed at UNT.

Table 4.A. Summary of Recommended Biosafety Levels for Infectious Agents				
Biosafety Level	**Agents**	**Practices**	**Safety Equipment (Primary Barriers)**	**Facilities (Secondary Barriers)**
1	**RG-1**; Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment	• Standard Microbiological Practices. • Lab-Specific safety manual required • Animals and plants not associated with the work being performed are not permitted in laboratory • Spill decon/procedure is developed and posted within the laboratory	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed, PPE: recommended laboratory coats; gloves; face protection as needed.	• Door required on lab • Handwash sink must be available • Eyewash available • Easily decontaminated, no carpets/rugs
2	**RG-2**; Associated with human disease, and pose moderate hazards to personnel and the environment; hazards are autoinoculation, ingestion, mucous membrane exposure.	BSL-1 practice plus: • Limited access to lab • Biohazard warning signs posted • Sharps precautions • Lab-specific biosafety manual defining any needed waste & surface decontamination or medical surveillance policies.	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE required: laboratory coats; gloves; face protection as needed.	BSL-1 plus: • Autoclave or other method for decontamination available. • Self closing, locking doors • Easily decontaminated, no carpets/rugs • Sink near exit
3*	**RG-3**; Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	BSL-2 practice plus: • Access limited to those with need to enter; • Viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; • All procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; PPE: Solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	BSL-2 plus: • Physical separation from access corridors; access through two consecutive self-closing doors; • hands-free sink near exit; • windows are sealed; • ducted air ventilation system with negative airflow into laboratory, w/visual verification; • autoclave available, preferably in laboratory
4*	**RG-4**; Dangerous or exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission.	BSL-3 practices plus: • Clothing change before entering; • Shower on exit • All material decontaminated on exit from facility.	All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus: • Separate building or isolated zone; • Dedicated supply/exhaust, vacuum, and decon systems; • Double-redundancy of facility systems • Other requirements outlined in BMBL.

Adapted from the Centers for Disease Control and Prevention BMBL & Yale University

*Note: No biosafety level 3 oFrer 4 work is currently permitted at UNT.

Table 4.B. Protection for the Routes of Transmission	
Route of Transmission	**Protection**
Mucous Membranes: Through mucous membranes or the eyes nose or mouth (splash, splatter).	**Achieve Face Protection**: • Wearing full-face shield or safety glasses and surgical mask • Working in a Biosafety cabinet or behind protective shields • Following good microbiological practices.
Ingestion: Mouth pipetting, eating, drinking, smoking in the lab.	**Prevent exposure via ingestion by**: • Not eating, drinking, chewing gum, smoking in lab • Mechanical pipettors • No storage of food items or utensils in lab • Following good microbiological practices
Inhalation: Breathing in respirable sized aerosols (<5µm), centrifuge leaks, spills, & aerosol-generating procedures such as pipetting, homogenizing, etc.	**Avoid exposure to aerosols by:** • Working in a Biosafety Cabinet • Using sealed rotors or safety caps for centrifuge buckets • Safety containment equipment • HEPA filtered respirator • Following good microbiological practices.
Percutaneous: Through intact or non-intact skin via needlestick, puncture with a contaminated sharp object, animal scratch, bite, through wounds, abrasions, or open sores.	**Avoid percutaneous exposures by:** • Using extreme precautions with sharps • Using needleless, retractable or safety sharp systems • Disposing sharps immediately in rigid leakproof sharps container placed near site of use • Using animal restraints • Using cut/bite resistant gloves • Using sleeve covers, water proof bandages over any open wounds • Using double gloves • Using good work practices
Contact (indirect transmission): Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment handles) or by failure to wash hands after working.	**Prevent indirect exposure by:** • Decontamination of work surfaces • Maintaining good personal hygiene (avoid touching your face or other unprotected skin with glove or non-gloved hands) • Avoid touching non-contaminated areas (e.g. phones) with contaminated hands. • Not applying cosmetics within the laboratory. • Removing gloves and wash hands before exiting laboratory • Frequent glove changing

(From Yale Biological Safety Manual http://ehs.yale.edu/sites/default/files/files/biosafety-manual.pdf)

LABORATORY PRACTICES

In this section, an attempt has been made to provide information regarding hazards involved with certain laboratory practices and methods for preventing them. Prevention is an important element to biohazard control. It is the responsibility of the PI, Clinical Director, and/or Instructional Course Designer or their designees to establish their laboratory-specific SOPs to address the risks within each laboratory. The PI of each laboratory is responsible for providing or arranging the appropriate training of personnel and for verifying each person's competence. This must be documented as part of the Biosafety Protocol (BSP) Registration process and these documents should provide a basis to begin laboratory-specific safety training for all laboratory personnel prior to initiation of work.

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. In addition, each PI 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.

Each PI in a biological laboratory must develop and maintain a laboratory-specific Biosafety Laboratory Manual for their lab as required by the BMBL and *NIH Guidelines*. This Biosafety Laboratory Manual should: 1) contain safety information relevant to the lab's specific hazards and research materials, 2) serve as a training tool for personnel and include documentation of such training, 3) be readily available to all research personnel in the lab and 4) be modified as needed to contain current laboratory SOPs and practices.

At a minimum, the Biosafety Laboratory Manual should include:

1. Lab contact information (PI name, phone number, including after-hours numbers);
2. A list of agents used and agent summary statement(s) or PSDS for each agent used in the lab; http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php
3. Reference sheets for biosafety guidelines/policies (NIH Guidelines/BMBL)
4. Project specific/Procedural SOPs;
5. Safety SOPs
6. Laboratory specific biosafety policies, including:
    - PPE required for working in the lab;
    - Infectious waste disposal procedures for:
        - Liquids
        - Sharps
        - Solids

- Research animals (if applicable);
- Biohazardous spill clean-up procedures;
    - Procedures for dealing with accidents;
    - Vaccination offer documentation for all lab users (e.g. HBV), if appropriate
    - Post exposure plans, if appropriate
    - Lab-specific exposure control plan(s), if applicable

7. Copies of: current IBC and approval letter; recent laboratory inspection report(s); and Approved personnel memo, and any amendments or annual updates.;
8. Training records for PI and staff (include topic and date of attendance)-both RMS trainings and lab-specific trainings should be included.

4.1.1 Human Factors and Attitudes in Relation to Laboratory Accidents

For the purpose of safety, an attitude can be defined as an accumulation of information and experience that predisposes an individual to certain behavior. Human factors and attitudes result in tendencies on the part of the individual to react in a positive or negative fashion to a situation, a person or an objective. Laboratory supervisors and PIs should understand the importance of attitudes and human factors in their own efforts to control biohazards in their laboratory. Some observations that may be of help to supervisors are listed below:

- The lack of accident perception ability is often a significant factor in laboratory accidents.
- Inflexibility of work habits, that tend to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.

- Working at an abnormal rate of speed is a significant causal factor.
- Intentional violations of regulations are a frequent cause of accidents. This is termed excessive risk taking.
- The performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
- Working when one is very tired is more likely to create a higher potential for accidents.
- Working at a well-organized and uncrowded laboratory bench will help in the prevention of lab accidents.
- Working alone or without supervision in a laboratory with higher hazard agents should be discouraged.

Each employee working with biohazardous agents must be constantly aware of the importance of the proper attitude in preventing accidents in the laboratory.

4.1.1.1 *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‡ in a laboratory where hazardous conditions exist should:

1. Obtain written permission (e.g., e-mail, letter) from the PI or Laboratory Supervisor to work alone in the laboratory;
2. Ensure that a means to contact emergency response personnel is available when working alone in the laboratory; and
3. 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.

4.1.2 Practices Associated with Biological Laboratories

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. 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. Examples of agents worked with at BSL-1 include: *E. coli*, murine cell lines and samples from lab mammals.
- **BSL-2** is required for work involving agents that pose moderate hazards to personnel and the environment. Examples of agents worked with at BSL-2 include: Human or non-human primate samples and cell lines, *Aspergillus fumigatus*, *Toxoplasma gondii*, *Salmonella typhimurium* and Influenza A.
- **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. Examples of agents worked with at BSL-3 include: *Coccidioides immitis* and *posadasii*, *Mycobacterium tuberculosis*, Chikungunya Virus and West Nile Virus. **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.

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

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

4.1.2.1 *Biosafety Level 1 (BSL-1/BL-1)*

As defined in:

- Section IV of the CDC BMBL:
- Appendix G-II-A for recombinant DNA BL-1:

  - Keep laboratory door closed when experiments are in progress. There should be floor-to ceiling physical separation from "laboratory areas" and "non-laboratory areas". Therefore, any doors leading into non-laboratory areas, such as offices or hallways must be kept shut when experiments are in progress.
  - A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible to all personnel within the laboratory.
  - Use procedures that minimize aerosols. For example, pipette gently along the sides of tubes to prevent aerosol production. Open vacuum pressured containers/vials carefully using protective covers to avoid exposures to any aerosols that may arise from the back-pressure that may result.

- 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.
- Wear appropriate PPE:
  - 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.
  - Laboratory gowns or coats should be worn over street clothes.
  - Protective eyewear should be worn 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 should be changed when contaminated, their integrity have been compromised and/or when otherwise necessary. Periodic glove-changing is recommended. Be aware that some petroleum-based hand moisturizers can impact the integrity of latex gloves and should be avoided.
- Gloves should be removed and hands washed after completing experimental procedures and before leaving the laboratory. Hand washing protocols must be rigorously followed. Soap should be used and hands washed for approximately 20 seconds prior to rinsing with warm water.
- Dispose of used gloves with other contaminated laboratory waste after removal. Do not wash, save and/or reuse disposable gloves.
- Do not mouth pipette. Use mechanical pipetting devices.
- Avoid using hypodermic needles, scalpels, blades, glass or other sharp items whenever possible. Consider alternatives or devices with safety features, for instance retractable blades, or substitute plastic for glass whenever feasible.
- Disinfect work surfaces daily and immediately after a spill with an appropriate disinfectant (as documented in the laboratory SOPs). Use of taped-down benchkote paper is discouraged, since this is often not changed daily. **A spill procedure is developed and posted within the laboratory.**
- Decontaminate all biological wastes before discarding. Decontaminate other contaminated materials before washing, reusing, or discarding.
- For off-site decontamination, package contaminated materials in closed, durable, leakproof containers before leaving the laboratory. See Section 4.1.8 for more information about appropriate transport procedures.
- Ensure that all containers which contain biological waste are labeled with the biohazard symbol. This includes intermediary beakers or bins used on bench-tops to contain pipette tips or small microcentrifuge tubes which may have been used with biological materials.
- Control insect and rodent infestations.
- Keep areas neat and clean.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures, and that appropriate records are maintained.
- Laboratory personnel must receive annual updates or additional training when equipment, procedural, or policy changes occur. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safety safeguards, and read and follow instruction on the practices and procedures.
- 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 their PI and healthcare provider for appropriate counseling and guidance.
- Animals and plants not associated with the work being performed are not permitted in the laboratory.

4.1.2.2 *Biosafety Level 2 (BSL-2/BL-2)*

As defined in:

- Section IV of the CDC BMBL:
- Appendix G-II-B for recombinant DNA BL-2:

Biosafety Level 2 containment practices are the same as those listed for Biosafety Level 1, with the following additions:

- Keep laboratory door closed and facilities must be locked after work hours and when unoccupied. There should be floor-to-ceiling physical separation from "laboratory areas" and "non-laboratory areas". Therefore, any doors leading into non-laboratory areas, such as offices or hallways must be kept shut.
- Only persons who have been informed of the research and its risks should be permitted to enter BSL-2 areas.
- Wear appropriate PPE (as documented in the laboratory SOPs):
  - Open wounds should be covered with occlusive bandages.
  - 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. Double-gloving may be necessary for some procedures.
  - Laboratory gowns or coats should be worn within the laboratory and must not removed from the laboratory. These should either be disposable or laundered by the institution— not taken home.
  - Protective eyewear should be worn 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.
  - Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.
- Use biological safety cabinets or similar containment equipment to contain aerosol-producing equipment.
- Maintain a biological spill kit within the laboratory.
- Vacuum aspirator lines need to be properly maintained to prevent overflow. It is recommended that vacuum traps never be allowed to collect liquids past half-full to enable full decontamination prior to disposal. Vacuum traps outside of biosafety cabinets should be placed in secondary containment sufficient in size to contain the contents of the trap, should the trap implode under vacuum pressure. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed.
- Laboratory equipment should be routinely decontaminated by staff properly trained and equipped to work with the material, after spills, splashes, or other potential contamination. Equipment must be fully decontaminated before repair, maintenance, or removal from the laboratory.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Report spills, accidents, potential exposures, near misses and disease symptoms related to laboratory acquired infection to the PI and the Biosafety Office. Additional reporting requirements may be required to comply with Human Resources and/or other governing bodies (See Section 6 for further information).
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory as determined by risk assessment. Some agents may require the collection and storage of serum samples from at-risk personnel prior to initiation of work within the laboratory. These measures must be documented in the laboratory Biosafety Manual and SOPs.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

4.1.3 Practices Associated with Activities Involving Animals

Laboratory animal facilities are a special type of laboratory. Although many of the practices followed under ABSL (or BL-N) containment levels are often comparable to those used *in vitro*, the animal room can present unique problems since the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. Necropsies of infected animals also present a risk of exposure to personnel. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol driven risk assessment in order to appropriately contain the risks of the agents and animals used in an experiment. The IBC works in close conjunction with the IACUC in order to ensure that the safety of human researchers and caretakers, the health of the animal colonies, and humane treatment of research animals are ensured. These considerations not only impact the safety, but the integrity and ethics of the science occurring in the facilities. Considerations are given to the proper handling of animals which may have been experimentally infected as part of a research protocol, those which may naturally harbor zoonotic infectious agents, or those which may inadvertently have been infected with animal pathogens which may pose hazards for colony maintenance and integrity. Animal Biosafety Levels as defined in the CDC BMBL (Section V) and the *NIH Guidelines* (Appendix M) presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations as described in:

- Institute of Laboratory Animal Research (ILAR), Commission on Life Sciences, National Research Council's *Guide for the Care and Use of Laboratory Animals and*
- U.S. Federal Laboratory Animal Welfare Regulations (*9 C.F.R.).*

Training videos are available through the American Biosafety Association (ABSA) web site to provide guidance to animal handlers at various Animal Biosafety Levels http://www.absa.org. In addition to the animal biosafety levels described in the CDC BMBL and *NIH Guidelines*, the USDA has developed facility parameters and work practices for handling agents of agriculture significance. These are described in Appendix D in the CDC BMBL. USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. The importation, possession or use of the following agents are either prohibited or restricted by law or by USDA regulations or administrative policies. These agents of agricultural significance include:

- African horse sickness virus
- Louping ill virus
- African swine fever virus
- Lumpy skin disease virus
- Akabane virus
- Malignant catarrhal fever virus (exotic strains or alcelaphine herpesvirus type 1)
- Avian influenza virus (highly pathogenic)
- Menangle virus
- *Bacillus anthracis*
- *Mycobacterium bovis*
- *Besnoitia besnoiti*
- *Mycoplasma agalactiae*
- Bluetongue virus (exotic)
- *Mycoplasma mycoides* subsp. mycoides (small colony type)
- Borna disease virus
- *Mycoplasma capricolum*
- Bovine infectious petechial fever agent
- Nairobi sheep disease virus (Ganjam virus)
- Bovine spongiform encephalopathy prion
- Newcastle disease virus (velogenic strains)
- *Brucella abortus*
- Nipah virus
- *Brucella melitensis*
- Peste des petits ruminants virus
- (plague of small ruminants)
- *Brucella suis*
- Rift Valley fever virus
- *Burkholderia mallei/Pseudomonas mallei* (Glanders)
- Rinderpest virus
- *Burkholderia pseudomallei*
- Sheep pox virus

- Camelpox virus
- Spring Viremia of Carp virus
- Classical swine fever virus
- Swine vesicular disease virus
- *Coccidioides immitis*
- Teschen disease virus
- Cochliomyia hominivorax (Screwworm)
- *Theileria annulata*
- *Coxiella burnetti* (Q fever)
- *Theileria lawrencei*
- Ephemeral fever virus
- *Theileria bovis*
- *Ehrlichia* (Cowdria) *ruminantium* (heartwater)
- *Theileria hirci*
- Eastern equine encephalitis virus
- *Trypanosoma brucei*
- Foot and mouth disease virus
- *Trypanosoma congolense*
- *Francisella tularensis*
- *Trypanosoma equiperdum* (dourine)
- Goat pox
- *Trypanosoma evansi*
- Hemorrhagic disease of rabbits virus
- *Trypanosoma vivax*
- Hendra virus d
- Venezuelan equine encephalomyelitis virus
- *Histoplasma* (Zymonema) *farciminosum*
- Vesicular exanthema virus
- Infectious salmon anemia virus
- Vesicular stomatitis virus (exotic)
- Japanese encephalitis virus
- Wesselsbron disease virus

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. Contact IBCprogram@unt.edu for additional information.

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 4.D. Animal Biological Safety - Personal Protective Equipment (PPE) Requirements*			
ABSL-I	ABSL-2	ABSL-3	ABSL-4
Protective laboratory coats, gowns, or uniforms recommended to prevent contamination of personal clothing. **Eye, face, and respiratory protection** should be used in rooms containing infected animals. **Protective eyewear** 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 **eye protection** when entering areas with potentially high concentrations or airborne particulates. **Gloves must be worn** to prevent skin contact with contaminated, infectious, and hazardous materials, and **when handling animals.**	**Protective laboratory coats, gowns, or uniforms** must be worn while in areas where infectious materials and/or animals are housed or manipulated. **Eye and face protection** (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 **eye protection** when entering areas with potentially high concentrations or airborne particulates. **Gloves must be worn** to prevent skin contact with contaminated, infectious and hazardous materials and **when handling animals.** **Eye, face, and respiratory protection** should be used in rooms containing infected animals.	**Not currently permitted at UNT.** Disposable personal protective equipment, 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. Eye, face, and respiratory protection 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 eye protection. Gloves must be worn to prevent skin contact with contaminated, infectious, and hazardous materials and when handling animals. Double-glove practices should be used. Boots, shoe covers, or other protective footwear, are used to prevent cross- contamination.	**Not permitted at UNT.** Please refer to the CDC/NIH BMBL for PPE requirements.

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

4.1.3.1 *Animal Biosafety Level 1 (ABSL-1, BL-1N)*

As defined in:

- Section V of the CDC BMBL and
- Appendix M-II-A for recombinant DNA BL1-N of the NIH Guidelines.

- The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations.
- Each institute must ensure that research staff and animal handler safety and health concerns are addressed as part of the animal protocol review.
- Prior to beginning a study animal protocols are reviewed and approved by the IACUC and the IBC, as appropriate.
- 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.
- 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.
    - The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    - The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
- Appropriate medical surveillance program is in place, as determined by risk assessment. An allergy prevention program is part of the medical surveillance.
- The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization).
    - Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance.
    - All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures.
    - An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
- 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 child-bearing 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 PI and their healthcare provider for appropriate counseling and guidance.
- Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
- A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated.
    - The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the 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.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. These should not be worn outside of the facility, and non-disposable PPE must be decontaminated and cleaned by the institution—not taken home to launder.

- Open wounds should be covered with occlusive bandages.
- Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
- Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.
    - Glove selection is based on an appropriate risk assessment.
    - Consider the need for bite and/or scratch-resistant gloves.
    - Gloves worn inside the animal facility are not worn outside the animal facility.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
- 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 and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
- 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:
    - Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
        - Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        - Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        - If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, or the use of forceps to hold the cap when recapping a needle).
        - 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.
    - 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. Plasticware should be substituted for glassware whenever possible.
- Equipment containing sharp edges and corners should be avoided.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. **A spill procedure is developed and posted within the animal facility**.
- Laboratory coats, gowns, or uniforms are the minimum recommended 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 animal facility.

- Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
- Facilities should be separated from the general traffic patterns of the building and restricted as appropriate.
    - 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 never propped open.
- The animal facility has a sink for handwashing.
    - Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
    - Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
    - If open floor drains are provided, the traps are filled with water and/ or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, ceilings) are water-resistant.
    - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
    - Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
    - External windows are resistant to breakage. Where possible, windows are sealed. If the animal facility has windows that open, they are fitted with fly screens.
    - Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Furniture can support anticipated loads and uses.
    - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    - Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
    - Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners
- Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.
    - Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
- Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washers have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.

4.1.3.2 *Animal Biosafety Level 2 (ABSL-2, BL-2N)*

As defined in:

- Section V of the CDC BMBL and
- Appendix M-II-B for recombinant DNA BL2-N.

In addition to the practices required by ABSL-1 containment, ABSL-2 requires that:

- Prior to beginning a study animal protocols are reviewed and approved by the IACUC and the IBC.
- Personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of pathogenic agents.
- Personnel entering the facility must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures.
- Procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, including animal necropsies, and injections of animals, should be conducted in BSCs or by use of other physical containment equipment.
- 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.
- Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc.).
- If used, actively ventilated caging systems are designed to contain microorganisms. Exhaust plenums for these systems are sealed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positively pressurized if the exhaust fan fails. The system is also alarmed to indicate operational malfunctions. Exhaust HEPA filters and filter housings are certified annually.
- Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are 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 contain a universal biohazard label.
- Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas. These should be periodically inspected to ensure that sharp edges are not present which may present a laboratory hazard.
- 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.
- 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 and the Biosafety Officer. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.
- Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.
- Protective clothing, such as gowns, uniforms, scrubs, or laboratory coats, and other PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated.
    - Scrubs and uniforms are removed before leaving the animal facility.
    - Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
    - Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
- Eye protection and face protection (e.g., safety glasses, goggles, mask, 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 when the animal or microorganisms is handled outside the BSC or another containment device. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.

- Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
- Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.
    - Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
    - Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
    - Decontamination processes are verified on a routine basis.
- ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate.
    - 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 are never to be propped open.
- A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are 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 is also available for handwashing at the exit from each segregated area.
    - Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
    - Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
    - If open floor drains are provided, the traps are filled with water and/ or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
    - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
    - Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
    - Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
    - External windows are sealed and resistant to breakage.
    - Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Furniture is minimized and can support anticipated loads and uses.
    - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    - Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
    - Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners
- Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.

    - Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
    - 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.
- BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
- An autoclave is present in the animal facility to facilitate decontamination of infectious materials and waste. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.

4.1.4 Plant Biosafety Levels

Plant biosafety levels are designated with a "P" after the containment level. These agents do not usually pose a threat to human health; however, they may pose a threat to plants and the environment. Plant pathogens can be spread by direct contact between plants, arthropods, soil borne nematodes, plant damage, and pollinators. Plants can be grown in the greenhouse, laboratory, growth chamber, and/or field. The *NIH Guidelines* define a greenhouse as a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment.

4.1.4.1 *BSL-1P*

Recommended for all experiments with transgenic plants and associated agents that have no or limited threat potential. For example: transgenic plants that are not noxious weeds or agents that have no recognized potential for rapid dissemination. Examples of agents worked with at BSL- 1P include: *Agrobacterium tumefaciens*, and *Rhizobium* spp..

Requirements at BSL-1P:

- Access to the laboratory and greenhouse shall be limited or restricted when experiments are in progress.
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.
- Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal.
- A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and federal laws.
- Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
- The greenhouse floor may be composed of gravel or other porous material. Impervious (e.g., concrete) walkways are recommended.
- Windows and other openings in the walls and roof of the laboratory and greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds). Screens are recommended.

- Laboratories and greenhouses must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids must be stored in locked containers.
- A record shall be kept* of experiments currently in progress in the greenhouse facility.

4.1.4.2 *BSL-2P*

Recommended for transgenic plants that are noxious weeds, plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent, plants associated with transgenic non-exotic microbe that has a recognized potential for serious detrimental impact on managed or natural ecosystems, or plant pathogens that have a recognized potential for serious detrimental impact on managed or natural ecosystems. Examples of agents worked with at BSL-2P include: *Meliodogyne incognita* (root-knot nematode), Pepino Mosaic Virus, and *Pseudomonas syringae*.

The following are required when working at BSL-2P:

- A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.
- A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagates of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagates of experimental organisms are readily disseminated through soil.
- Materials containing experimental microorganisms must be transferred in a closed non-breakable container.
- An autoclave must be available for the treatment of contaminated plant material including soil.
- If intake fans are used, measures shall be taken to minimize the ingress of arthropods.
- Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.
- BSL-2P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.
- Laboratories and greenhouses must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids and BSL-2P agents must be stored in locked containers. All material in the open bay or common use areas must be secured when not in use.
- A record shall be kept* of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
- A record shall be kept* of experiments currently in progress in the greenhouse facility.
- A greenhouse practices manual shall be prepared or adopted*. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.
- All incidents regarding BSL-2P agents that have a USDA/APHIS permit must be reported to the supporting regulatory agency.

*These records/documents must be available for review within the laboratory.

4.1.5 Arthropod Containment Levels

This section describes the arthropod handling practices, safety equipment, and facilities constituting Arthropod Containment Levels 1–4 (ACL-1 to -4). These are recommended by the ASTMH/ACME for work with a variety of uninfected arthropods and those carrying infectious agents, and for work with transgenic vector arthropods in laboratory settings. The principles of risk assessment, specific practices, and equipment will also be useful in nontraditional arthropod research settings such as tents, greenhouses, and outdoor cages. Readers should refer to the appropriate BMBL section for standard and special microbiological practices appropriate for the agents with which they work. These have been repeated here when they would apply to the vector alone as well as the agents. **Note**: ACL-3 and ACL-4 containment-level work is not permitted at UNT.

Table 4.E.: Summary of Arthropod Containment Levels					
Arthropod containment level:	**I**		**2**	**3**	**4**
Arthropod distribution, escaped arthropod fate	Indigenous/no change in local fauna	Exotic/inviable or transient only	Exotic with establishment potential, indigenous, or transgenic		
Infection status	Uninfected or infected with non-pathogen (up to BSL-1)		Up to BSL-2	Up to BSL-3	BSL-4
Active VBD cycling	No		Irrelevant		
Practices	ACL-1 Standard Arthropod-Handling Practices		ACL-2 and BSL-2 limited access, training, signage, containment, and disposal	ACL-3 and BSL-3 restricted access, training, appropriate PPE, signage, containment, disposal, record-keeping	ACL-4 with BSL-4 isolation, training, appropriate PPE, signage, containment, disposal, record-keeping
Primary Barriers	Species-appropriate containers		Appropriate PPE, escape-proof containers	Appropriate PPE, escape-proof containers, pesticide available for emergency use	Appropriate PPE, escape-proof containers, pesticide available for emergency use
Secondary Barriers			BSL-2 facilities, breeding sites, and harborage minimized, pest control	BSL-3 facilities, biological safety cabinets, other physical containment devices, pest control	BSL-4 and facility-specific procedures and equipment for arthropod handling while wearing positive pressure containment suit

General guidelines for best laboratory containment practices are shown for vector species of arthropod that are uninfected (above the bold line) or infected (below the bold line) according to biosafety and ACLs. Indigenous species are those species whose current range includes the research location. All others are considered exotic. For uninfected arthropods, containment guidelines take into account the consequences of accidental escape from a laboratory, in which the arthropod would be (1) inviable as a result of exposure to unfavorable conditions; (2) transient because conditions vary such that the arthropod would die during typical year climate cycle; or (3) has potential for establishment because escaped arthropods could reasonably be expected to persist through a typical climatic year. Arthropod containment specifics for each BSL should always be reviewed in the context of a laboratory-, vector-, and pathogen-specific risk assessment that is based on consultation between the investigator and the appropriate institutional oversight committee(s) and according to the constraints of the infrastructure available. (Vector-Borne and Zoonotic Diseases, Vol 19, No. 3, 2019)

4.1.5.1 *Arthropod Containment Level 1 (ACL-1)*

As described in

- Arthropod Containment Guidelines, Version 3.2 (2019)

ACL-1 is suitable for work with uninfected arthropod vectors or those infected with a non-pathogen, including (1) arthropods that are already present in the local geographic region regardless of whether there is active vector-borne disease transmission in the locale and (2) exotic arthropods that on escape would be nonviable or become only temporarily established in areas not having active vector-borne disease transmission. This category would include most educational use of arthropod vectors. A summary of the containment levels is provided in Table 4.E.

- Standard practices
    - Location of arthropods. Furniture and incubators containing arthropods are located in such a way that accidental contact and release are minimized. This may be achieved by locating arthropods out of the flow of general traffic, avoiding hallways, or placing them in closets.
    - Supply storage. The area is maintained to allow detection of escaped arthropods. For example, materials unrelated to arthropod rearing and experimentation (e.g., plants, unused containers, clutter) that provide breeding sites and harborages are minimized.
    - General arthropod elimination. Accidental sources of arthropods from within the insectary are eliminated. This may be accomplished by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. For example, personnel in mosquito laboratories should immediately eliminate any standing water.
    - Primary container cleaning and disinfestation. Practices should be in place such that arthropods do not escape by inadvertent disposal in primary containers. Cages and other culture containers are appropriately cleaned to prevent arthropod survival and escape (e.g., heated to, or chilled below, lethal temperature).
    - Primary container construction. Cages used to hold arthropods effectively prevent escape of all stages. Screened mesh, if used, is durable and of a size appropriate to prevent escape. Non-breakable cages are recommended. Bags, rearing trays, and so on effectively prevent leakage and escape.
    - Disposal of arthropods. All life stages of arthropods must be killed before disposal. Arthropods may be killed with hot water or freezing before flushing down drains or placed into trash bags.
    - Primary container identification and labeling. Arthropods are identified with descriptive labels to include the species, strain/origin, date of collection, responsible investigator, and so on; labels are firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g., eggs, pupae, hibernating adults) are likewise labeled and (if applicable) housed or stored to prevent progression to, and escape of, a mobile life stage.

- Prevention of accidental dispersal on persons or via sewer. Personnel take appropriate precautions to prevent transport or dissemination of live mobile arthropods from the insectary by practicing appropriate disposal methods and preventing escapees at every level of containment (primary container, environmental chamber, laboratory, etc) to prevent dispersal on persons.
- Escaped arthropod monitoring. Investigators assess whether escapes are occurring. An effective arthropod trapping program is recommended to monitor the escape prevention program.
- Pest exclusion program. A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.
- Source and harborage reduction. Harborage and breeding areas are reduced as appropriate. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods.
- Notification and signage. Persons entering the area are made aware of the presence of arthropod vector species by signage.

- Special practices: vertebrate animal use
  - Institutional approval. Investigators should consult with their institutional research oversight office if vertebrate animals will be used to feed hematophagous arthropods. The requirement for IACUC and/or IBC review is an institutional decision, although highly recommended.
  - Housing of vertebrate animals. Animals used as hosts or blood sources should be housed according to institutional laboratory animal guidelines. If necessary, vertebrate animals may be housed within the insectary but need to be adequately protected from access by escaped arthropods. Animals not necessary for maintaining arthropods should not be accessible to hematophagous arthropods in the laboratory setting.
  - Containment during blood feeding. Special considerations should be taken when hematophagous arthropods are fed on host animals. The primary container must be sufficiently robust to prevent escape during feeding. When handling/removing vertebrate animals after exposure to arthropods, precautions must be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g., concealment in fur, ears, axillae, or other possible hiding places). Finally, all precautions should be taken to prevent arthropods fed on host animals from accidental transfer to host cages and therefore dispersal outside of containment, if animals and their cages are returned to a holding room.
  - Blood source. The blood source should be considered a possible source of inadvertent arthropod infection and transmission. Whenever feasible, use of sterile blood or blood from sources known to be specific pathogen free is recommended, whereas use of blood from animals or humans whose disease status is uncertain should be avoided. In some instances, a vector colony is specifically adapted to and will not propagate without human blood acquired directly by feeding on a volunteer. Such arthropods should not be fed a second time on a different volunteer; those fed initially by membrane on animal or human blood should not be allowed to subsequently feed on a human volunteer.
- Safety equipment (primary barriers)
  - Gloves.* Latex or nitrile gloves should be used when handling host animals or blood used to feed the arthropods, but local risk assessment and institutional policy may provide exceptions.
  - Torso apparel. White laboratory coats, gowns, and/or uniforms should be worn at all times in the insectary when handling blood and vertebrate animals, but local risk assessment and institutional policy may provide exceptions.
  - Arthropod-specific personal protective equipment. Personal protective equipment is worn as appropriate, for example, respirators for arthropod-associated allergies, particle masks, and head covers, but local risk assessment and institutional policy may provide exceptions.
  - *UNT biosafety manual states that gloves are to be worn when handling all animals and hands are to be washed upon removal of gloves and before leaving the lab.
  - Facilities (secondary barriers)

- Location of insectary. The insectary area is separated, if possible, from areas that are used for general traffic within the building.
- Insectary doors. Door openings, whether covered by rigid panels, glass, screens, plastic sheets, or cloth, minimize escape and entrance of arthropods or pests.
- Insectary windows. Windows, if present, effectively prevent escape of the smallest arthropods contained within as well as prevent entry of wild arthropods and pests.
- Lack of an insectary. Arthropods may be maintained at ACL-1 in rooms other than those specifically designed as insectaries. If the facility does not have secondary barriers that would minimize escape or entry of pests, and is not separated from general traffic, specific operating procedures must be developed and tested to mitigate such risks. For example, mosquitoes might be held by a "cage within a larger cage"; removal of adult mosquitoes accomplished by the aspirator manipulated through cage sleeves placed perpendicular to each other and the sample container loaded entirely within the outer cage. Alternatively, entire mosquito containers may be chilled before aspirating individual mosquitoes. Plexiglas glove boxes might also be used for manipulations, particularly if exotic species are maintained. Nonflying species may be manipulated on designated tables or benches in pans within moats of water, and housed in vials or other containers held within a secondary storage container such as a lidded plastic food container.

4.1.5.2 *Arthropod Containment Level 2 (ACL-2)*

ACL-2 should be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are reasonably suspected of being infected with such agents (diagnostic samples). The PI must perform a risk assessment when deciding whether arthropods are reasonably suspected of being infected with a pathogen. For example, live mosquitoes collected during the course of a disease outbreak and maintained in the laboratory would present more of a risk to laboratory personnel than those that are cold-immobilized or killed before sorting and identifying them for standard surveillance purposes. Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no or only negative effects on viability, survivorship, host range, or vector capacity. ACL-2 builds on the practices, procedures, containment equipment, and facility requirements of ACL-1. It is more stringent in physical containment, disposal, and facility design requirements. Moreover, access is more restricted than ACL-1. The decision to propagate infected exotic arthropods under ACL-2 conditions in active transmission areas or in cases in which establishment is a possibility typically requires that measures that otherwise would only be recommended or preferred must be instituted as policy.

- Standard practices
  - Location of arthropods. Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons are unlikely. This may be achieved by locating arthropods in dedicated rooms, closets, incubators located out of the traffic flow, or similar measures. Nonflying arthropods such as ticks are typically held in primary containers (vials) that are placed within an environmentally controlled container such as a desiccator or plastic food container; often, this in turn is held within an environmental chamber. Although a dedicated space is recommended for long-term storage of ticks, appropriate risk assessment by the local IBC or other institutional entities, informed by the PI or other experts, may allow for the housing of ticks in non-insectary settings.
  - Supply storage. The area is designed and maintained to enhance detection of escaped arthropods. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary are located in a designated area and not on open shelves.
  - Primary container cleaning and disinfestation. In addition to cleaning cages and culture containers to prevent arthropod escape as in ACL-1, containers are disinfected chemically and/or autoclaved if used for infected material, according to an IBC-approved protocol and/or laboratory standard operating procedures.

- Primary container construction. Cages used to hold arthropods are shatter-proof and screened with mesh of a size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during removal and introduction of arthropods are recommended.
- Disposal of arthropods. All life stages of arthropods must be killed before disposal by freezing or other suitable methods. Infected arthropods should be autoclaved, or decontaminated with chemical disinfectants such as 10% bleach or other EPA-approved disinfectant, based on an agent-specific risk assessment. The lack of an autoclave or means of incineration should be evaluated by local risk assessment and appropriate substitutes sought.
- Isolation of uninfected arthropods. Spread of agents to uninfected arthropods is usually a low risk, given that most infections occur via hematophagy. Containers must be clearly marked to easily distinguish infected from uninfected arthropods.
- Primary container identification and labeling. As per ACL-1.
- Prevention of accidental dispersal via sewer or on persons. Before leaving the insectary and after handling cultures and infected arthropods, personnel wash their hands. Care should be taken to not disperse viable life stages into the drainage system. No infected material is disposed through the sewer unless it is decontaminated.
- Pest exclusion program. As per ACL-1.
- Escaped arthropod monitoring. Investigators assess whether escapes are occurring by instituting an effective arthropod trapping program to monitor the escape prevention program. Oviposition traps, ground-level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes, and so on are recommended. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained. Any evidence of escape should trigger a review of practices and procedures before resuming work.
- Source and harborage reduction. Harborage and breeding areas are eliminated. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods. Equipment in which water is stored or might accumulate (e.g., humidifiers) is screened to prevent arthropod access, or contains chemicals to prevent arthropod survival.
- Laboratory sharps. Disposable sharps should be discarded in puncture-proof containers or as mandated by institutional policy.
- Routine decontamination. Equipment and work surfaces in the insectary are routinely decontaminated with an effective chemical disinfectant.
- Notification and signage. Persons entering the area should be made aware of the presence of BSL-2 agents in arthropod vectors.
- Procedure design. All procedures are carefully designed and performed to prevent arthropod escape.
- Safety manual. A site-specific safety manual is prepared, approved by the IBC or other institutional review entities, and adopted. The manual contains emergency procedures, standard operating procedures, waste disposal, and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary.
- Training. Laboratory personnel are advised of special hazards and are required to follow instructions on practices and procedures contained in the safety manual. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.
- Medical surveillance. An appropriate medical surveillance program should be considered, although institutional policy may vary with this requirement. At the minimum, all personnel should be educated by the PI about the risks associated with the specific tasks and experiments, as well as the signs and symptoms of any illness caused by the agent(s) under study. In general, persons who may be at increased risk of acquiring infection, or for whom infection may be unusually hazardous (e.g., immunocompromised), are not allowed in the insectary unless special personal protection procedures are in place to eliminate extra risk.

- Access restrictions. Routine access is limited to trained persons and accompanied guests. Service persons are made aware of the hazards present and the consequences of arthropod release and contact with agents that may be present.
- Special arthropod handling containers and areas. Infected arthropods are prevented from release into the laboratory area. Additional physical barriers (e.g., glove box, biosafety cabinet) or procedures (incapacitated arthropods, e.g., removing a wing from a mosquito) may be required depending on the local risk assessment.
- Safe transport in the laboratory. All infectious and potentially infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of arthropods between manipulation and holding areas is in non-breakable secure containers.

- Special practices
  - IBC and IACUC approval: as for ACL-1. Microbial agents classified at BSL-2 require at the minimum registration with the appropriate institutional entity (Biosafety Office). Work with recombinant organisms requires review and approval.
  - Housing of non-arthropod animals. Other animals are not accessible to the arthropods.
  - Containment during blood feeding. Recommendations for ACL-1 containment of arthropods during blood feeding are more stringently assured by special practices and container design, as recommended by the local risk assessment.
  - Blood source: as per ACL-1. To prevent inadvertent contamination of the clean colony, sources of infection, such as a tube of infected blood, should not be stored in the same refrigerator as a tube of uninfected blood for maintaining uninfected colonies by membrane feeding.
  - Escaped arthropod handling. Loose arthropods must be killed and disposed, or recaptured and returned to the container from which they escaped. Infected arthropods must not be killed with bare hands and must be manipulated using filtered mechanical or vacuum aspirators or other appropriate means (e.g., forceps, paintbrushes, gloved hands).
  - Accidental release reporting. A release procedure is developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in release of infected arthropods from primary containment vessels or that result in overt exposure to infectious material must be reported immediately to the insectary director (PI) who is responsible for ensuring that appropriate and documented action is taken to mitigate the release as well as the Biosafety Office. The room where the incident occurred is closed off, a warning sign indicating the location, number, and type of material released is prominently posted, and other laboratory personnel are informed until the source is eliminated.
  - Follow-up medical evaluation, surveillance, and treatment are provided as directed by institutional policy and local risk assessment, and written records are maintained.
  - Movement of equipment. All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.
- Safety equipment (primary barriers)
  - Personal protective equipment should be evaluated as part of the local risk assessment. Clothing (primary as well as safety) should conform to institutional policy and to the risk assessment by the local IBC. As an example, entering a room containing an environmental chamber holding plastic food containers with tick vials would not require personal protective equipment (PPE), unless the vials were opened and the ticks manipulated.
  - Eye and face protection. Appropriate face/eye and respiratory protection is worn by all personnel entering the insectary, if recommended by the local risk assessment.
  - Gloves. Gloves (latex or nitrile) are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.
  - Torso apparel. White laboratory coats, gowns, and/or uniforms are typically worn at all times in the insectary when handling vertebrate animals and infected materials.
  - Personal clothing. Clothing should minimize the area of exposed skin, since this can increase the risk of attracting and being bitten by a loose arthropod.

- Arthropod-specific personal protective equipment. Other equipment may be required as determined by the local risk assessment.

- Facilities (secondary barriers)
  - An insectary may simply be a room with a door that may be closed tightly; it may or may not have environmental controls. Dedicated spaces to be used as insectaries are highly recommended, but resources may not exist to permit such arrangements. The use of infected arthropods may be permitted after risk assessment by the local IBC even in the absence of a dedicated space. Ticks, for example, may be safely manipulated within general BSL-2 laboratory settings that are otherwise not considered to be insectaries.
  - Location of insectary. The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent passage of the arthropods.
  - Insectary doors. Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling arthropod escape. Alternative arrangements may be specified by local risk assessment in the absence of a dedicated insectary.
  - Insectary windows. Windows are not recommended, but if present cannot be opened and are well sealed. Windows should be resistant to breakage (e.g., double paned or wire reinforced).
  - Vacuum systems. If a central vacuum system is installed, each service outlet is fitted with suitable barriers/filters to prevent arthropod escape. Filters are installed to permit decontamination and servicing. Other vacuum devices are appropriately filtered to prevent transfer and exhausting of arthropods.
  - Interior surfaces. The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are preferably light colored so that a loose arthropod can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Light-colored floors are also highly recommended, smooth and uncovered. Ceilings are as low as possible to simplify detection and capture of flying insects.
  - Floor drains. Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate chemical treatment to prevent survival of all arthropod stages (e.g., mosquito larvae).
  - Plumbing and electrical fixtures. Internal facility appurtenances (e.g., light fixtures, pipes, ducting) are minimal since these provide hiding places for loose arthropods. Penetrations of walls, floors, and ceilings are minimal and sealed/caulked.
  - Heating, ventilation and air conditioning (HVAC). Ventilation is appropriate for arthropod maintenance, but does not compromise containment of the agent or arthropod. Examples include the following: exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/ barriers are installed to prevent escape of arthropods; the direction of airflow in the insectary is inward; a progressively negative pressure gradient is maintained as distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent escape of flying arthropods; and hanging or air curtains are located in vestibules and doorways.
  - Sterilization equipment. An autoclave is available, conveniently located in rooms containing arthropods within the insectary building.
  - Sink. The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.
  - Illumination. Illumination is appropriate for arthropod maintenance, and does not compromise arthropod containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped arthropods are avoided.
  - Facility compliance monitoring. The facility should be evaluated annually for compliance to ACL-2. The PI or insectary director inspects the facility at least annually to ensure that alterations and maintenance have not compromised the containment characteristics. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods is considered.

4.1.6 Tissue, Cell, and Microbiological Culture Practices

4.1.6.1 *Tissue/Cell Culture Practices*

Adhering to appropriate tissue culture techniques is intended to contain the risks of the biological agents used in an experiment, but also intends to improve the integrity of the science. The following measures are general guidelines for improved safety while performing cell culture techniques within a biosafety cabinet (BSC, also commonly called a "tissue culture hood").

Wear long sleeved gowns with knit cuffs and long gloves when working in the biosafety cabinet. Maintain a clean lab coat reserved solely for cell culture work. Cover any open wounds with occlusive bandages prior to donning PPE.

Refer to the Biosafety Cabinet Guidance document available on the Biosafety website for additional information on BSCs. Contact the biosafety officer for BSC training.

- Avoid causing unnecessary air disturbances in and around the BSC. Avoid moving ones hands in and out of the biosafety cabinet or sweeping side-to-side motions. Avoid talking during culture manipulations as aerosols may be drawn into the work area. BSCs should be placed in low traffic areas away from doors to prevent disturbance of the air curtain protecting the containment and sterility in the BSC.
- Place all reagents and supplies inside the Biosafety cabinet before the experiment to reduce the disturbance of air within the BSC during use.
- Work from a clean to a dirty side. Place all unused (clean) materials and reagents on one side of the work surface, and waste containers on the other (dirty) side.
- Do not over-crowd your biosafety cabinets, which may interfere with proper laminar air flow required for BSC function.
- Do not block the grates in the front or the back of the BSC which would prevent proper laminar air flow required to maintain containment and sterility within the BSC. Work approximately 4 inches back from the front of the BSC work area.
- Allow the BSC to run for fifteen (15) minutes prior to use and fifteen (15) minutes after use to purge the air within the BSC of any contaminants.
- Clean all work surfaces, interior vertical surfaces and face shields before and after use with an appropriate disinfectant.
- Do not use open flames inside the BSC. Heat currents generated from the flame may interfere with the laminar airflow of the BSC. In addition, the heat can damage the HEPA filters or the adhesives in the filter units or other components of the BSC. Lastly, flames pose a high flammability risk when used in the vicinity of alcohols, which are often used in tissue culture situations and have been known to cause explosions within BSCs. Alternative devices or measures should be utilized, such as glass bead sterilizers, microincinerators, use of disposable instruments, or having multiple packets of sterile instruments on-hand. If no appropriate substitute can be found for a flame, only the use on flame-on-demand devices, such as properly-used touch-plate microburners, or small alcohol burners. Gas and air balance should be set in the microburner such that only a small pilot light is visible most of the time; flame is only produced after depressing the plunger when needed.
- Do not use volatile chemicals in unducted Class II, A2 Biosafety Cabinets. After HEPA filtration (which will not filter out chemicals), these BSC typically recirculate 70% of the air and exhaust from the BSC into the room. If volatile chemicals are used within the BSC, recirculation will serve only to concentrate the chemicals within the BSC, and pose a spark hazard; in addition, the chemicals will be inappropriately exhausted into the room posing health risks. Minute amounts of volatile chemicals can only be used in BSCs which are

equipped with ducted exhaust ventilation—either via a thimble/canopy connection (a "ducted" Class II, A2 BSC); while somewhat larger amounts of volatile chemicals can be used in hard-ducted Class II, B2 BSCs. Please be aware, as of January 2021, all BSCs on the UNT campus are unducted Class II, A2 types and therefore inappropriate for the use of volatile chemicals; please contact the Biosafety Office (IBCprogram@unt.edu) if you are not sure of what type of BSC you possess.

- Radiological materials should not be used in unducted Class II, A2 Biosafety Cabinets unless approved by the Biosafety Office and Radiation Safety Office. Contact the Radiation Safety Office for further information about protection devices for use in unducted BSCs. Radiological materials should not be used in these BSCs for similar reasons that volatile chemicals should not be used (see above); in addition, radiological material which adheres to dust particles may also contaminate the HEPA filters and lead to inadvertent exposures of BSC maintenance and certification personnel. Minute amounts of radiological materials can only be used in BSCs which are equipped with ducted exhaust ventilation—either via a thimble/canopy connection (a "ducted" Class IIA2 BSC); while somewhat larger amounts can be used in hard-ducted Class IIB2 BSCs. Please be aware, as of January 2021, all BSCs on the UNT campus are unducted Class II, A2 types and therefore inappropriate for the use of radiological materials without additional protective measures.
- Liquid wastes should be collected in order to decontaminate using an appropriate method which should be described in the laboratory SOPs. Typically, liquid wastes are collected in vacuum aspirators into which some disinfectant has been placed. To ensure appropriate decontamination, subsequent disinfection measures should be followed prior to disposal.
    - Do not allow vacuum traps to become overfull (recommended not greater than half-full). This not only prevent liquids from being inadvertently drawn into the vacuum line, but will allow for full decontamination of the liquid wastes prior to disposal
    - HEPA filters or equivalents should be placed in the vacuum lines of any laboratory requiring BSL-2 containment or higher.
    - Do not leave pipettes in the ends of the vacuum aspirator hoses. After use, remove them from the hose and place in disinfection tray/container prior to disposal. Leaving pipettes within the hoses only presents additional exposure or contamination risks.
    - Rinse vacuum tubing with disinfectant after use. This will prevent backflow of contaminated liquids within the vacuum line and subsequent contamination.
    - If the vacuum traps are outside of the Biosafety Cabinet, place in sufficient secondary containment to hold the volume of liquid which may be spilled if implosion of the vacuum flask should accidentally occur.
- Glassware/plasticware and other contaminated items should be disinfected or autoclaved before washing, reuse, or disposal. Glassware should be thoroughly cleaned and rinsed, by washing repeatedly with tap water and distilled water.
- Place dirty pipettes, tips and tubes in a decontamination tray or container in which disinfectant has been placed on the "dirty side" of the BSC rather than move hands in and out of the BSC to dispose of these. This will avoid disrupting the protective air curtain when hands are removed from the BSC. Discard empty tubes immediately into the disinfection tray or similar containment device; after the experiment, drain the disinfectant from the plastic wastes then dispose of the wastes in the biohazard waste containers.
- Keep open tubes parallel to the airflow.
- After transferring inoculums, always recap vials.
- Do not place tubes on work surface.
- Pipette gently along the sides of tubes to prevent production of aerosols
- Work with one specimen at a time; recap before going to the next.
- Autoclave verification should be performed routinely.

If a problem with contamination develops please contact the Biosafety Office (IBCprogram@unt.edu) for further assistance.

4.1.6.2 *Microbiological Practices*

In the absence of definite accidents or obvious spillage, it is not certain that the opening of plates, tubes, and bottles of other microorganisms has caused laboratory infection. However, it is probable that some infections have occurred by this means. Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a biological safety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is resuspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause splattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture of colony.

Placing an inoculating loop, wire, or needle directly into a flame after use can also cause splattering and release of an aerosol. Following use of inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop or needle. Disposable inoculating loops are also commercially available. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water arising from syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. Vented plastic Petri dishes, where the lid touches the rim at only three points, are less likely to pose this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent dispersal. If plates are obviously wet, they should be opened in the biological safety cabinet.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long sleeved laboratory garment.

Dried, infectious culture material may also collect at or near the rim or neck of culture tubes/flasks and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened in a biological safety cabinet.

4.1.6.3 *Glass Ampules*

When a sealed ampule containing a lyophilized or liquid culture is opened, an aerosol may be created. Aerosol creation should be prevented or minimized; opening of ampules should be done in biological safety cabinets. When recovering the contents of an ampule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a biological safety cabinet and wear gloves.
Nick the ampule with a file near the neck. Wrap the ampule in disinfectant wetted cotton. Snap the ampule open at the nick, being sure to hold the ampule upright. Alternatively, at the file mark on the neck of the ampule, apply a hot wire or rod to develop a crack. Then wrap the ampule in disinfected wetted cotton, and snap it open. Discard cotton and ampule tip into disinfectant. The contents of the ampule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw the contents into a fresh container.
Some researchers may desire to use commercially available ampules pre-scored for easy opening. However, there is the possibility to consider that this may weaken the ampule and cause it to break during handling and storage. Ampules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, non-breakable leakproof secondary containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel. The secondary container must be labeled with a biohazard label bearing the name of the infectious material.

4.1.6.4 *Cryovials and Cryopreservation in Liquid Nitrogen*

There is a foreseeable risk that when samples that have been stored in liquid nitrogen are removed from the liquid phase and warmed they might explode. Clearly there is potential for significant physical injury to anyone nearby and in many cases there might also be an associated infection risk. Therefore, all personnel working with liquid nitrogen storage vessels or near them should be made aware of the explosion and infection risks and appropriate control measures should be taken to protect personnel from these risks.

When plastics are placed in liquid nitrogen, they become brittle and shrink due to the effects of the extremely low temperatures. This applies even to those plastics used for cryogenic vials sold specifically for storage of samples in liquid nitrogen. While the use of tubes with internal threads and a gaskets will lower the risk of leakage, it is virtually impossible to achieve a leakproof vial once the specimen is placed in liquid phase liquid nitrogen. Inevitably, some seepage in to the vial will occur. When the vial is removed from liquid nitrogen for thawing, the quick warming and expansion of air within the vials can cause the tube to explode.

Therefore, cryopreserved materials should be stored in the vapor phase of liquid nitrogen in a cryotank. If it is placed in the liquid phase, commercially available plastic tubing may be sealed around the cryovial like a sausage skin.
Special caution should be taken when removing cryovials from liquid nitrogen storage, and should include cryoprotective gloves to protect against liquid nitrogen burning, and full eye and face protection. Cryovials should be placed into a secondary container with a closed lid and into a Biosafety Cabinet as soon as possible to further protect the handler should the vial explode.

4.1.6.5 *Vacuum Packed Tubes/Vials*

When a vacuum-sealed tube or vial (e.g., Vacutainer blood tubes or septum bottles) are opened, the lyophilized material or liquid culture may become aersolized due to the sudden influx of air within the tube/vial. Protective measures to protect personnel from exposures to these aerosols should be incorporated into the laboratory Standard Operating Procedures. Ideally, opening of vacuum-sealed tubes/vials should be done in biological safety cabinets. Covering the lid or plug with a disinfectant-soaked gauze pad will also provide a barrier against exposures to the aerosolized material that may be generated upon opening. Commercial "cap" devices are also available to limit the spread of aerosols generated when opening tubes or vials.

4.1.6.6 *Embryonated Eggs*

Harvesting cultures from embryonated eggs is a hazardous procedure and leads to heavy surface contamination of the egg trays, shells, the environment, and the hands of the operator. It is essential that operations of this type be conducted in a biological safety cabinet. A suitable disinfectant should be at hand and used frequently.

4.1.7 Storage of Biological Materials

- A biological hazard sign must be clearly posted on storage areas such as refrigerators, freezers, cabinets, etc. containing recombinant and/or biohazardous materials
- All containers and/or racks are to be clearly labeled to identify the stored agent.
- Storage containers must be intact (no tears or cracks), leak-proof, and covered or closed to avoid spills or contamination. Secondary containment must be used when possible.
- To prevent unnecessary handling of specimens, all materials should be inventoried and organized.
- Any substance being stored in a freezer must be placed in a labeled container designed for low temperature storage.
- If flammable materials are used, they must be stored in equipment that is designed for this purpose.
- No personal items may be stored in refrigerators, freezers or incubators (e.g. food, medication, beverages) within laboratories.
- When storage equipment needs repair, calibration, or transport, it must be completely decontaminated prior to starting work or being removed. Biohazard stickers must be removed from the equipment once it has been decontaminated.

Each PI is responsible for performing a physical inventory of their long term storage (i.e. freezers and liquid nitrogen tanks) at least once a year. This annual physical inventory must be documented and the records kept available for inspection.

4.1.8 Transport of Biological Material on Campus (between Labs or Buildings)

To prevent exposure to non-authorized personnel (with unknown health status) and potential environmental release and contamination, biological materials which must be transported between laboratory areas by authorized personnel only through common hallways or walkways must be properly packaged, contained, and labeled. Here are the guidelines which should be followed:

- Biological materials must be contained inside two leakproof containers prior to removal from the laboratory. The IBC standard for intramural transport is:
    - Inside a sealed, leakproof primary container. A container is considered "leakproof" if it can be filled with water, turned upside down without holding the lid in place and leakage does not occur.
    - The primary container must be placed inside a sealed, leakproof, durable secondary container. The IBC standard for "durable" is puncture-resistant. Ziplock bags are not considered "durable" by this standard. Sealed 15 ml or 50 ml plastic centrifuge tubes or "burpable" household plastic storage containers (e.g., Tupperware®, Rubbermaid® or similar brands) are considered "durable.
    - Absorbent material (*e.g.,* paper towels) must be placed between the primary and secondary containers suitable for the volume transported.
    - A biohazard sticker and label must be affixed on the outside of the secondary container with agent name, lab address, and emergency contact phone number.
- Utilize plastic containers whenever feasible. Avoid glass. If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.
- The outside of the primary container should be decontaminated before placing into the secondary container. The outside of the secondary container should be decontaminated before leaving the laboratory.
- Biological materials may not be transported through non-UNT. Live animals must not be transported through non-UNT areas.
- Transport of biological materials in a private vehicle is discouraged. Keep in mind that transport of hazardous materials (which may include biological material, dry ice and liquid nitrogen) is against the terms of most private automobile insurance policies. Check with your insurer.
- Any extramural transport of biological materials must comply with the IATA/DOT shipping standards as described in Section 11 of the UNT Biosafety Manual.

4.1.9 Housekeeping

Well-defined housekeeping procedures and schedules are essential in reducing the risks associated with working with pathogenic agents and in protecting the integrity of the research program. This is particularly true in the laboratory operating under less than total containment concepts and in all areas used for the housing of animals, whether or not they have been intentionally infected. A well conceived and well executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminates in the event of inadvertent release of an etiologic agent. Less immediately evident are the benefits of establishing among personnel of widely varying levels of education some concepts of the nature and sources of contamination.

4.1.9.1 *Objectives of Housekeeping*

The objectives of housekeeping in the laboratory are to:

- Provide an orderly work area conducive to the accomplishment of the research program.
- Provide work areas devoid of physical hazards.
- Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems under study.

- Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.
- Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that may:

- Harbor microorganisms potentially a threat to the integrity of the biological systems under investigation.
- Enhance the survival of microorganisms inadvertently released in experimental procedures.
- Retard penetration of decontaminants.
- Be transferable from one area to another on clothing and shoes.
- With sufficient buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement
- Cause allergenic sensitization of personnel (e.g., to animal dander).

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other area in the laboratory has the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

4.1.9.2 *Scope of Housekeeping*

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological materials are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The following list outlines a portion of the terms requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the laboratory complex must be viewed.

- Aisles
- Eyewashes
- Lab Entry and Exit Ways
- Bench Tops
- Floors
- Lab Equipment Cleanup
- Biological Safety Cabinets
- Glassware
- Refrigerators
- Cold Rooms
- Supply Storage
- Deep Freezer Chests
- Incubators
- Waste Accumulations
- Dry Ice Chests
- Insect and Rodent Control
- Work Surfaces
- Instruments

4.1.9.3 *Assignment of Responsibilities*

Housekeeping in the laboratory is one avenue that leads to safely accomplishing the research program. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research environment. The recommended approach to housekeeping is the assignment of

housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need to be accomplished. The supervisor should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by a contracted cleanup crew; delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements; and the location of concentrated biological preparations, as well as contaminated equipment used in their preparation and application, will be known.

4.1.10 Standard Operating Procedure (SOP) Development

Central to the concept of risk assessment and management is the development of laboratory-specific Standard Operating Procedures (SOPs). These function within the laboratory to train all personnel on the "rules of the laboratory" and provide easy future reference for all staff members. These SOPs also serve as documentation to the IBC that appropriate practices are in place to contain the biological risks posed in a particular experiment. All mitigation methods specific to the agents, locations, and operations, which are required to contain their risks, must be documented. The BSO has many safety SOP templates available for use.

At **minimum**, laboratory SOPs should address the following:

- Risk communication information. The risks of the agents and operations which may present possible routes of entry should be documented. Symptoms of any illnesses which may arise due to inadvertent exposure to the materials should also be documented, and instructions provided to personnel about communication of this information to any healthcare provider, should they present symptoms (even if the personnel was not knowingly exposed to the materials). Prophylactic measures (e.g., vaccinations) and possible post-exposure health measures should be documented.
- Emergency Response Procedures. The procedures documenting the response of the personnel and supervisors should an incident, accident, exposure, spill, release, or equipment failure involving biological materials should be documented. This includes spill response, centrifuge tube breakages, exposure responses, reporting requirements, etc.
- Personal Protective Equipment (PPE) and Protective Equipment. The typical PPE to be worn by the personnel in the laboratory and any special PPE and/or equipment which must be used during particular agents (*e.g.,* animal or biotoxin handling) or during particular operations which may present additional splash, spray or aerosol risks must be documented and followed by personnel.
- Disinfection/Decontamination/Disposal Procedures. Documentation should be provided to personnel related to the appropriate disinfection, decontamination agents and procedures for all surfaces (*e.g.,* benchtops, stainless steel surfaces of equipment), equipment, liquid wastes, solid wastes, spills and instruments. Disposal procedures for sharps, solid waste, liquid waste, and pathological waste (e.g., animal carcasses or anything identifiable as a body part) should also be documented. If autoclaves are used as a method of decontamination, autoclave maintenance and certification procedures and records should be documented. Any special deactivation procedures for biological toxins must also be included in the SOPs.

- Standard Prudent Practices for Laboratories (e.g., no eating, drinking, gum chewing, mouth pipetting, hand-washing) as well as those particular to the PIs laboratory (e.g., closing the doors to inner laboratories).
- Storage and Access Control measures. The procedures the laboratory is expected to follow to ensure no unauthorized personnel accesses the biological materials.
- Transport/shipping procedures which should be followed by all personnel removing biological agents from the laboratory to transport to another UNT laboratory or shipping. This includes training requirements which may be required by these personnel.
- Any training requirements of personnel.

No particular template is required, however, RMS/BSO can provide a template if requested . This form is not intended to be comprehensive; PIs are encouraged to modify this form as appropriate for their laboratories. New or amended SOPs must be submitted to the Biosafety Office as part of the IBC Biosafety Protocol Registration, and as part of the lab specific safety manual. The Biosafety Office encourages researchers to seek assistance from the biosafety office in SOP development.

LABORATORY EQUIPMENT

Along with practices and facilities, proper use of laboratory equipment provides containment for work with biological materials. Equipment can refer to PPE, as well as operational equipment, which are often used for the dual purposes of maintaining scientific integrity as well as maintaining safety. Laboratory staff should be trained by the PI, Laboratory Director, or Instructional Course Director in the proper use of laboratory equipment.

4.1.11 Personal Protective Equipment (PPE)

Multidisciplinary research conducted in UNT laboratories requires that PPE (protective clothing and safety apparatus/equipment) be used to protect the researcher from contact with infectious, toxic and corrosive agents, excessive heat, cold, fire and other physical hazards. Suitable PPE also protects the experiment from contamination. The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research operations and levels of risk associated with the research. While PPE is an important component of any biological safety program, PPE is used with the understanding that PPE serves as a second line of defense. Good laboratory techniques, procedures, and appropriate laboratory equipment are the primary barriers against potential exposure to hazardous agents.

For additional information you are urged to consult the Biosafety Office (IBCprogram@unt.edu). In the event the Biosafety Office does not have a listing of the kind of protective devices you are seeking, efforts will be made to acquire the information needed.

4.1.11.1 *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 should 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.

Laboratory clothing serves to protect the wearer, the experiment, and environment against contamination. If proper precautions are not taken, contaminated clothing may carry infectious materials outside the laboratory and into other work areas, cafeterias, or the home. Infectious agents can remain viable on cotton and wool fabrics and be disseminated from these fabrics.

Some additional points:

- Overt exposure to agents at all level of risk should be followed by immediate decontamination of the PPE and change into clean PPE to protect the worker, the experiments, and the environment.
- Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.
- PPE worn within the laboratory must not be worn outside the facility to the library, cafeteria, or other places accessible to the public.
- PPE should be placed in an appropriately designated area or container for storage, washing, decontamination, or disposal.
- All PPE should be decontaminated before being sent to the laundry or discarded. Treat contaminated areas of PPE with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
- Do not take PPE home to launder; select a laundry service that follows universal precautions.
- Change PPE as soon as feasible whenever it is compromised, soiled, or torn.
- Wear appropriate sizes and keep an adequate supply of PPE available in the laboratory.
- Wash hands whenever PPE is removed.
- Do not touch door handles, elevator buttons, telephones, computers, or other clean surfaces or items with gloved hands.
- Wear closed-toe shoes and long pants to guard against skin contamination or chemical exposure. **Do not wear sandals or shorts in the laboratory.**

4.1.1.1.1 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, animals, and microbiological material. When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment should 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.

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. Quality assurance is an important consideration.

No one glove can be expected to be satisfactory for all intended uses. Gloves may be fabricated of cloth, leather, natural and synthetic rubbers, or plastics. New formulations of synthetic rubber and plastic continue to be developed as research makes varied and changing demands on the protective capabilities of gloves. Changing applications lead to improved capabilities of impermeability, strength, flexibility, tactile sense, and control.

Disposable (single use) gloves provide a barrier between infectious agents and the skin. Glove use is a basic precept of preventing infectious agent transmission. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common.

Gloves shall be removed and hands washed before exiting the laboratory. Use the one glove method, or an appropriate secondary container, when transporting materials through common use areas.

The UNT Division of Environmental Health and Safety (EHS) can provide information on gloves needed for various tasks, such as working with animals, dry ice, heat, acids, etc. Consult the Division of EHS with details of your work to receive further information about the type and availability of gloves that will best meet your requirements.

Considerations for the selection and use of gloves:

- Gloves are not 100% leakproof; change gloves periodically and when soiled and always wash hands after removing gloves or other PPE.
- Gloves will not prevent needle sticks or other puncture injuries.
- Check gloves for visible tears before use.
- Avoid wetting disposable examination gloves as water or disinfectants will encourage wicking and leaking which may lead to exposure.
- Do not reuse disposable gloves; discard contaminated gloves in a biohazard bag immediately after use.
- Double glove or use household utility gloves when cleaning spills. Household utility gloves may be decontaminated and reused (replace when compromised.)
- Latex allergies among laboratory and clinic personnel are common and can become extremely severe (see Section 5.2.3, Allergies). Alternatives to latex should be considered.

4.1.1.1.2 Procedure for Removing Gloves

The exterior of the gloves should be considered contaminated and exposure to skin should be avoided. Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out. When removing PPE, remove lab coat or solid front gown first, then remove gloves aseptically), remove face protection last to avoid touching your face with contaminated hands. If wearing double gloves, remove outer gloves before removing lab coat or solid front gown.

(https://www.globus.co.uk/how-to-safely-remove-disposable-gloves)
Figure 4.A. Procedure for Removing Gloves

4.1.1.1.3 Shoes

Shoes worn in the laboratory must have closed-toes. Open-toed shoes are not permitted in the laboratory. Special protective shoes (*e.g.,* steeltoed shoes) may be required for certain work activities (*e.g.,* when working in areas where there is a danger of foot injuries due to falling or rolling objects, or objects piercing the sole, and where feet are exposed to electrical hazards). 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.

4.1.1.1.4 Gowns, Lab Coats, Jumpsuits, Aprons, and Other Protective Clothing

Gowns, lab coats and jumpsuits protect the wearer's clothing and skin from contamination. As with all PPE, the type of clothing needed depends on the task being performed and the degree of exposure anticipated.

Solid front wrap-around clothing offers better protection than pull-over type clothing or clothing with front closures. Lab coats are not 100% leakproof; change PPE when soiled, and always wash

your hands after removing any PPE. Lab coats or other protective clothing will also not provide protection against needle sticks or other punctures.

Spills and splashes occur most often in the chest or lap area. The contaminated surface must be touched during removal of a front closing jacket or lab coat. The contaminated portion often ends up in the wearer's face during removal of pullover clothing. Many workers prefer not to button up front closing jackets, which leaves street clothing exposed. If front closing jackets must be worn, strict measures should be implemented to assure the clothing is closed at all times when performing procedures or tasks that may cause exposure.

Long-sleeved garments with snug fitting cuffs are preferred over open or short sleeves. Snug fitting cuffs prevent splashes, splatters, and aerosols from making contact with exposed skin on the lower arms. Longer single-use gloves can be pulled over snug fitting cuffs to seal out any infectious materials.

Plastic, vinyl, or rubber aprons are usually worn over other protective clothing when extra protection is desired. Aprons are necessary for protection against liquids spilling or splashing on clothing. It is recommended that appropriate aprons be worn to protect against the potential harmful effects of liquid waste. Aprons may also be used to provide protection from steam and hot water in locations such as animal handling facilities, autoclave rooms, and laboratory glasswashing rooms.

4.1.1.1.5 Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid, to splash on the head, face, and eyes or contact lenses. 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. A variety of face shields, head covers/hoods, protective goggles, and lenses are available from safety supply houses. The selection is dependent upon materials of construction, fit, comfort, and compatibility with the work and the overall facial area requiring protection.

Some of the considerations for selection and use of face and eye protection are indicated below:

- Face shields and hoods protect the face and the neck from flying particles and sprays of hazardous material; however, they do not provide basic eye protection against impacting objects.
- Shields should cover the entire face, permit tilting back to clean the face if desired, and be easily removed in the event of an accident.
- If an eye hazard exists in a particular operation or experiment, the soundest safety policy would be to require that eye or face protection, or both, be worn at all times by all persons entering or working in the laboratory.
- Contact lenses do not provide eye protection; in fact, they may present more risk to the eyes by holding hazardous materials in contact with the eye for a longer period of time until they can be removed. It is recommended that contact lenses not be worn when working around chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses

are worn, eye protection, such as tight fitting goggles, must be worn. Contact lens wear is also not recommended with some pathogens.

4.1.1.1.6 Respiratory Protection

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to ten organisms, when inhaled, may cause infection. Particles with an effective aerodynamic diameter of between 0.5 and 5.0 μm (therespirablefraction) aremost effectiveat penetration and retention in the deep pulmonary spaces. Particles larger than 5 micrometers are generally trapped in the upper respiratory tract and eventually cleared or swallowed.

Engineering controls, such as the use of biological safety cabinets, should always be considered as a first line of defense against respiratory infection when working with infectious organisms.
Respirators should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.

Respirators vary in design, application, and protective capability. Respirators can be placed into two categories:

- air purifying
- supplied air

By far, the most commonly used respirators in laboratories for protection against biological materials are air purifying respirators. These protect by purifying the existing breathing air through a filter (for particulates) or cartridge (for gases and vapors), or both. Standard air purifying respirators used at UNT are half-mask or full face respirators. These rely on the proper cartridge selection to filter out the contaminant.

Dust masks that have been approved by the National Institute of Occupational Safety and Health (NIOSH) are also considered to be air purifying respirators. Approved dust masks will have one of the following designations – N95, N99, N100, R95, R99, R100, P95, P99, or P100. The selection of N-, R-, and P-series filters depends on the presence or absence of oil particles, as follows:

- If no oil particles are present in the work environment, use a filter of any series (i.e., N-, R-, or P-series).
- If oil particles (e.g., lubricants, cutting fluids, glycerine, etc.) are present, use an R- or P- series filter. Note: N-series filters cannot be used if oil particles are present.
- If oil particles are present and the filter is to be used for more than one work shift, use only a P-series filter.

> *Note: To help you remember the filter series, use the following guide: N for Not resistant to oil, R for Resistant to oil P for oil Proof*

Selection of filter efficiency (i.e., 95%, 99%, or 99.97%) depends on how much filter leakage is acceptable. Higher filter efficiency means lower filter leakage. There are online sources of information to help select appropriate respirators: the NIOSH Guide to Industrial Respiratory

Protection and OSHA also has an online electronic tool for assisting in the proper selection of appropriate respirators.

Federal OSHA regulations (29 CFR 1910.134) require initial and annual training and fit testing, and well as medical surveillance of all respirator wearers. If a respirator is to be used in the course of research with biological materials, the UNT IBC will require adherence to these OSHA standards.

Please make sure that UNT Risk Management Services (RMS) is notified whenever the use of a respirator is being considered. Proper selection of cartridges and respirators is very important and should not be made without input from RMS. In addition, RMS will assist in guiding researchers through the evaluation process and provide the required training and fit testing.

4.1.11.2 *Selection of PPE*

Use the following PPE to minimize exposure via mucous membrane OR non-intact skin:

- For face protection, wear safety glasses and a mask, or a chin length face shield whenever splashing, splattering, or droplets may be anticipated (any work with liquids on the open bench). An impact resistant face shield should be used when working with cryogenic materials. Impact resistant face shields will protect the user's face against splatters of hot liquids or broken glass or plastic fragments.
- Gloves and a lab coat are worn to protect the skin and clothing from contact with potentially infectious materials. Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists so no bare skin is exposed. Consider double gloving when working with cultures of infectious agents or handling spills. Thicker household utility gloves can be worn for cleaning blood or BSL-2 spills. Utility gloves can be decontaminated and reused until the integrity of the glove is compromised. Temperature resistant gloves should be worn to protect hands from physical damage when working with very hot (autoclave) or cold (liquid nitrogen tank, -70°C freezer) materials.
- Sleeve covers may be worn over lab coat and gown sleeves to provide protection to the sleeves and wrists from contamination when working in the biological safety cabinet. Disposable sleeve covers have tight fitting grips at both ends.
- Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin conditions or wounds. Avoid working with Risk Group 2 infectious materials if non-intact skin cannot be adequately covered.
- Solid front gowns provide more protection to clothing and skin than lab coats. Solid front gowns are worn for high hazard infectious agent work. The tight fitting cuffs of the gown help to minimize wrist contamination.
- Impervious lab coats, gowns or aprons are worn when heavy contamination or soiling is likely.
- Head covers are worn to protect the hair and scalp from splatter or droplets when working with heavy contamination or when contact with the head is likely. When choosing a head cover make sure it is impervious to liquids (some head covers are not impervious).
- Shoe covers are worn over the shoes to protect shoes from contamination when working in heavily contaminated areas (such as large spills, dissection areas, or surgical operation areas).
- Gowns, head, and shoe covers also help keep contaminants from entering the sterile area in clean rooms, surgical suites and barrier animal facilities.

Use the following PPE to minimize exposure via cuts, slices, or scratches:

Kevlar gloves and sleeves are cut resistant and will help guard against slices, scratches or cuts, but will not prevent direct puncture or needlestick injuries. Steel mesh gloves also protect against slices, cuts, and scratches but will not eliminate punctures. Neoprene and other abrasive resistant gloves are cut resistant, but significantly reduce dexterity.

Use the following PPE to minimize exposure via aerosols:

HEPA filtered respirators (air purifying or powered air purifying) are worn to prevent exposure to potentially infectious aerosols when cleaning spills of concentrated infectious material or responding to centrifuge incidents. Employees who wear a respirator must enroll in the UNT Respiratory Protection Program through RMS before using a respirator.

Table 4.F. PPE Requirements		
PPE	**Biosafety Level 1**	**Biosafety Level 2**
Gloves	Recommended to prevent skin or clothing contact with biological materials. Note: work that may involve radioactive materials or chemicals will require the use of a lab coat and gloves	Required
Lab Coat	Recommended to prevent skin or clothing contact with biological materials. Note: work that may involve radioactive materials or chemicals will require the use of a lab coat and gloves	Recommended to prevent skin or clothing contact with biological materials.
Face Protection		Wear protective eyewear and surgical mask or chin-length face shield whenever splashing, splattering or spraying is anticipated to prevent contact with mucous membranes of the eyes, nose and mouth. Researchers may choose to augment eye protection by performing experiments behind a protective splash shield
Respiratory Protection		The UNT IBC may recommend respiratory protection on a case-by-case basis if the potential for aerosol generation is high and alternate containment devices, such as a biosafety cabinet, cannot be used
Other		Other PPE, such as Tyvek coveralls, booties, sleeve guards, plastic aprons, and household rubber gloves will be recommended on a case-by-case basis. Generally, additional protective clothing is required whenever there is a high potential for splashing of potentially infectious materials, such as organ harvesting or large spill response and clean-up.

4.1.11.3 *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.

4.1.12 Biological Safety Cabinets (BSCs)

Please refer to the BSC guidance document for additional information. A biosafety cabinet (BSC) is a primary containment device used with biological material. The most common cabinet is the Class II Type A2 biosafety cabinet. While handling biological agents, it is the biological equivalent of using hazardous chemicals inside a chemical fume hood. Like a chemical fume hood, a biosafety cabinet protects the user from hazardous material using directional air flow.

A key distinction is that biosafety cabinets have an internal blower motor which recirculates potentially contaminated air through HEPA filters. HEPA filters are designed to remove any biological agent from the air passing across the filter. Air leaving the chamber passes through the exhaust HEPA filter which prevents contamination of the lab or environment. Air recirculating through the chamber passes through the supply HEPA filter. This creates a sterile environment inside the chamber which is ideal for doing tissue culture work or sterile microbiology. Thus, biosafety cabinets are sometimes referred to as Tissue Culture hoods (though biosafety cabinet is the proper term).

The Class II Type A2 biosafety cabinet is the most common cabinet on campus. It uses a curtain of air and HEPA filters to provide both containment and a sterile environment.

- Provides 3 levels of protection
    - Personnel - Air curtain and HEPA filters protect users from biohazardous aerosols generated inside the chamber

    - Sample Protection – Recirculating and unidirectional HEPA filtered air protect samples from contamination from unsterile lab air
    - Lab/Environmental protection – HEPA filtered exhaust from top of cabinet protects lab environment from contamination by biohazardous aerosols generated inside the chamber
- Suitable for use with any biological agent
    - Bacteria, viruses, viral vectors, fungi, parasites, human/animal tissue and cell lines, prions, etc.
- Must not be used with
    - Large amounts of volatile or toxic chemicals
    - Concentrated flammable chemicals
    - Volatile radionuclides
    - Open flames

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.
- 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 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 RMS at AskRMS@unt.edu and the BSO at IBCprogram@unt.edu.

Moving and Installation

Disinfect biosafety cabinet work surfaces prior to moving them to new facilities and remove and biohazard labeling. Biosafety Cabinets used for work with pathogenic organisms may require gaseous decontamination before being moved. Contact RMS AskRMS@unt.edu for additional information prior to relocation.

Each biological safety cabinet must be recertified for correct air flow and filter integrity after it has been moved and placed in its final location. Contact AskRMS@unt.edu , or contract an outside company, for biosafety cabinet certification.

Decontamination and Maintenance

The PI is responsible for arranging and funding cleaning, decontamination, and maintenance of their biological safety cabinet. Facilities Management personnel will disconnect the cabinet and label when the cabinet was disconnected and decontaminated. If the safety cabinet is equipped with a UV light, do not use this as your primary disinfectant. No maintenance work is to be conducted on BSCs without prior decontamination. Review the BSC guidance document available on the biosafety website.

For additional information on BSCs, please refer to the BSC Guidance document available on the UNT Biosafety website.

4.1.13 Laminar Flow Hoods/Clean Benches

Laminar Flow Hoods (a.k.a. Clean Benches) differ from Biosafety Cabinets in that they offer only product protection, not personnel protection. They function by blowing HEPA filtered air through the metal baffle in the back of the unit directly across the work surface *toward the user*. While this provides a sterile work surface, this may increase the exposure risk of the individual using the unit to the materials on the work surface. Therefore, LFHs should not be used for work with hazardous materials. These function well for media preparation or similarly low hazard operations which may require sterile conditions.

4.1.14 Fume Hoods

Unlike Biosafety Cabinets or Laminar Flow Hoods, chemical fume hoods offer personnel protection to the user, but no product or environmental protection. Air drawn from the front of the unit passes directly across the work surface of the fume hood, thereby potentially compromising the sterility of any materials exposed on the work surface. In addition, the exhaust air from the fume hood is not filtered in any way before expelling the air via the building's exhaust systems. This may result in environmental contamination, and potential exposure of personnel who may be near the exhaust outtakes. Fume hoods should be used for working with hazardous materials, such as biological toxins; however, not if sterility is needed, and not when working with pathogenic materials.

4.1.15 Centrifuges

Hazards associated with centrifuging include mechanical failure (e.g. rotor failure, tube or bucket failure) and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions and users must be properly trained. The greatest aerosol hazard is created if a tube breaks during centrifugation. All centrifugation of Risk Group 2 agents or higher shall be done using centrifuge safety buckets with

safety caps or in

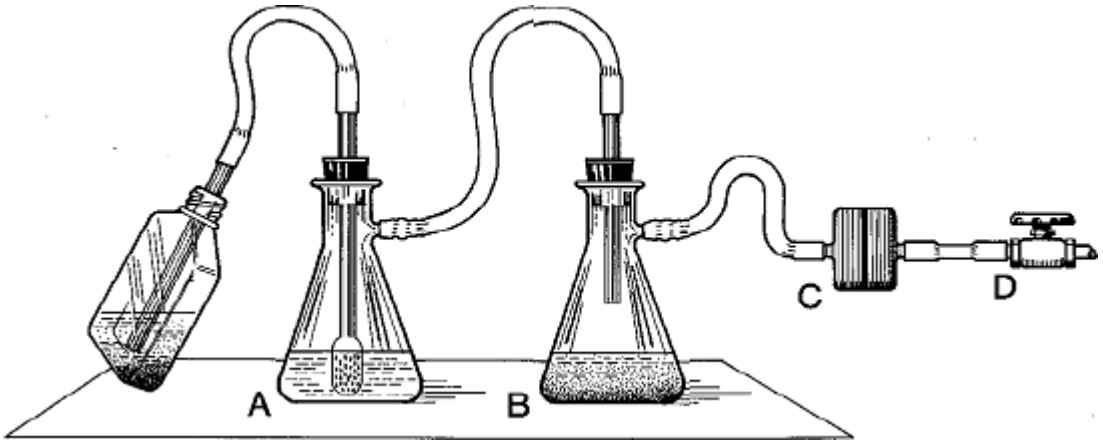
sealed centrifuge tubes in sealed rotors. If a small centrifuge is used and centrifuge safety cups are not available, the centrifuge should be operated in the biological safety cabinet.

Each person operating a centrifuge should be trained on proper operating procedures. Keep a log book detailing operation records for centrifuges and rotors to assist in determining service requirements.

The following procedures for centrifugation are recommended safety measures to consider while using biological materials in centrifuges :

- Use sealed tubes, safety cups, or sealed rotors that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet.
- Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- Always cap tubes before spinning.
- Place all tubes in safety buckets with safety caps or in sealed rotors. Correct rough walls caused by erosion or adhering of matter and remove debris from the rubber cushions.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket. Wipe the exterior of the rotor or safety buckets before removal from Biosafety Cabinet.
- Never exceed safe rotor speed.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- Wait five to fifteen minutes after the run before opening the centrifuge. This will allow aerosols to settle in the event of a breakdown in containment.
- Decontaminate safety carriers or rotors and centrifuge interior after each use.
- Open sealed tubes, safety cups, or sealed rotors inside a biosafety cabinet.
- Work in a biosafety cabinet when resuspending sedimented material from a biohazardous source. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a biosafety cabinet during use to reduce the aerosol escape.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.
- If construction of the centrifuge permits, the centrifuge chamber is to be connected to a vacuum pump with a HEPA filter installed between the centrifuge and the vacuum pump.

4.1.16 Vacuum Line Chemical Traps and Filters



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 or other, EPA approved, disinfectant. 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.

- Liquid wastes should be collected in order to decontaminate using an appropriate method which should be described in the laboratory SOPs. Typically, liquid wastes are collected in vacuum aspirators into which some full-strength disinfectant has been placed.
- The aspiration of tissue culture media from cultures and supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure. Protection against pulling biological aerosols or overflow fluid into the vacuum system is necessary. An overflow flask and a cartridge type filter are required to provide protection for the vacuum line.
- For assembling the apparatus, flexible tubing is used of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flask of capacities from 250 to 4,000 ml may be used for the overflow flask depending on the amount of fluid that could be aspirated out of the collection flask.
- When setting up a vacuum aspirator system, make sure the tubing inside the vacuum flasks extend far below the vacuum arm of the flask to prevent liquid wastes from being drawn through the flask's vacuum arm and contaminating the vacuum line.
- The overflow flask contains a disinfectant solution appropriate for the recombinant and/or biological material in use. Bubbling of air through the disinfectant can cause foam which can shut off the vacuum if it reaches the filter.
- Do not allow vacuum traps to become overfull (recommended not greater than half-full). This not only prevent liquids from inadvertently drawn into the vacuum line, but will allow for full decontamination of the liquid wastes prior to disposal
- To ensure appropriate decontamination, subsequent disinfection measures should be followed prior to disposal.

- Do not leave pipettes in the ends of the vacuum aspirator hoses. After use, remove them from the hose and place in disinfection tray/container prior to disposal. Leaving pipettes within the hoses only presents additional exposure or contamination risks.
- Rinse vacuum tubing with disinfectant after use. This will prevent backflow of contaminated liquids within the vacuum line and subsequent contamination.
- If the vacuum traps are outside of the Biosafety Cabinet, place in sufficient secondary containment to hold the volume of liquid which may be spilled if implosion of the vacuum flask should accidentally occur
- Vacuum line filters shall be examined and replaced if clogged or if liquid makes contact with the filter. Used filters shall be discarded in the medical waste stream.
- Change the filter if it becomes contaminated.

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

4.1.17 Syringes and Needles

The hypodermic needle is a dangerous instrument. To lessen the chance of accidental injection, aerosol generation, or spills, the use of syringes should be avoided when alternate methods are available. For example, use a blunt needle or cannula on the syringe for oral or intranasal inoculations and never use a syringe and needle as a substitute for a pipette in making dilutions.

The following practices are recommended for hypodermic needles and syringes:

- Use the syringe and needle in a biological safety cabinet and avoid quick and unnecessary movements of the hand holding the syringe.
- Examine glass syringes for chips and cracks, and needles for barbs and plugs. This should be done prior to sterilization before use. Use needle-locking syringes only, and be sure that the needle is locked securely into the barrel. Replace glass syringes with plastic disposable syringes whenever possible.
- Whenever possible, use safer needle systems. These might include retractable needle systems or shielded needle systems.
- Wear latex or nitrile gloves for all manipulations with needles and syringes.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Expel excess air, liquid, and bubbles from a syringe vertically into a cotton pad moistened with an appropriate disinfectant, or into a small bottle of sterile cotton.
- Do not use the syringe to forcefully expel a stream of infectious fluid into an open vial for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the syringe is held below the surface of the fluid in the tube.
- If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in the transfer of infectious material to the fingers.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pad moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pad may be used and immediately discarded into a biohazard bag.
- When inoculating animals, position the hand that is holding the animal "behind" the needle or use a pair of forceps to hold the animal in order to avoid puncture wounds.

- Be sure the animal is properly restrained prior to the inoculation and be on the alert for any unexpected movements of the animal.
- Before and after injection of an animal, swab the injection site with an appropriate antiseptic.
- Discard syringes into an authorized sharps container, which should always be kept near the site of use. DO NOT bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable, use a one handed method. DO NOT discard syringes into biohazard bags.

4.1.18 Pipettes

Pipetting is the act of transferring, measuring or dispensing a liquid typically through a narrow tube. Pipets can be constructed of a variety of glass or plastic materials. Liquids can be drawn into the pipet through the use of hand-held bulbs, manual pipet aids, motorized pipet aids, or various other vacuum sources. Pipetting is a routine function in most laboratories; therefore, the safety concerns must not be overlooked.

The following is excerpted from Laboratory Safety, Principles and Practices 2nd Ed., ASM Press.:

- Never suction or pipette by mouth; always use some type of pipetting aid when pipetting infectious materials. Preferably, all activities should be confined to a biosafety cabinet.
- Pipetting of toxic chemicals should be performed in a chemical fume hood.
- Infectious or toxic materials should never be forcefully expelled from a pipette. Mark-to-mark pipettes are preferable to other types because they do not require expulsion of the last drop.
- Infectious or toxic fluids should never be mixed by bubbling air from a pipette through the fluid.
- Infectious or toxic fluids should never be mixed by alternate suction and expulsion through a pipette.
- Gently discharge from a pipette as close as possible to the fluid or agar level, and the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.
- Pipettes used for transferring infectious or toxic materials should always be plugged with cotton, even when safety pipetting aids are used.
- Avoid accidentally dropping infectious or toxic material from the pipette onto the work surface. Place a disinfectant dampened towel or other absorbent material on the work surface, and autoclave before discard or reuse. Plastic backed bench paper is suitable for this purpose. This paper should be changed daily.
- Contaminated pipettes should be placed horizontally into a pan or tray containing enough suitable disinfectant, such as hypochlorite, to allow complete immersion of the pipettes. Pipettes should not be placed vertically in a cylinder that, because of its height, must be placed on the floor outside the biosafety cabinet. Removing contaminated pipettes from the biosafety cabinet and placing them vertically in a cylinder provides opportunity for dripping from the pipette onto the floor, or the rim of the cylinder, thereby creating an aerosol, and the top of the pipettes often protrude above the level of disinfectant.
- Place discard pans for used pipettes within the biosafety cabinet.
- After suitable contact time, excess disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together, and replaced by a clean pan with fresh disinfectant.

4.1.19 Aerosol-generating Equipment

The use of blenders, ultrasonic disrupters, grinders, and lyophilizers can result in considerable aerosol production. This equipment and any other device that may generate an aerosol must be used in a biosafety cabinet when working at BSL-2, or may require assessment for the use of respiratory protection. Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding or disrupting biohazardous materials. Even the use of a mortar and pestle can be a hazardous operation. Other devices that may produce aerosols are ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices/sonicators, homogenizers, ultrasonic cell disintegrators, French Presses, and shakers.

Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Wherever sonicators are used in the cleaning process; such as in dishwashers, animal cage washers, etc.; all items should be sterilized prior to cleaning.

The laboratory practices generally required when using equipment that may generate aerosols with biohazardous materials are as follows:

- Operate blending, cell disruption, and grinding equipment in a biological safety cabinet.
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leakproof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline, or methylene blue solution is recommended prior to use.
- If the blender is used with infectious material, place a towel moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use.
- Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break.
- Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product.
- Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.
- Grinding of infected tissues or materials with any open device is to be done within a biological safety cabinet.

4.1.19.1 *Blenders*

Safety blenders are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. Test blender rotors with sterile saline or dye solution to determine if they are leak-proof prior to use with recombinant and/or biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars must be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant must be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle and then open in a BSC. The entire device and jar must be decontaminated promptly after use.

4.1.19.2 *Sonicators and French Presses*

Sonication of living microorganisms is potentially a source of aerosols. Whether using a sonicating bath or probe sonicator, precautions must be taken to protect personnel. Ordinarily, this will be done by performing the sonication in a biosafety cabinet or glove box. It is prudent to consider all surfaces in the vicinity of the sonicator to be contaminated following its use, and they must be thoroughly disinfected. Modern sonicators have containment mechanisms. Ensure these mechanisms are utilized if available.

The use of French presses requires similar caution. The greatest potential for aerosols is at or near the end of a pressing cycle, when air bubbles at the top of the column of suspension can escape with little or no warning. This can result in microaerosols, which will contaminate the work area, but also in macroaerosols which can effectively inoculate the mucus membranes and conjunctivae of the operator. Due to the size of the press, it is usually impractical to perform this operation inside a biosafety cabinet. The pressing of human pathogens outside of a biosafety cabinet is restricted.
Operators must use face shields or other eye protection.

Arcing, which sometimes occurs during electroporation of bacteria, can also cause aerosols. These range from minimal spattering of the bacteria/DNA solution to major broadcast of potentially infectious material when a cuvette shatters. The shields on most electroporators are usually sufficient to protect the operator from flying plastic and gross contamination, but will not contain microaerosols. Thus, if one must electroporate bacteria at BSL-2, it must be done in a biosafety cabinet.

4.1.19.3 *Cell Concentrators*

Cell concentrators are also employed in laboratories as a means of handling viable organisms. There are two principal types of cell concentrators. The first involves the removal (through evaporation) of liquid from solid material thereby increasing the concentration versus volume. The second involves the retention of the solid material on the surface of a filter and the subsequent harvesting of the material from the filter surface. The following safety rules must be applied when using such an apparatus:

- Before starting, check all of the equipment to be used for signs of stress or fatigue. Pay close attention to tubing and glassware.
- When possible conduct the procedure in a biosafety cabinet.
- Upon the completion of the run, thoroughly sanitize the apparatus before the next experiment.
- For rotary type concentrators, make sure the load is balanced.
- If a vacuum is to be used, make sure the appropriate exhaust filter is present on the vacuum line to prevent contamination (normally a 0.22μm hydrophobic filter).
- Do not exceed recommended pressures or speed for operation of equipment.

4.1.19.4 *Lyophilizers*

Specimens snap-frozen in ampules are dried on a vacuum manifold or in a chamber-type drier at low negative pressure. If the glass neck of the ampule is sealed off while the ampoule is still under vacuum, it may cause implosion, either during the sealing or later when the evacuated ampule is being opened. To avoid this, after drying is completed and before sealing is done, bring the pressure within the ampule back to normal by gradually introducing dry nitrogen, avoiding turbulent disturbance of the dry product.

The narrow or constricted neck of the ampoule is contaminated if the specimen is allowed to run down the wall of the neck during filling. Subsequently, when the ampule is sealed with a torch, the dried material on the wall becomes charred or partially decomposed; residues of this material may adversely affect the dried material when it is reconstituted. To avoid this, a syringe with a long cannula or a Pasteur-type pipette should be used to fill the vial. Do not allow the delivery end of the cannula or pipette to touch the neck of the vial.

All ampules used for freeze-drying of cultures, toxins, or other biohazardous material should be fabricated of Pyrex-type glass. This type of glass requires a high-temperature torch using an air-gas or oxygen-gas mixture for sealing. These hard glass ampoules are much less apt to form gas bubbles that burst inwardly during sealing under vacuum than the soft glass ampoules and are more resistant to breakage during handling and storage.

The filling of ampules and vials with infectious specimens, the subsequent freeze-drying, and sealing or closing of ampules and vials in the preparation of dry infectious specimens should be performed in a biological safety cabinet. The same is true for the preparation of ampules and vials containing liquid specimens not subject to freeze-drying.

Safety precautions to be taken will depend on the agents, equipment, and containment available. Therefore, before initiating this procedure, the PI should work out the protocol for each machine in consultation with the BSO. All persons using the procedure must then follow the protocol.

4.1.19.5 *Microtome/Cryostat*

Due to the very sharp blade and the nature of the materials used with the microtome/cryostat, training is essential in the use of the equipment and in the hazards of the materials used with the equipment. Users should be informed of the need to prevent cuts and scrapes as well as protect the eyes, nose, mouth, and skin from exposure to the materials being used.

New personnel must be trained in the proper use and maintenance of the equipment, and demonstrate proficiency prior to use.

If using human tissue, microtome/cryostat users are required to attend Bloodborne Pathogens training. Fixatives take time to penetrate tissue; the fixatives may not inactivate pathogens deep in the tissue. Freezing and drying do not inactivate most pathogens, so, as with fixative use, the pathogens that may be present in the tissue should be considered capable of causing infection.

Microtome/cryostat users will likely need to also complete Chemical Safety training due to the fixatives and dyes used in histology.

When purchasing new units the available safety features should be taken into consideration prior to deciding on a manufacturer or model. Some available safety features are:

- Auto-decontamination cycle
- Easy blade release for installing and changing blades.
- Retractable knife/blade to permit safe entry into chamber for cleaning, retrieving specimens, etc.
- Disposable blades.

Never retrieve samples, change blades, or clean equipment by hand with the blade in place; always use appropriate engineering controls (i.e. forceps, tweezers, dissecting probes, and small brushes).

Things to remember when using and maintaining microtomes/cryostats:

- Always keep hands away from blades.
- Use extreme caution when aligning blocks, the blocks may be close to the blades. If available, make sure block holder is in locked position when loading/aligning blocks.
- Use knife-edge protectors/guards. Do not leave knife-edges that may extend beyond microtome knife holder unprotected.
- Keep blocks wet when in the microtome to minimize airborne shavings during slicing.
- Use brushes to clean/brush equipment.
- Use engineering controls such as forceps when removing or changing the blade.
- Dislodge stuck blocks using mechanical means such as forceps and/or dissecting probes.
- Wear appropriate PPE such as a lab coat or gown, mask, safety glasses or goggles, surgical grade Kevlar gloves that provide dexterity and cut protection, and examination gloves to protect against biohazards.
- When changing blades, wear stainless steel mesh gloves to provide additional protection from cuts and scrapes.
- Avoid freezing propellants that are under pressure as they may cause splattering or droplets of infectious materials.
- Decontaminate equipment on a regular schedule using an appropriate disinfectant.
- Consider trimmings and sections of tissue as contaminated and discard in the appropriate waste stream.
- Do not move or transport microtome with knife in position.
- Do not leave knives out of containers when not in use.
- Do not leave motorized microtomes running unattended.

4.1.20 Fluorescence Activated Cell Sorters (FACS) and FACS analyzers

In 1994 the International Society of Analytical Cytology (ISAC) first recognized the need to formulate safety guidelines for sorting and analysis of unfixed cells to provide laboratories with recommendations for practices to reduce the potential for biohazard exposure of instrument operators. These standards are periodically updated by ISAC, most recently in 2019 (See: https://isac-net.org/page/Biosafety).

The following is excerpted from Schmid, I, Lambert, C. Ambrozak D and Perfetto SP (2007) Standard Safety Practices for Sorting of Unfixed Cells. *Current Protocols in Cytometry* 3.6.1-3.6.20. John Wiley & Sons, Inc.:

> Biological particles 0.1 μm to 60 μm in size (*e.g.*, aerosols) have been found to be important in the spread of infectious diseases. Submicrometer particles formed through dehydration of small droplets (droplet nuclei) can contain inorganic material, organic material, or infectious agents and may stay suspended in air

for prolonged periods of time. During inhalation, larger particles are deposited mainly into the nasal passages, 3- to 7-μm particles into the tracheal area and pharynx, and ≤3-μm particles into the lungs of the exposed individual. Droplets that fall out of suspension in air will land on surfaces, and pathogens they may contain can then be transmitted by exposure to broken skin or mucous membranes, or by ingestion.

Consequently, protection of all laboratory workers from exposure is critical, in particular during high-risk procedures such as droplet-based cell sorting using instruments with high system pressures.

Jet-in-air technology utilized for cell sorting involves a liquid stream carrying the cells through a nozzle vibrating at high frequency. At a given distance from the nozzle orifice the stream is broken into individual droplets. These droplets are then passed between high voltage plates. Droplets containing cells of interest with parameters preselected by the operator are electrostatically charged and deflected into sort sample receptacles. Overall droplet size depends on the instrument operating pressure and the size of the nozzle orifice and its vibration frequency. High-speed cell sorters utilize higher system pressures and sort frequencies and thus produce more smaller droplets compared to older instruments designed for low speed separations. All sorters also generate microdroplets, i.e., satellite droplets, 3 to 7 μm. Owing to the high fluid pressure produced in high-speed cell sorters large amounts of secondary aerosols of various and undefined droplet sizes can occur during instrument failures, for instance, when a partial clog in the nozzle causes a deflection in the fluid stream that is hitting a hard surface, e.g., the waste catcher. Droplets larger than 80 μm constitute the majority of droplets generated during sorting and settle quickly out of the atmosphere; smaller droplets, however, may be aerosolized, particularly when they are elevated by air currents. Because of the potential health risk to sorter operators and the environment if aerosols escape into the room, aerosol containment of a sorter, whether free standing or enclosed in a biological safety cabinet, must be verified routinely using appropriate testing methods, such as the use of highly fluorescent Glo-Germ® (5-μm melamine copolymer resin beads in a 5-ml volume of ethanol) under the same conditions as the cell sort.

Although FACS analyzers are typically a more contained unit that the FACS sorter, it is important to note that some functional FACS analysis measurements on cells (e.g., evaluation of calcium flux or membrane potential, certain apoptosis assays, cytokine assays, or live DNA or RNA staining) preclude cell fixation, and when performed on jet-in-air flow cytometers, can also expose operators or bystanders to potentially hazardous aerosols and sample splashes. Therefore, the safety practices outlined here apply whenever unfixed samples are run through a jet-in-air flow cytometer or a cell sorter that combines a flow cell with jet-in-air sorting.

When sorting any infectious or hazardous material, even if it is classified as BSL-2, it is critical to understand that **droplet-based sorting procedures are considered BSL-3 practices**.

It is therefore recommended that viable, unfixed samples that are potentially infectious be sorted at a minimum on a sorter which has been tested for aerosol containment located in a modified BSL-2 facility using practices and containment equipment recommended for BSL- 3 by the CDC. However, because of the increased hazard of a sudden quick release of large amounts of fluid or aerosols into the environment, it is highly recommended that high-speed sorting be performed in a BSL-3 laboratory facility under complete BSL-3 containment.

All risks associated with materials being used or presented for sorting or analysis in any UNT FACS facility should be fully disclosed to the facility manager before use and documented on the PI's Biosafety Protocol for risk assessment. Currently, the UNT core facilities do not offer facilities capable of adequately containing BSL-2 materials when FACS sorting. Please refer to https://cdn.ymaws.com/isac-

net.org/resource/resmgr/docs/2014_isac_cell_sorter_biosaf.pdf for additional guidelines.

Table 4.G. Biosafety Level Determination for Cell Sorting

Table 1. Biosafety level determination for cell sorting

	BSL2	BSL-2 WITH ENHANCED PRECAUTIONS (DURING SORTING OPERATIONS)	BSL3	BSL4
Risk Assessment Condition	Uninfected non-primate cells	Non-infectious Human/NHP cells; Infectious but with low risk assessment	Infectious samples with high risk assessment; All samples containing known aerosol pathogens	Extremely Dangerous Pathogens
Example sample type or agents[a]	Normal murine cells third-generation Lentivirus (non-human cells)	Normal human blood; Human cell lines[a]; An example agent is: Influenza A[a]; second-generation Lentivirus or third-generation in human cells	Example agents include[a]: Mycobacterium Tuberculosis, Monkeypox	Example agents include[a]: Ebola, Marburg
Containment System Validated	Periodically (monthly or with filter change)[b]	Periodically (monthly or with filter change)[b]	Weekly or before Every Sort[b]	Weekly or before Every Sort[b]
Aerosol Containment Operational	Required	Required	Required	Required
Respirator	Optional	N-95, FFP2 or better[c]	PAPR	Special Suit
Eye protection	Safety Glasses	Face shield or safety goggles	N/A	N/A
Lab Coat	Front Closure lab coat	Wrap around, solid-front	Coveralls	Special suit
Separate Room and Environmental controls	Optional	Required or limited access to room[d]	Required[e]	Required[e]

[a]Example Sample type or Agents—the samples and/or agents listed represent only a partial list of agents, which may be included in each category. A risk assessment should be conducted for all samples/agents before sorting, and the appropriate biosafety level determined in collaboration with safety specialists, cell sorter operators, subject matter experts and the Institution's IBC or equivalent. For additional information please consult the following web sites: http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php; http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

[b]Frequency of testing will be dependent upon the risk assessment and consultation with biosafety professionals and/or the IBC or equivalent. For more detail see Section 3.1.1.1.

[c]Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened. Note that respirator protection may otherwise be removed during the sorting process providing the aerosol management system is active and all sort chamber and collection chamber doors are closed. For human pathogens, i.e. Risk Group 2 agents, which are classified as BSL2 and are not respiratory hazards, but which may pose a risk if exposed to mucous membranes, only mucous membrane protection is required. Examples of agents in this category include Leishmania and toxoplasmosis in murine cells.

[d]Enclosure of the cell sorter within a certified (see Section 3.1.1.2) Class II BSC may abrogate the need to house the sorter in a separate room within the BSL2 lab space; PPE (as detailed above) is optional, but strongly encouraged for the operator during procedures requiring manipulation of instrument. Cell sorters located within a shared laboratory may be operated under BSL2 with enhanced precautions if during the operation of the sorter, access to the room is limited and PPE as detailed above is worn by all occupants.

[e]Enclosure of cell sorter within a certified (see Section 3.1.1.2) Class II BSC required.

https://cdn.ymaws.com/isac-net.org/resource/resmgr/docs/2014_isac_cell_sorter_biosaf.pdf

For further information on FACS Biosafety, please contact the Biosafety Office (IBCprogram@unt.edu).

4.1.21 Miscellaneous Equipment (Waterbaths, Cold Storage, Shakers, etc.)

Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended. Sodium azide should not be used as a bacteriostatic. It creates a serious explosion hazard.

Whenever possible, the use of dry heat/incubator blocks and dry incubators should be considered instead of water baths since this may reduce the risks of water intrusion upon the samples and contamination of water in baths or shakers. Dry heat blocks come with modular adapters designed to fit tubes of multiple sizes as well as rectangular 12-, 24- or 96-well plates.

Deep freezer, liquid nitrogen, and dry ice chests as well as refrigerators should be checked and cleaned out periodically to remove any broken ampules, tubes, etc. containing infectious material, and decontaminated. Annual inventories of biologicals should be conducted.

Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deep freezers should be properly labeled. If equipment failure occurs, immediate action to prevent specimen loss and contamination of equipment and facilities may be required. Security measures should be commensurate with the hazards.

The degree of hazard represented by contaminated liquid nitrogen reservoirs will be largely dependent upon the infectious potential of the stored microorganisms, their stability in liquid nitrogen, and their ability to survive in the airborne state. Investigations suggest that storing tissue culture cell lines in containers other than sealed glass ampoules might result in potential inter- contamination among cell lines stored in a common liquid nitrogen repository.

Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as noninfectious until culture or other tests have proven their sterility.

Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

No person should work alone while performing any extremely hazardous operation.

LABORATORY FACILITIES

Laboratory Facility design and function is the third element, along with practices and equipment, which provides appropriate containment for work with biological materials. The unique design and construction of laboratory facilities contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Keep in mind, conversion of non-laboratory spaces into laboratory spaces is not always possible without significant monetary investment, since the design, ventilation requirements, and furnishings within the non- laboratory areas may not comply with those required by safety standards.

Laboratory directors are responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the ASHRAE Laboratory Design Guide published by the American Society of Heating, Refrigerating, and Air- Conditioning Engineers (ASHRAE).

Considerations based on the risk assessments of the biological, chemical, and radiological materials agents to be used in the facility and anticipated operations should be incorporated into any laboratory construction or renovation project during the design phase of the project.

Animal facilities, in particular, present significant design and construction challenges. Small disruptions (loud or unusual noises, odors, illumination, etc.) can greatly impact animal research operations. From a biosafety perspective, special considerations should be made in determining the locations of these facilities, as well as the equipment and mechanical, electrical and plumbing (MEP) features in and around these facilities. These design issues can be particularly challenging for architects and engineers unfamiliar with these issues.

In addition, considerations should be made during design to ensure that the facility can be properly maintained without disruption of operations which may lead to inadvertent hazardous material exposure of maintenance personnel, laboratory personnel, ongoing research activities (including research animals), the environment, or the surrounding community. Proper facility operation and maintenance is key to maintaining containment in any laboratory.

Some general biosafety considerations for design and operations of facilities are discussed below.

4.1.22 Physical Separation of Laboratory Spaces from Non-Laboratory Spaces

A physical barrier must exist which distinguishes "laboratory areas" from "non-laboratory areas". Basically, a room is either considered a laboratory or it is not. Laboratory areas are where hazardous materials may be handled. "Non-laboratory" areas may include offices, hallway areas, kitchens, or library where unauthorized personnel (*i.e.,* those who have not been properly informed of the hazards and provided precautionary medical screening) may be present and in which activities not permitted within the laboratory (*e.g.,* food or drink consumption/storage, storage of utensils for food/drink consumption, application of cosmetics, gum chewing, etc.) are permitted.

All laboratories require floor-to-ceiling physical separation between these two areas. Cubicles, study carrels and/or artificially demarcated areas (e.g., taped-off areas) physically located within a laboratory do not satisfy the physical separation criteria. Therefore, all restrictions within laboratories (*e.g.,* food/drink consumption and storage restrictions) apply to all desks physically located within laboratories which do not have floor-to-ceiling separation from the laboratory space. During laboratory design, accommodation for the needs of the laboratory research staff, particularly for separated areas for storage and consumption of food or drink, should be considered.

Also, consider that gloves must be removed and hands must be washed before exiting the laboratory areas, so hand-washing facilities near the exit doors is a convenient feature that should be considered during design of facilities.

4.1.23 Doors and Locks

Doors leading from laboratories into non-laboratory areas (*e.g.,* offices, hallways) should be kept closed after entering or exiting from the laboratory to maintain the physical barrier between laboratory and non-laboratory areas. Doors to hallways, offices or other "non-laboratory" area (as discussed in Section 4.3.1) should be kept closed. If doors open inward into laboratory spaces, consideration should be made to include a window in the door to prevent collisions into personnel handling biological (or other) hazardous materials. Ventilation grilles are not appropriate for laboratory doors.

Laboratory doors in BSL-2 facilities must be self-closing and have locks to provide security for/from the materials stored and handled within the facility. Animal facility doors which open to the exterior must not only be self-closing, but also self-locking. Doors to areas where infectious materials and/or animals are housed must open inward and must be kept closed when experimental animals are present. These doors must never be propped open. Doors to cubicles inside of animal rooms may open outward or slide horizontally or vertically.

4.1.24 Laboratory and Biological Material Security

Measures should be taken to secure biological materials within the laboratory to prevent inadvertent exposure of personnel who have not been informed of the risks and received appropriate health screening/vaccinations, theft, or inadvertent release. Whenever possible, accommodations for storage of higher risk agents (≥ Risk Group 2 and biological toxins) should be made within the most secure area of the PI's laboratory rather than in common or shared storage rooms.

If materials must be stored in common/shared facilities, additional measures to secure the materials may be required, including freezer/cryotank locks or lockboxes within refrigerators or freezers.

Security for any Select Agent or Toxin (See Section 2.6.4) is even more critical and is required by Federal Law.

4.1.25 Windows

Laboratory windows that open to the exterior are strongly discouraged. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

In animal facilities, external windows are not recommended, since the presence of windows may impact facility security. Exterior windows in animal facilities should therefore be assessed by security personnel. However, if windows are present, they must be resistant to breakage, and where possible, sealed.

4.1.26 Floor Coverings, Walls, and Ceilings

Laboratory facilities in which biological materials are housed should be designed to be easily cleaned and decontaminated. Since organic material, including cloth and unvarnished wood, and porous materials are virtually impossible to properly disinfect, the use of these materials in construction or furnishing biological laboratories is not permitted. Carpets and rugs in laboratories are not permitted in any laboratory.

In animal facilities, all interior surfaces (walls, floors, and ceilings) need to be assessed for cleanability. These surfaces need to be water-resistant, and floors must be slip-resistant, impervious to liquids, and resistant to chemicals. Penetrations in floors, walls, and ceiling surfaces should be sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Ceilings should be smooth, impervious to moisture, able to withstand cleaning with detergents and chemical disinfectants, and be free of imperfect junctions.

4.1.27 Furnishings

All furnishings within any laboratory must be capable of supporting anticipated work loads and uses and should be easily cleaned and disinfected. Since organic material, including cloth or unvarnished wood, and porous materials, such as unsealed cement, are virtually impossible to properly disinfect, the use of these materials in construction or furnishing biological laboratories is discouraged.

Chairs must be covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant. Sharp edges and corners should be avoided.

Spaces between benches, cabinets, and equipment should be accessible for cleaning. Storage of cardboard boxes, particularly on the floor, is discouraged because this may impede cleaning and disinfection efforts. In addition, cardboard may harbor insects or may become moldy (particularly when used in damp areas, such as cold rooms). In BSL-2 and ABSL-2 facilities, a method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). No plants or animals which are not associated with the experiments in the laboratory are permitted.

4.1.28 Lab Benches/Cabinetry

Again, because they must be durable and cleanable, bench tops and cabinets in all laboratories 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.

4.1.29 Exhaust Systems/Directional Airflow/HVAC

Although there are no specific requirements on ventilation systems at BSL-1 or -2 facilities, the CDC/NIH recommends that in planning new facilities, considerations should be made in the mechanical ventilation systems to provide an inward flow of air without recirculation to spaces outside of the laboratory. This is often required by the Chemical Safety regulations that are also often present in UNT laboratories.

In addition, any specific ventilation requirements related to the proposed use or equipment in the laboratory should also be addressed during laboratory design (*e.g.,* compressed gas tanks, particularly tanks containing compressed cryogenic liquids, are permitted only in well-ventilated areas due to the

potential asphyxiation risk associated with the rapid displacement of oxygen within an enclosed spaces).

Hazardous components of exhausted laboratory air can potentially contaminate the environment or inadvertently expose personnel (particularly maintenance personnel, who are more likely to be working on the ventilation systems on rooftops, etc.). This should also be considered when designing the exhaust system of these facilities. Stacked exhaust systems which expel the discharged air at high velocities and/or HEPA filtration should be considered based on the risks present.

Animal facilities have stricter requirements for ventilation to comply with the Guide for Care and Use of Laboratory Animals. No recirculation of exhaust air should occur in any animal facility and should be discharged to the outside without being recirculated to other rooms. While it is recommended that all animal rooms have inward directional airflow compared to adjoining hallways or rooms; this is required for compliance with ABSL-2 standards. Ducted exhaust air ventilation systems are also an ABSL-2 standard. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms, and especially the cage washing areas.

4.1.30 Plumbing/Eyewashes/Showers

Handwash sinks should be present in each laboratory room to enable personnel to wash their hands just prior to exiting from the facility. In BSL-1 and BSL-2 containment laboratories, the sink may have a manual, hands-free, or automated operation. The location of the handwash sink should be near the exit door, and additional sinks for hand-washing should be located in other appropriate locations within the facility as well. For instance, if an 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. Handwash sinks must be stocked with hand soap and paper towels.

Emergency safety showers are required in all animal facilities and within a 10 second walking distance from any location within an UNT laboratory, since hazardous chemicals are often present in the laboratories, in addition to biological materials.

BSL-1 and -2 laboratories and all animal facilities must also have eyewashes. Contact the RMS for recommendations of makes and models of eyewashes. Eyewashes must be flushed out regularly (weekly) by laboratory staff to prevent the growth of microorganisms within the water lines, which could expose personnel to further mucous membrane damage during emergency eyewash use. Sink or floor drain traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases into the laboratories.

4.1.31 Biosafety Cabinets

BSCs 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, windows that can be opened, 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.

Refer to the BSC Guidance document available on the UNT biosafety website for additional information.

4.1.32 Lighting, Air Ducts, Utility Pipes

Adequate illumination is required for all activities in the laboratory. Care should be taken to avoid reflections and glare that could impede vision.

To facilitate cleaning efforts, all internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, should be arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites. This is a requirement of animal facility design.

Air ducts should not be located over lab benches, but should be positioned over aisles so airflow readings may be taken periodically to monitor the laboratory's HVAC performance and air balance.

4.1.33 Additional Animal Facility Considerations

Laboratory animal facilities pose unique challenges to balance biosafety and animal care and use issues. In general, laboratory animal facilities requirements can be found in the Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations; however, assessments should also be made based on biosafety, occupational health, and safety risks to address potential hazards associated with the conduct of laboratory animal research as outlined in the BMBL.

Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross contamination should be incorporated into the facility design. Animal areas should be as restricted as possible. Special containment equipment or facility design may be required as determined by appropriate risk assessment.

In addition, provisions should be made for animal-specific operations. At ABSL-1, cages may be washed manually or preferably in a mechanical cage washer. At ABSL-2 containment, the cages must first be autoclaved or otherwise decontaminated prior to washing using a mechanical cage washer.
The mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should also 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.

SECURITY

4.1.34 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.

4.1.35 Information Security

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

5 OCCUPATIONAL HEALTH

The purpose of an Occupational Health program is to conduct periodic health assessments of personnel handling biological materials with particular attention devoted to factors or conditions associated with a particular biohazardous agent a given individual might handle. For a particular person, the medical surveillance program might call for any of a number of precautionary measures, including immunizations, a periodic physical examination, or collection of a serum specimen. Each laboratory should have the requirements for Employee Health Screening and vaccinations as part of their Standard Operating Procedures. The purpose of the medical surveillance program is to:

- recommend appropriate medical precautions to be followed, and
- do periodic reassessment of employees to determine if medical conditions associated with employment are prevalent and, if so, to undertake definitive measures to alleviate them.

The extent of medical surveillance for a given employee will vary greatly and be dependent upon:

- the nature of the research project in which involved,
- the biohazardous agents to which directly or potentially exposed, and
- certain additional factors relating to the current or previous health status of the individual.

The CDC BMBL standard for Biosafety Level 2 places the responsibility on the laboratory supervisor to provide Employee Health services and education pertaining to their health risks while working as indicated: "The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered… Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance." (CDC BMBL, Biosafety Level 1 and 2) Employee Health services are also a requirement of the OSHA Bloodborne pathogen standard 29 CFR 1910.1030.

If you have an underlying health condition which is known to weaken your immune system (e.g. HIV-positive), or if you are taking certain medications which cause immunosuppression or weakening of the immune system, or if you are pregnant, etc. you may be at increased risk of infection. If you have any concerns about your health, you are advised to consult with your medical care provider, or, if you are a student, at the UNT Student Health Center.

As a condition of authorization, personnel who work with animals, are required to be enrolled in an Occupational Health and Medical Surveillance Program related to their potential risks. The goal of an Occupational Health and Medical Surveillance Program is to prevent occupational injury and illness, and protect the vulnerable populations of students, employees, and research animals.

It is the policy of the University of North Texas to prevent exposure to airborne hazards while on the job. A respiratory protection program is one means to provide protection when other controls are not available or are ineffective. The primary objective is to prevent harmful exposure to airborne contaminants. Where feasible, this shall be accomplished through engineering controls (i.e., enclosure or isolation, general or local ventilation, and substitution of less toxic materials). When effective engineering controls and/or administrative controls are not feasible, or while they are being instituted or evaluated, the use of appropriate respiratory protection will be required.

All employees required to wear a respirator must complete an OSHA Respirator Medical Evaluation Questionnaire before using a respirator. The respiratory protection program is overseen by Risk Management Services.

Individuals with compromised immune systems (either due to a medical condition or medications) are more susceptible to infection which may be associated with research materials or animals.

These potentially "immunocompromising" conditions include the following:

- Diabetes
- HIV infection
- Pregnancy
- Autoimmune diseases being treated with immunosuppressive drugs
- Splenectomy
- Primary immunodeficiency syndromes
- <2 years post-bone marrow transplant
- Graft versus host disease
- Leukemia, lymphoma, myeloma, congenital primary or complement deficiencies
- Corticosteroid therapy >20 mg per day prednisone or equivalent for 2 weeks or more
- Cancer chemotherapy (currently undergoing or <3 months after cancer chemotherapy)
- Immunosuppressive drugs for a transplant
- All persons being treated with TNF-inhibitor monoclonal antibodies

Other medical conditions are associated with higher consequences of exposure to hazardous materials, including:

- Heart conditions (e.g., valve disease, heart failure)
- Iron-overload conditions (e.g., hemochromatosis)
- Chronic liver disease (e.g., cirrhosis, hepatitis, fatty liver disease)
- Hemoglobinopathies (e.g., sickle cell disease, beta thalassemia)
- Gastrointestinal conditions (or medications used to treat these)
- Eczema or other skin diseases which may present with open lesions

UNT policies for seeking any appropriate health counseling and vaccination differ based on whether the laboratory personnel is an employee or a non-employee (which would include any student who is not an UNT employee). A medical surveillance program should be offered by the employer to

personnel engaged in biohazardous research; UNT students who may be working with biohazardous materials in the course of their education should seek health counseling and surveillance services with the Student Health Center. It is beholden upon UNT to offer any personnel who are not UNT employees (volunteers, visitors) who may be working with biological materials to further the UNT research or educational mission while at UNT the appropriate Occupational Health services prior to authorizing them to work within the laboratory or clinic, or subsequent to an incident, exposure, or accident involving biological materials. It is the responsibility of the PI, Clinic Director, and/or Instructional Course Director to map out how these personnel will receive the appropriate health counseling, vaccination, and post-exposure follow-up health care as part of the laboratory SOPs.

Employee Health screening and medical surveillance should be provided without charge for any UNT employee whose job may result in potential exposure. As UNT has no university-wide health program, these costs are typically incurred at the departmental level.

Plans to address how a biological exposure incident will be addressed should be prepared by the laboratory, included in the laboratory safety manual, and provided to the Biosafety Office for review prior to working with these agents.

This must include identifications of any post-exposure prophylaxis options and/or medical monitoring plans for those who may have been exposed to the agents.

Refer to Section VII of the BMBL for additional information on Occupational Health Support for Biomedical Research.

RESPIRATORY PROTECTION PROGRAM

UNT policies for seeking any appropriate health counseling and vaccination differ based on whether the laboratory personnel is an employee or a non-employee (which would include any student who is not an UNT employee). A medical surveillance program should be offered by the employer to personnel engaged in biohazardous research; UNT students who may be working with biohazardous materials in the course of their education should seek health counseling and surveillance services with the Student Health Center. It is beholden upon UNT to offer any personnel who are not UNT employees (volunteers, visitors) who may be working with biological materials to further the UNT research or educational mission while at UNT the appropriate Occupational Health services prior to authorizing them to work within the laboratory or clinic, or subsequent to an incident, exposure, or accident involving biological materials. It is the responsibility of the PI, Clinic Director, and/or Instructional Course Director to map out how these personnel will receive the appropriate health counseling, vaccination, and post-exposure follow-up health care as part of the laboratory SOPs.

Employee Health screening and medical surveillance should be provided without charge for any UNT employee whose job may result in potential exposure. As UNT has no university-wide health program, these costs are typically incurred at the departmental level.

Contact Risk Management Services (RMS) for a copy of the Respiratory Protection Program.

MEDICAL RESTRICTIONS

As previously described in Section 3.1.1 Risk Groups, all risk group and containment recommendations presented in the CDC/NIH BMBL, the NIH Guidelines, the WHO Biosafety Guidelines, etc., are based on the risks to healthy human adults. These recommendations do not account for individual health considerations, such as allergies, pregnancy, breast feeding, medication effects, a compromised immune system (due to illnesses or medical treatments such as steroids or chemotherapy) or other illnesses which may make individuals more susceptible to agents.

For this reason, for their own safety, any individual with special health concerns is strongly encouraged to discuss these with the PI, Clinical Director, or Instructional Course Director prior to initiation of work within the laboratory.

5.1.1 Pregnancy

It is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Therefore, if pregnancy is possible while you are working in an infectious disease laboratory or laboratory engaged in work with infectious agents, you should consult your PI or supervisor. Student Health Offices should also be made available for answering questions regarding the potential harm from the biological agents present within your laboratory.

Women who are pregnant or become pregnant are encouraged to inform their supervisors, PIs, Clinic Directors, and/or Instructional Course Directors, who should, in turn, encourage them to seek appropriate health counseling through a medical professional or Student Health Offices.
Personnel are urged to discuss exposure issues with their supervisors or PIs regarding associated risks of research being conducted and pregnancy. Student Health will give advice about precautions that might be necessary.

The Student Health Offices are resources for pregnant women to ask about any questions or concerns they may have regarding risks in their work environment. The Student Health Offices may require additional information about the agents and on-going operations within the laboratory beyond what the laboratory personnel is able to offer. The Student Health Office may need to discuss these matters with the PI, Clinic Director, or Instructional Course Director, or they may contact the Biosafety Office to discuss the agents and operations documented in the laboratory's Biosafety Protocol. The Student Health Office may also act as a liaison between pregnant laboratory personnel and their respective supervisors or PIs.

5.1.1.1 *Reproductive Biological Hazards*

Reproductive biological hazards include, but are not limited to the following:

- Cytomegalovirus (CMV)
- Hepatitis B virus (HBV)
- Hepatitis E virus
- Human Immunodeficiency virus (HIV)
- Human parvovirus B19
- Rubella (German Measles)

- Lymphocytic Choriomeningitis virus
- *Toxoplasma gondii* (Toxoplasmosis)
- *Listeria monocytogenes*
- Varicella-zoster virus (chicken pox)
- *Coxiella burnetii* (Q fever)
- Vaccinia virus

Whenever necessary, the Biosafety Office will offer an opportunity to review work procedures in the lab to ensure that potential exposure is minimized. Consideration for reassignment to other tasks that do not involve exposure to the reproductive hazard (generally with actual pathogens, not necessarily for only other potentially infectious materials such as blood or body fluids) should be given. Also, PIs actively working with reproductive hazards should explain these risks at the time of hire.

5.1.2 Work with Animals

Occupational Health programs play an important part of both animal use regulations as well as biosafety guidelines. Special hazards exist for workers who are exposed to animals, and therefore guidance is provided by the Institute for Laboratory Animal Research (ILAR) Commission on Life Sciences, National Research Council related to Occupational Health issues in:

- Occupational Health and Safety in the Care of Research Animals

5.1.3 Allergies

5.1.3.1 *Allergies to Laboratory Animals (ALAs)*

Allergic reaction to animals is among the most common condition 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 allergen tend to have progressively more frequent and severe symptoms, and an estimated 10% develop asthma. Studies have shown that about 50% of those with symptoms will eventually stop working with animals permanently or temporarily because of the discomfort involved in ALA.

Initial allergy symptoms are usually:

- Runny nose (allergic rhinitis),
- Itchy eyes (allergic conjunctivitis)
- Rashes (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.

Hence, it is critical that all workers seek to minimize their exposure to animal allergens and that PIs and Laboratory supervisors discuss these risks with their laboratory workers. Supervisors are also responsible to ensure that appropriate operating procedures have been established to prevent undue exposure of workers to animal allergens and provide Employee Health/Student Health screening for workers who may be developing hypersensitivity to assess their risks of further work with animals. These risks should also be communicated to others in the laboratory to inform them of the potential consequences of exposure to their fellow laboratory staff member to prevent inadvertent exposure.

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.

Of particular importance is wearing a face mask to reduce inhalation and hand-to-face spread of allergens and covering all exposed skin (i.e. gloves, lab coat, sleeve protectors) to prevent allergen contact. In some cases, respirators may be recommended; in which case the employee must be enrolled in UNT's respirator program, and undergo annual fit-testing as per OSHA standards (29 CFR 1910.134).

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. Contact your supervisor or biosafety further information regarding PPE.

5.1.3.2 *Latex Gloves and Related Allergies*

Allergic reactions to natural rubber latex have been increasing since 1987 when the Center 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.

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). The full text of this publication is available at the NIOSH web site, http://www.cdc.gov/niosh/topics/latex/.

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 (for instance, nitrile gloves)
- Wash hands with mild soap and water after removing latex gloves

5.1.3.3 *Antibiotic Allergies*

Allergic reactions have been described to a large number of medicines, and those against antibiotics is one of the most common of these. Reactions to antibiotics can range from a rash or hives starting a few days after exposure to

sudden onset of rashes, difficulty breathing, stomach upset and anaphylaxis soon after exposure. Because of the potential severity of these reactions, any personnel with known allergies to antibiotics should discuss their personal health risks in working in a laboratory with medical professionals or Student Health Offices.

In the laboratory setting, antibiotic allergies may impact the risks in two ways:

1) Should exposures occur to biological materials for which the medical treatment modality is administration of an antibiotic against which the laboratory personnel is allergic, an alternate post-exposure treatment plan should be made prior to exposure in conjunction with the PI, Clinical Director, and/or Instructional Course Director. These supervisors should be also be made aware of the potential for allergic reaction, so this can be communicated to health care providers in an emergency situation
2) Biomedical laboratories often use antibiotics for research purposes. For instance, antibiotic selection is often used during culture operations in both microbiological and mammalian cell/tissue culture settings. The risks of exposure of allergic personnel to the antibiotic should be communicated to the PI, Clinic Director, and/or Class Instruction to:
    a) Enable development of any operating practices which would help limit the exposure any allergic personnel to the antibiotic
    b) Enable communication of the exposure risks to others in the laboratory to consider the exposure risks to the allergic personnel.

5.1.3.4 *Mold Allergies*

Some people are very sensitive to molds. For these people, exposure to molds can cause symptoms such as nasal stuffiness, eye irritation, wheezing, or skin irritation. Some people, such as those with serious allergies to molds, may have more severe reactions. Severe reactions may occur among workers exposed to large amounts of molds in occupational settings. Severe reactions may include fever and shortness of breath. Some people with chronic lung illnesses, such as obstructive lung disease, may develop mold infections in their lungs.

Molds can be found almost anywhere; they can grow on virtually any substance, providing moisture is present. There are molds that can grow on wood, paper, carpet, and foods. There is no practical way to eliminate all mold and mold spores in the indoor environment; the way to control indoor mold growth is to control moisture and humidity which may provide ideal conditions for mold growth. In buildings, conditions which can favor mold growth can include roof and plumbing leaks or floods, condensation, and excess humidity. In large buildings, mold and mildew are commonly found on the exterior wall surfaces of corner rooms in heating climate locations. In laboratories, mold is often found in areas where moist conditions are present and condensation is likely to occur such as cold rooms, inside refrigerators, or in thawed freezers or decommissioned warm rooms-- particularly when organic materials (like cardboard) are placed in these areas.

Because of the potential health consequences to personnel with mold allergies, care should be taken by all UNT personnel (i.e., not just laboratory staff), to take measures to prevent mold growth in UNT facilities. Prudent preventative measures include:

- Not storing organic materials which can serve as growth medium to mold in moist atmospheres. Do not store cardboard boxes in cold rooms, freezers, or refrigerators.

- Inspect the building for signs of mold, moisture, leaks, or spills routinely. Clean up mold and eliminate sources of moisture as soon as possible.
- Fix the source of water problems or leaks to prevent mold growth.
- Reduce indoor humidity (to 30-60%) to decrease mold growth by: venting bathrooms, dryers, and other moisture-generating sources to the outside; using air conditioners and de-humidifiers; increasing ventilation; and using exhaust fans whenever steam-producing operations occur (such as glassware or cage washing or shower facilities).
- Clean and dry any damp or wet building materials, floors/carpeting and furnishings within 24-48 hours to prevent mold growth.
- Clean mold off hard surfaces with water and detergent, and dry completely. Absorbent materials, such as ceiling tiles, that are moldy may need to be replaced.
- Prevent condensation: Reduce the potential for condensation on cold surfaces (i.e., windows, piping, exterior walls, roof, or floors) by adding insulation.
- In areas where there is a perpetual moisture problem, do not install carpeting (i.e., by drinking fountains, by sinks, or on concrete floors with leaks or frequent condensation).

Further information about mold in the indoor environment, please see the EPA Mold Resources web page at: http://www.epa.gov/mold/ .

5.1.4 Other Restrictions

Restrictions or recommendations will be made on an individual basis after discussion with the employee's personal medical doctor. Examples of conditions that might warrant special precautions are HIV infection, immunosuppressive conditions, and drug therapy that suppresses the immune system (e.g. steroid therapy).
Therefore, if you are suffering from any of the above conditions, you must inform your physician or Student Health Offices about the situation.

MINORS IN LABORATORIES AND OTHER HAZARDOUS AREAS

The Standard Operating Procedure (SOP) for Minors in Labs must be followed if minors are involved in any University laboratory or shop program.

All risk group and containment recommendations presented in standard Biosafety Guidelines (CDC/NIH BMBL, the NIH Guidelines, the WHO Biosafety Guidelines, etc.), are based on the risks to healthy human adults and do not account for differential effects on immature bodies/systems. Persons under the age of 18 are prohibited from entering laboratory areas or other areas where hazardous materials or conditions may be present, except when such entry is a pre-authorized, escorted, scheduled open house tour. During these times, no work with Risk Group 2 organisms will be permitted and the facility should be decontaminated to prevent inadvertent exposure to minors to any residual hazardous material while visiting.

Because of their biological, social, and economic characteristics, young workers have unique and substantial risks for work-related injuries and illnesses. For this reason, both the Federal and State Departments of Labor have issued special requirements for work of minors under the age of 18 years old. These laws specifically generally restrict minors under the age of 18 from working in hazardous areas, and specifically restrict any minor under the age of 18

from working in areas where this is exposure to radiation or storage/manufacture of explosives. CDC/NIOSH has also issued some recommendations for protecting the health and safety of minors while in occupational settings.

By definition, any laboratory is a potentially hazardous area, whether these hazards are presented by biological materials, chemicals, or radioactive substances. Few studies exist which adequately characterize potential differential health consequences of exposure of minors to these hazards. While the Biosafety Office recognizes that paid or volunteer employment within a laboratory can be valuable learning experience, minors under the age of 18 years should be restricted from working in these areas, unless provisions can be made and followed to address the following concerns:

- Risk communication and comprehension of those risks and mitigation methods is a crucial safety measure within the biomedical laboratory. This may require a certain level of education background and maturity to understand. General biosafety training modules assume a base level of comprehension of biological and chemical concepts, and the EH&S and Biosafety Office would not be equipped to provide special training sufficient to compensate for the lack of education and experience.
- Signed liability waivers, normally obtained from parents and guardians to permit minors to enter laboratories require that the parent or guardian be fully informed of the risks faced by their child in these areas. Again, the amount of education/training required to bring parents or guardians of minors who wish to work in a laboratory may greatly vary, and cannot be guaranteed.
- Adequate supervision of the minor worker within the laboratory would have to be ensured. Contact Nadia Guevara Nadia.Guevara@unt.edu for Youth Protection Program. PPE and all necessary safety precautions must be always followed. This means all activities engaged by the minor within the laboratory would have to be monitored at all times, which requires the physical proximity (i.e., within the supervisor's line-of-sight) and attentiveness of the supervisor at all times while the minor is within the laboratory. This level of supervision is difficult to guarantee within the busy working laboratory. Minors should not be allowed in the lab spaces after office hours and during holidays or allowed to work alone.
- Appropriate occupational health screening and vaccinations would be required of all personnel within the laboratory. Some vaccinations require multiple administrations over time, and completion of the vaccination series may not be possible within the time frame for the minor's employment/volunteer opportunity. In addition, special health care provisions would have to be made for the minor in case of incident or potential exposure or illnesses which may have resulted from a potential exposure. If the minor is in the laboratory on a volunteer basis, and is not an UNT student, special provisions may be required to address the minor's occupational health issues.
- Laboratories/projects requiring IBC registration/approval must obtain explicit approval from the IBC for minors to work in their laboratories.

6 ACCIDENTS, EXPOSURES, SPILL RESPONSE

Laboratory-specific SOPs should address any emergency response procedures, including those required if an accident, exposure, potential exposure, an illness which may have resulted from a possible laboratory exposure, release from primary containment; or environmental contamination of any biologically hazardous material.

Each PI and laboratory manager is responsible for developing, training in appropriate emergency procedures for his/her work area and limiting access to authorized individuals only.

PIs must be aware of the provisions for emergency procedures and preparedness. Emergency procedures and

preparedness should 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, and the *NIH Guidelines* require the preparation of emergency plans for laboratories and facilities involved in biohazardous activities.

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

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, and posted safety point of contacts (POCs) in case of emergency. KNOW WHAT TO DO BEFORE AN EMERGENCY OCCURS.

Guidelines for the development of SOPs are discussed below.

EMERGENCY PROCEDURES FOR EXPOSURE INCIDENTS

An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of an employee's duties or commission of the person's responsibilities in conjunction with the research or educational mission of UNT. A person who sustains a known or potential exposure incident must remove their gloves and treat the affected area immediately by following the appropriate exposure incident response below.

6.1.1 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 PI and/or RMS/BSO immediately.
5. Submit an Incident Report form within 24h in case of an injury.

6.1.2 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 PI and/or RMS/BSO immediately.

5. Submit an Incident Report form within 24h in case of an injury.

6.1.3 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 PI and/or RMS/BSO immediately.
5. Submit an Incident Report form within 24h in case of an injury.

6.1.4 Ingestion or Inhalation

Do not inhale. Immediately leave room. Remove Personal Protective Equipment (PPE) carefully. When removing PPE, make sure to turn the exposed areas inward but away from your body. Wash hands well with soap and water. Post a spill sign on the laboratory entry doors to prevent others from entering. The laboratory should remain evacuated for at least 30 minutes to allow for the droplets to settle and/or aerosols to be purged by the air exchange rate within the laboratory.

The PI must clear the laboratory before re-entry and spill clean-up to commence. For *extensive* BSL-2 contamination (*i.e.* an incident involving a centrifuge), the Biosafety Office must be notified immediately, and, in conjunction with the PI, will be responsible to clear the laboratory for re-entry.

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 PI and/or RMS/BSO immediately.
5. Submit an Incident Report form within 24h in case of an injury.

REPORTING AN INCIDENT

The employee must report the incident to his/her supervisor. Because the health and safety issues of the injured personnel is of primary importance, if the injury is an emergency, the supervisor should take (or make arrangements for the injured person to be taken) to the nearest care clinic or emergency room and reporting requirements should be completed *post-hoc*.

> **Please note**, there are no consequences for reporting an incident, and reporting all incidents is strongly encouraged, even near misses. This helps Risk Management, Environmental Health and Safety, and Biosafety work to improve all safety on campus

The supervisor should take measures to ensure that additional personnel are restricted from areas to prevent further inadvertent exposures. Any incident, accident, exposure, possible exposure, illness which may have resulted from exposure, releases from primary containment or environmental contamination involving biological materials which occurred in the course of accomplishing the research and/or educational missions of the university should be reported as soon as possible to the UNT Biosafety Office (IBCprogram@unt.edu). It is the responsibility of the Biosafety Office to assist PIs, Clinical Directors, and/or Instructional Course Directors in completion of any

funding agency-specific, public health, or environmental safety reporting requirements and to review the incident with the supervisor and the injured person to discuss whether alterations in the laboratory's or institution's SOPs would be prudent to prevent future similar occurrences.

Injured personnel and their supervisors should also keep in mind that the IBC and Biosafety Office are primarily concerned with ensuring that the appropriate prevention, treatment, and post-exposure follow-up measures are implemented to ensure the health and safety of the exposed personnel and the environment and prevention of future incidents. Other UNT offices may require the injured person and/or their supervisor to report incidents, accidents or exposures for other purposes, such as completion of State Workman's Compensation requirements and/or to maintain campus safety statistics. Therefore, it is recommended that PIs, Clinic Directors, and Instructional Course Directors review these requirements and incorporate these additional reporting requirements in any laboratory SOP to assist laboratory personnel in completion of all reporting requirements.

6.1.5 All accidents shall be reported as follows

Each person involved in or supporting biohazard work shall report to his/her PI 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 (near misses).

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 (IBCprogram@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 9-1-1 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. If you are injured on the job and need medical treatment, you 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. (Emergencies should call 9-1-1).

Students may receive treatment at:

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

If exposure or incident occurs with r/sDNA, work with the PI, supervisor, and Biological Safety Officer to report accident to the NIH Office of Biotechnology Activities as required by the NIH Guidelines.

An injured employee is not required to seek medical treatment if they do not wish to do so. 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.

Personnel who have been potentially exposed on the job will be provided with post-exposure evaluation and follow-up at no cost to employees who experience "exposure incidents". The post-exposure monitoring periods are dependent on the type of exposure. This time period is related to the various incubation periods of the infectious agents.

Employees can obtain copies of their Employee Health records by contacting the UNTSHC. UNT must retain medical records for your duration of employment plus 30 years.

SPILL CLEAN-UP PROCEDURES

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, but should be replaced daily. 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.

This section is intended to outline the basic procedures for dealing with some of the biological spills that may be encountered in a research laboratory. All lab personnel should refer to the relevant spill response procedures before initiating their experiments.

Spills involving infectious materials are to be contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is to be developed and **posted** within the laboratory.

6.1.6 Composition of a Basic Biohazard Spill Kit

Microbiological and biomedical research laboratories should prepare and maintain a biological spill kit. A spill kit is an essential safety item for labs working with agents which require Biosafety Level 2 and for groups working with large volumes (> 1 liter) under Biosafety Level 1 containment. The following items should be included in the spill kit:

- Concentrated household bleach (< 1 year old) or other EPA-registered disinfectant
- A spray bottle for making 10% bleach solutions

- Forceps, disposable broom/dust pan, and/or other mechanical devices for handling sharps or removing solid objects within the spill
- Paper towels or other suitable absorbent (diapers, disposable shop towels)
- Biohazard bags for the collection of contaminated spill clean-up items
- Utility gloves and disposable gloves
- Hand sanitizing wipes
- Face protection (eye wear and mask, or full face shield)
- Signage for warning others to avoid the area

Additional personal protective equipment, such as a disposable cover-gown or disposable booties may also be recommended equipment in some spill clean-up situations

Although household bleach is recommended as a standard disinfectant in the spill kit, other suitable disinfectants may be used provided the disinfectant is effective against the agents in use at the appropriate dilutions and contact time. Disinfectants should be registered with the Environmental Protection Agency as tuberculocidal for compliance with the Occupational Health and Safety Administration Bloodborne Pathogens Standard (29CFR 1910.1030). EPA-approved disinfectants may be found here.

Representatives from the IBC Office are available if you have any questions regarding biological spill response procedures or decontamination (IBCprogram@unt.edu).

6.1.7 Spills Occurring Outside of Laboratory Areas

Because laboratory facilities are designed to contain hazards within the confines of the laboratory, where all who are likely to encounter the material have knowledge of the risks, any spill outside of the laboratory area poses a particular risk for exposure of the general public or environmental contamination. Therefore, the procedures for addressing a spill which occurs outside the confines of a laboratory are:

- Attend to any injuries or exposures
- Alert others to avoid the area to prevent contamination of additional personnel and environment
- Contact PI, EH&S, the Biosafety Office to assist in spill clean-up if needed.

6.1.8 Spills within the Laboratory Areas

Because laboratory facilities are designed to contain hazards within the confines of the laboratory, spills within the laboratory are generally not as potentially problematic as those which occur outside the laboratory. Of course, the overall risk will depend on the agents, operations, and personnel involved in the spill and clean-up measures.

6.1.8.1 *Biosafety Level 1 (BSL-1) Spills*

Immediate Procedures

- Notify others in the area to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing and wash exposed skin with soap and water.
- Isolate the spill area.

Clean-up of BSL-1 Spill

- Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill allowing it to mix with spilled material. Allow suitable contact time, at least 15 min.
- Pick up any pieces of broken glass with forceps or other mechanical device(s) (never use hands) and place in a sharps container.
- Discard all disposable materials used to clean up the spill into a biohazard bag.
- Wash hands with soap and water.

6.1.8.2 *Biosafety Level 2 (BSL-2) Spills*

Immediate Procedures

- Remain calm and make note of whether your person has been contaminated.
- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave.
- Close door, and post a warning sign.
- Remove contaminated clothing, turning exposed areas inward but away from your body, and place in a biohazard bag.
- Wash all exposed skin with soap and water.
- Inform Supervisor, and, if assistance is needed, consult the Biosafety Office.

6.1.8.3 *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 PI and to RMS/BSO immediately.
8. Resume work if deemed safe by supervisor/manager.
9. Submit Incident Report

6.1.8.4 *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, then leave the room.
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 PI, 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 Incident Report

6.1.8.5 *Small Spill (<500 ml) of r/sDNA 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 Incident Report

6.1.8.6 *Large Spill (>500 ml) of r/sDNA 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 PI, 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. Wear appropriate Personal Protective Equipment (PPE) (e.g., disposable gown, protective eyewear, gloves, shoe coverings and respiratory protection if needed) before initiating spill cleanup.
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; place in a biohazard bag for autoclaving.
    g. Wash your hands with soap and water after removing your gloves.
11. Submit Incident Report

6.1.8.7 *Blood Spills*

For blood or other material with a high organic content and low concentration of infectious microorganisms:

1. Wear appropriate PPE (gloves, eye protection, and a lab coat) before initiating spill cleanup.
2. Absorb blood with paper towels and place in a biohazard bag. Collect any sharp objects with forceps or other mechanical device (never use your hands) and place in a sharps container.
3. Using a detergent solution, clean the spill site of all visible blood.
4. Spray the spill site with 10% household bleach and allow to air-dry for 30 minutes.
5. After the 30 minute contact time, wipe the area down with disinfectant-soaked paper towels.
6. Discard all disposable materials used to decontaminate the spill and any contaminated personal protective equipment into a biohazard bag.
7. Wash your hands with soap and water after removing your gloves.
8. Submit Incident Report

6.1.8.8 *Spill in a Biological Safety Cabinet*

1. Remain calm and secure research samples.
2. Alert the other laboratory employees of the spill.
3. Leave the biological safety cabinet blower on and begin cleanup immediately.
4. While wearing PPE (gloves and gown) cover the spill area with paper towels or disinfectant soaked paper towels. Do not place your head in the cabinet to clean the spill, keep your face behind the view screen.
5. If necessary, flood the work surface as well as the drain pans and catch basins below the work surface with disinfectant. Be sure the drain valve is closed before flooding the area under the work surface.
6. Allow a contact time of at least 20 minutes.
7. Wipe cabinet walls, work surfaces, and inside the view screen with disinfectant.
8. Lift the front exhaust grill and work surface; wipe all surfaces with disinfectant. Be sure no paper towels or soiled debris are blown into the area under the spill tray
9. If the work surface, as well as drain pans and catch basins under the work surface, have been flooded with disinfectant, soak up the disinfectant in the work surface. Place a container under the drain valve and drain the disinfectant under the work surface into the container.
10. Wipe the areas under the work surface to remove residual disinfectant.
11. Wash hands and exposed skin with soap and water.
12. Dispose cleanup materials in the biohazard waste container.
13. Notify your PI or supervisor.
14. If the spill overflows the drain pan/catch basin under the wok surface into the interior of the biological safety cabinet, notify the Biosafety Office. A more extensive decontamination of the biological safety cabinet may be required.
15. Resume work if deemed safe by supervisor/manager
16. Submit Incident Report

6.1.8.8.1 *Large Spill (>500 ml) of r/sDNA Molecules in a Biological Safety Cabinet*

1. Biological safety cabinets must run during cleanup to contain aerosols and to filter exhaust air.
2. Wear appropriate PPE before initiating cleanup. Close the lab until clean up is completed.
3. Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is not acceptable.
4. If the spill is contained on a bench pad, remove the contaminated bench pad 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 (never use hands). Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
9. Place contaminated re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re- use.
10. If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required.
    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 PI, 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 Incident Report

6.1.8.9 *Centrifuge Spill*

- Always use sealed safety-cap sealed buckets or sealed rotors with O-rings. Examine O-ring and replace if worn, cracking or missing. Check tubes and bottles for cracks and deformities before each use.
- Wait five minutes before opening the centrifuge following the end of a run with potentially hazardous biological material if using safety caps or sealed rotors. If a spill is identified after the centrifuge lid is opened, carefully close the lid and evacuate the laboratory and close the laboratory door. Remain out of the laboratory for at least 30 minutes. Post a sign on the laboratory door indicating there is a biohazard spill and do not enter.
- Remove any contaminated protective clothing and place into a biohazard bag. Wash hands and any exposed skin surfaces with soap and water.
- Notify your supervisor, EH&S and the Biosafety Office (IBCprogram@unt.edu).
- Submit Incident Report

6.1.8.9.1 *Spill of Biohazards (Including r/sDNA 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.

6.1.8.9.2 *Unsealed Centrifuge 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 (never use hands). 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.
12. Wash your hands with soap and water after removing your gloves.
13. Notify PI, supervisor, and/or RMS/BSO.
14. Submit Incident Report

6.1.8.9.3 *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 PI, supervisor, and RMS/BSO if there was a potential for any material escaping the centrifuge.
4. Submit Incident Report.

6.1.8.10 *Spill of a Biohazardous Radioactive Material*

A biohazardous spill involving radioactive material requires emergency procedures that are different from the procedures used for either material alone. Use procedures that protect you from the radiochemical while you disinfect the biological material.

Before any clean up, consider the type of radionuclide, characteristics of the microorganism, and the volume of the spill. Refer to the Radiation Safety Manual or contact the UNT Radiation Safety Office (RSO@UNT.EDU) for isotope cleanup procedures.

Immediate Procedures

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave.
- Close door, and post a warning sign.
- Remove contaminated clothing, turning exposed areas inward but away from your body, and place in a biohazard bag labeled with a radioactive materials label or a radioactive waste container labeled with a biohazard label.
- Wash all exposed skin with soap and water; follow with a three-minute water rinse.
- Inform supervisor and Radiation Safety Office of spill, and monitor all exposed personnel for radiation.

Clean-Up of a Biohazardous Radioactive Material

- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, autoclavable containers, forceps, towel, and sponges), and confirm with Radiation Safety (RSO@UNT.EDU) representative that it is safe to enter the lab.
- Put on protective clothing (gown, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA-filtered respirator instead of a surgical mask.
- Cover the area with disinfectant-soaked towels, and carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least 30 minutes contact time.

Do Not use bleach solutions on iodinated material, radioiodine gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.

- Handle any sharp objects with forceps (never use your hands). Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- Soak up the disinfectant and spill, and place the biologically decontaminated waste, along with all protective clothing contaminated with radioactive materials, into an approved radioactive waste container and label it according to Radiation Safety Guidelines.
- Contaminated protective clothing should also be biologically decontaminated, if possible, prior to disposal as radioactive waste.

Do Not autoclave the waste unless this action is approved by the Radiation Safety Officer. If waste cannot be autoclaved, add additional disinfectant to ensure biological decontamination of all the materials.

- Wash hands and exposed skin areas with soap and water; monitor personnel and spill area for residual radioactive contamination.
- If skin contamination is found, repeat decontamination procedures under the direction of the Radiation Safety Officer.
- If the spill area has residual activity, determine if it is fixed or removable and handle accordingly.
- Discarding items contaminated with radioactive materials:
- Place the contaminated item(s) on absorbent paper.
- Spray EPA-approved disinfectant on the contaminated areas and allow 20 minute contact time.
- Wrap the item(s) inside the paper and dispose of as radioactive waste.
- Wash hands with soap and water.

INVESTIGATION OF LABORATORY ACCIDENTS

Environmental Health and Safety (EHS) and the IBC, in cooperation with the PI and staff, will conduct the necessary investigation of a laboratory accident. The goal of the investigation is the prevention of similar accidents as well as obtaining information concerning the circumstances and number of employees who have been exposed to the agent in question. In addition, RMS/EHS, in consultation with Human Resources might institute further steps to monitor the health of those who may have been exposed to the agent in question.

It should be emphasized that the reporting of accidents to the PI or laboratory supervisor is the responsibility of the employee who has the accident. The PI or the laboratory supervisor should then report to the EH&S or Biosafety Office (IBCprogram@unt.edu) if IBC protocol research is involved.

We encourage PIs and employees to also report incidents that did not result in an exposure ("near misses") to the Biosafety Office. Evaluation of near misses can lead to alternative work practices and implementation of engineering controls to minimize future incidents.

Whenever an injury involves a sharp and human material (body fluid, tissue, cell line, etc.) the Biosafety Office must perform an investigation to determine if a safe sharps device is available to prevent future occurrences of the injury. If safe sharps devices are available, they must be evaluated by the biosafety office in conjunction with the Group or Department. The incident must also be recorded on the University's Sharps Injury Log. The confidential log will include the type and brand of device involved in the incident; the Department or work area where the exposure incident occurred; and an explanation of how the incident occurred.

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

7 DISINFECTION AND DECONTAMINATION OF BIOLOGICALS

7.1 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. BMBL Appendix B details decontamination and disinfection of laboratory surfaces and items.

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.

7.1.1 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.

7.2 DECONTAMINATION METHODS

Decontamination methods fall into three main categories: heat, application of chemical decontaminants (including vapors and gases), and physical methods (such as filtration or irradiation).

7.2.1 Heat

The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121°C under pressure in the autoclave is the most convenient method of rapidly achieving sterility under ordinary circumstances. Dry heat at 160°C to 170°C for periods of two to four hours is suitable for destruction of viable

agents on an impermeable non-organic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat for decontamination. Incineration serves as an efficient means of disposal for human and animal pathological wastes.

7.2.1.1 Autoclave Use and Maintenance

Moist heat causes the denaturation of proteins at lower temperatures and shorter times than dry heat. One of the most effective physical decontamination controls is steam sterilization (autoclave) which generates moisture and high temperature pressurized steam within a sealed chamber. Autoclaves can sterilize all items that are heat stable. In gravity autoclaves, a cycle of 250°F (121°C) at 15 to 18 pounds per square inch (psi) of pressure for one hour may be required for decontamination. In the newer vacuum autoclaves, decontamination may require a cycle of 270°F (132°C) at 27 to 30 psi for 45 minutes. Cycle temperature and time is dependent on loads. For an entire waste load to reach and maintain the proper time, temperature, and pressure, the cycle needs to be set at 123°C for at least 70 minutes.

Table 7.A. Criteria for Autoclaving Typical Materials		
Material	**Temperature**	**Time**
Laundry	121 C°	30 minutes
Trash (biohazard bags, <2/3 full, containing waste	**121 C°**	**45 minutes**
Glassware	121 C°	60 minutes
Liquids	121 C°, each gallon	60 minutes

The hazards of handling hot solids and liquids are reasonably familiar. Laboratory personnel should be cautioned that steam under pressure, such as that found in autoclaves, could be a source of scalding jets if the equipment is misused. When preparing to use an autoclave, check the door seal gaskets each time to ensure these are intact prior to using the autoclave. Also, before use, check to make sure the drain at the bottom of the autoclave is unobstructed. Prepare to autoclave loads of manageable size only. Do not overfill autoclaves.

Fluids treated by steam under pressure may be superheated if removed from the sterilizer too soon after treatment, which may cause a sudden and violent boiling of contents from the containers that can splash scalding liquids onto personnel handling the containers. Therefore, slow exhaust cycles should be used to autoclave liquids. In addition, bottles with liquids should allow for the liquid expansion during autoclaving; "head room" should be left in the vessels before autoclaving to avoid over-pressurization occurring inside of vessels with liquids if gas is not able to freely enter/exit the bottle during the cycle. Therefore, only loosely fit covers or caps on these vessels prior to autoclaving.

For solid materials, keep in mind that steam must be in contact with the materials to efficiently sterilize them. Therefore, autoclave bags should be left partially opened and/or some additional water should be placed on the inside of the bag prior to autoclaving.

Because of the air and liquid exchange inside of bags or vessels, other hazardous materials should not be included in autoclave loads. Mixed waste—either chemicals (such as phenol: chloroform, or bleach) or radiological material mixed with biological materials should never be autoclaved due to the chemical and radiological hazards present.

In addition, some materials should never be autoclaved. Nitrocellulose materials (tubes/filters), for instance, can explode under autoclave temperatures. Make sure any plastic materials that are autoclaved are guaranteed "autoclavable" by the manufacturer. Most plastics will melt inside the autoclave and produce irritating odors.

Red polypropylene, preprinted, biohazard autoclave bags (FisherScientific 01-828 A through E) should be utilized in UNT autoclaves if biohazardous waste. However, DO NOT use red bags for non-biohazardous waste! Do not use polyethylene bags, as these will melt at higher temperatures. DO NOT enclose the 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!

The outer collection container must be durable, leak proof, have a lid and be of such a design so as not to be mistaken by Housekeeping 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. 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.

Always place materials to be autoclaved inside of a metal or *autoclavable* plastic autoclave tray to prevent spillage of agar or melted plastics into the bottom of the autoclave. (Note: most plastics are **not** autoclavable unless specially formulated, so check the manufacturer's specifications to ensure your plastics are autoclavable!). Melted and re-solidified agar or plastic plugs the drain at the bottom of the autoclave, which prevents proper function of the autoclave and often requires maintenance to repair.

Wear closed toe shoes, pants, lab coat, and long sleeved insulated gloves when operating an autoclave. Allow time for loads to cool before removing them from autoclaves after a run. Take proper precautions when first opening the door; do not stand directly in front of the door when opening.

All autoclaves should be on a preventative maintenance program and certified regularly to ensure proper function. Heat-sensitive autoclave tape can be used to ensure that an autoclave got warm; however, this is insufficient to tell the user that the appropriate temperature and pressure were maintained over a sufficient period of time to provide full decontamination.

If you experience any problems or unusual occurrences during autoclave use, please report these to your supervisor and/or building/department manager to enable them to contact the autoclave maintenance provider. For autoclaves attached to "house" steam lines, ensure that steam pressure is sufficient to operate the autoclave prior to contacting external repair offices

7.2.1.2 Decontamination Cycle Testing and Verification

Please note: UNT does not have a campus-wide autoclave certification and maintenance program. Responsibility for certifying and maintaining autoclaves falls to the owner—the department or PI who owns the autoclave. *Geobacillus stearothermophilus* biological indicators with waste using average spore populations of 10^4 to 10^6 organisms must be used bi-monthly for autoclaves that are utilized to treat special biohazardous waste (see section 8.2), quarterly for all other autoclaves. There are many commercially available biological indicators with a choice of spore ampoules or spore strips with growth media. Contact the EH&S with any questions.

Follow the instructions provided by the manufacturer of the biological indicators. Some 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 during the incubation period (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 and autoclaved, 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 at AskRMS@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.

7.2.1.3 Autoclave Preventative Maintenance

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

- A service contract with the vendor is recommended.
- 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.

7.2.2 Chemical Decontaminants

In general, chemical decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems if allowed (contact RMS prior to disposal). A list of EPA-approved disinfectants may be found on the UNT biosafety website here.

7.2.2.1 Liquid Chemical Decontaminants

There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, contact time, pH, the presence and state of dispersion, penetrability, and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in the effectiveness of decontamination. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids and alkalis, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant, the more likely it will possess undesirable characteristics such as corrosivity. In addition, some of the chemical disinfectants will require disposal as chemical waste after disinfection.

None is equally useful or effective under all conditions for all infectious agents. Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned to the task of making up use-concentrations from stock solutions must be informed of the potential hazards and trained in the safe procedures to follow and appropriate personal protective equipment to use as well as the toxicity associated with ocular, skin, and respiratory exposure.

7.2.2.2 Vapors and Gases

A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde, vapor-phase hydrogen peroxide (VHP), chlorine dioxide, and ethylene oxide. When these can be employed in a closed system and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating biological safety cabinets and associated air-handling systems and air filters; bulky or stationary equipment that resists penetration by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; and rooms, buildings, and associated air-handling systems.

Only those who have received appropriate training in vapor and gas decontamination should attempt these operations. They should avoid inhalation of vapors of any of these vapors of gasses. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas. In preparing use-dilutions and when applying them, personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary.

Mutagenic potential has been attributed to ethylene oxide; toxic and hypersensitivity effects are well-documented for formaldehyde. Ethylene oxide use is very limited and is generally used in surgical and clinical areas.

If you have a BSC, equipment, or space that requires vapor or gas decontamination, please contact the Biosafety Office to discuss options.

7.2.3 Physical Methods

Alternative measures for decontamination which rely on physical properties of decontaminants are often used as precautionary measures, such as HEPA filtrations on BSC or exhaust systems, rather than a primary mode of disinfection.

Gamma-wave irradiation is another method used for decontamination; however, this often requires a high energy irradiator, currently making it impractical as an everyday method for decontamination of most materials at UNT. UV irradiation is a somewhat controversial method of disinfection for surfaces. While it is an effective measure, time of exposure, distance, presence of dust or debris and UV lamp intensity can all affect the germicidal effect of the UV lamp. Users should recognize that the visible blue-violet glow of the UV lamp does not indicate there is germicidal effect. The UV lamp needs to be cleaned periodically to remove dust. UV lamps may damage eyes, skin, and laboratory equipment and must be shut off while the room is occupied.

Because users tend to over-rely on UV irradiation for surface decontamination in lieu of thorough chemical surface decontamination and the compounding factors which may impede proper decontamination using UV irradiation, the Biosafety Office discourages the use of UV lamps. For further information related to the use of UV lamps for surface decontamination, please see the following references:

Burgener, J (2006) Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. *Applied Biosafety 11*(4): 228-230.
Meecham, P.J. and Wilson, C. (2006) Use of Ultraviolet Lights in Biological Safety Cabinets: A Contrarian View. *Applied Biosafety 11*(4): 222-227.

7.3 CHARACTERISTICS OF CHEMICAL DECONTAMINANTS

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, or liquid concentrates. These may be added to water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperature are useful as space-penetrating decontaminants. Others become gases at elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways:

- Coagulation and denaturation of proteins
- Lysis
- Binding to enzymes or inactivation of an essential enzyme by oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants may be altered substantially by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending on how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay system employed. Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms rather than failure of the decontaminant to act. If an item is placed in a liquid decontaminant, tiny bubbles are visible on the surface of the item. The area under the bubbles is dry and microorganisms in these dry areas will not be affected by the decontaminant. If there are spots of grease, rust or dirt on the item, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful. A decontaminant should have, and most do have, incorporated surface-active agents.

7.3.1 Properties of Some Common Decontaminants

7.3.1.1 Chlorine/Hypochlorites

This halogen is a universal decontaminant active against a broad spectrum of microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in the presence of protein. Free available chlorine is the active element. Chlorine solutions must be prepared frequently because of its instability in water. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25%, or 52,500 ppm, available hypochlorite. If diluted 1 to 10, the resulting solution will contain 0.525% or 5,250 ppm of available hypochlorite. The addition of a 0.7% (v/v) of nonionic detergent can improve the disinfection properties.

Because hypochlorite is a strong oxidizing agent, it can be corrosive to metals. Therefore, caution should be taken to prevent etching of stainless surfaces cleaned with bleach solutions. This effect can be limited if disinfection if followed by a sterile water rinse or ethyl alcohol (EtOH).

7.3.1.2 Alcohols

Ethyl or isopropyl alcohol in a concentration of 70-85% by weight is often used; however, both lose effectiveness at concentrations below 50% and above 90%. Alcohols denature proteins and are somewhat slow in germicidal action. However, alcohols are effective decontaminants against lipid-containing viruses. A contact time of ten minutes is generally employed in efficacy tests with disinfectants. Due to the high evaporation rate of alcohols, repeated applications may be required to achieve the required ten-minute contact time for decontamination. Because of this, the OSHA Bloodborne Pathogens Standard does not recognize alcohol as an effective decontaminant for surfaces. Given this and recent studies, **UNT and OSHA do not accept alcohols as appropriate primary disinfectants**.

Isopropyl alcohol is generally more effective against vegetative bacteria; EtOH is a more virucidal agent.

Alcohols are also very flammable, so precautions should be taken to prevent exposure to spark or flame sources.

7.3.1.3 Aldehydes (Formaldehyde, Glutaraldehyde)

Aldehydes are commonly-used fixative agents in laboratories; however, during the process of fixation, materials are decontaminated. Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration, referred to as formalin, or as a solid polymerized compound called paraformaldehyde. Glutaraldehyde is commonly used in concentrations of 2-4%.

Formaldehyde at a concentration of 5% active ingredient is an effective liquid decontaminant. A glutaraldehyde-based commercial disinfectant (Cidex®) is used in some hospital settings. Aldehyde disinfectants lose considerable activity at refrigeration temperatures, and the pungent, irritating odors and health risks make formaldehyde solutions difficult to use in the laboratory. The use of Cidex® has been restricted in the U.K. because of the carcinogenic effects. All materials disinfected with aldehydes must be disposed as chemical wastes to adhere to EPA standards.

Formaldehyde vapors generated from solution is an effective space decontaminant for buildings or rooms, but in the vapor state in the presence of water it tends to polymerize on surfaces to form paraformaldehyde, which is persistent and unpleasant. Heating paraformaldehyde to depolymerize can liberate formaldehyde gas. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than is the case in the vapor state.

7.3.1.4 Phenols

Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants, such as the original Lysol® and Amphyl®. Phenolic compounds are effective decontaminants against some viruses, fungi, and vegetative bacteria, including rickettsiae. Phenolics are not effective in ordinary use against bacterial spores.

7.3.1.5 Quaternary Ammonium Compounds (a.k.a. "Quats")

After 40 years of testing and use, there is still considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses and often vegetative gram positive bacterial, however, they have variable activities against gram negative bacteria and fungi, and are not very effective against non-lipid enveloped viruses. The Quats will attach to protein so that dilute solutions will quickly loose effectiveness in the presence of proteins. Quats tend to clump microorganisms and are neutralized by anionic detergents such as soap. They have the advantages of being nontoxic, odorless, stable, non-staining, non-corrosive to metals, and inexpensive. Many common household and laboratory disinfectants are Quats (e.g., Roccal®, Germex®, Coverage Plus®).

7.3.1.6 Iodine

The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants for laboratory use are the iodophors, including Wescodyne®, Betadyne® and Providone®.

The range of dilution of Wescodyne® recommended by the manufacturer is 1 oz. in 5 gal. of water (25 ppm available iodine) to 3 oz. in 5 gal. of water (75 ppm available iodine). The small amount of free iodine available in this range can rapidly be taken up by extraneous protein that may be present. Clean surfaces or clear water can be effectively treated with 75-ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For iodophors such as Wescodyne®, it is critical that the manufacturer's written instructions are followed. Higher concentrations of iodophores are actually less effective, as the iodine is bound to itself or the carrier molecule. For washing the hands or for use as a sporicide, it is recommended that Wescodyne® be diluted 1 to 10 in 50% ethyl alcohol (a reasonably good decontaminant itself.) This will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

7.3.1.7 Peroxygens

Peroxide-based disinfection has become more popular in recent years with the increasing popularity of vapor-phase hydrogen peroxide generators, which are an effected area decontamination method. Liquid peroxygen disinfectants, such as Virkon-S® are also available as surface decontamination methods. Peroxygens have broad-spectrum disinfectant properties and are generally effective against vegetative bacteria, viruses, and some spores. Peroxygens have variable efficacy in the presence of organic matter. However, peroxygens can be incompatible with some materials (such as Aluminum, Copper, Zinc, Brass, Natural rubber, and some plastics), which should be considered when selecting disinfectants.

7.3.2 Selecting Chemical Disinfectants

No single chemical disinfectant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical disinfectants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

- What is the target organism(s)?
- What disinfectants, in what form, are known to, or can be expected to, inactivate the target organism(s)?
- What degree of inactivation is required?

- In what menstruum is the organism suspended (i.e. simple or complex, on solid or porous surface, and/or airborne)?
- What is the highest concentration of organisms anticipated to be encountered?
- Can the disinfectant, either as a liquid, vapor, or gas, be expected to contact the organism and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility of materials?
- What is the stability of the disinfectant in use concentrations, and does the anticipated use situation require immediate availability of the disinfectant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?
- Will conditions permit safe use of some disinfectants (*e.g.,* is ventilation sufficient to safely use aldehydes)?

The primary target of decontamination in the laboratory is the organism(s) under investigation. Laboratory preparations or cultures usually have titers in excess of those normally observed in nature. Inactivation of these materials presents other problems since agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active components of chemical disinfectants. Such interference with the desired action of disinfectants may require higher concentrations and longer contact times than those shown to be effective in the test tube. Similarly, a major portion of the contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information with which to predict the probable virulence of these more resistant cells. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting disinfectants and procedures for their use.

Organisms exhibit a range of resistance to chemical disinfectants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid-containing viruses are relatively susceptible to chemical disinfection. The non-lipid-containing viruses and bacteria with a waxy coating, such as tubercule bacillus, occupy a mid-range of resistance. Spore forms and unconventional (slow) viruses are the most resistant.

A disinfectant selected on the basis of its effectiveness against organisms on any range of the resistance scale generally will be effective against organisms lower on the scale. Therefore, if disinfectants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other organism generated by laboratory operations, even in higher concentrations, would also be inactivated.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in the table on the following pages. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic content. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. Individual investigators should conclusively determine the efficacy of any of the disinfectants. It is readily evident that each of the disinfectants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of disinfectants.

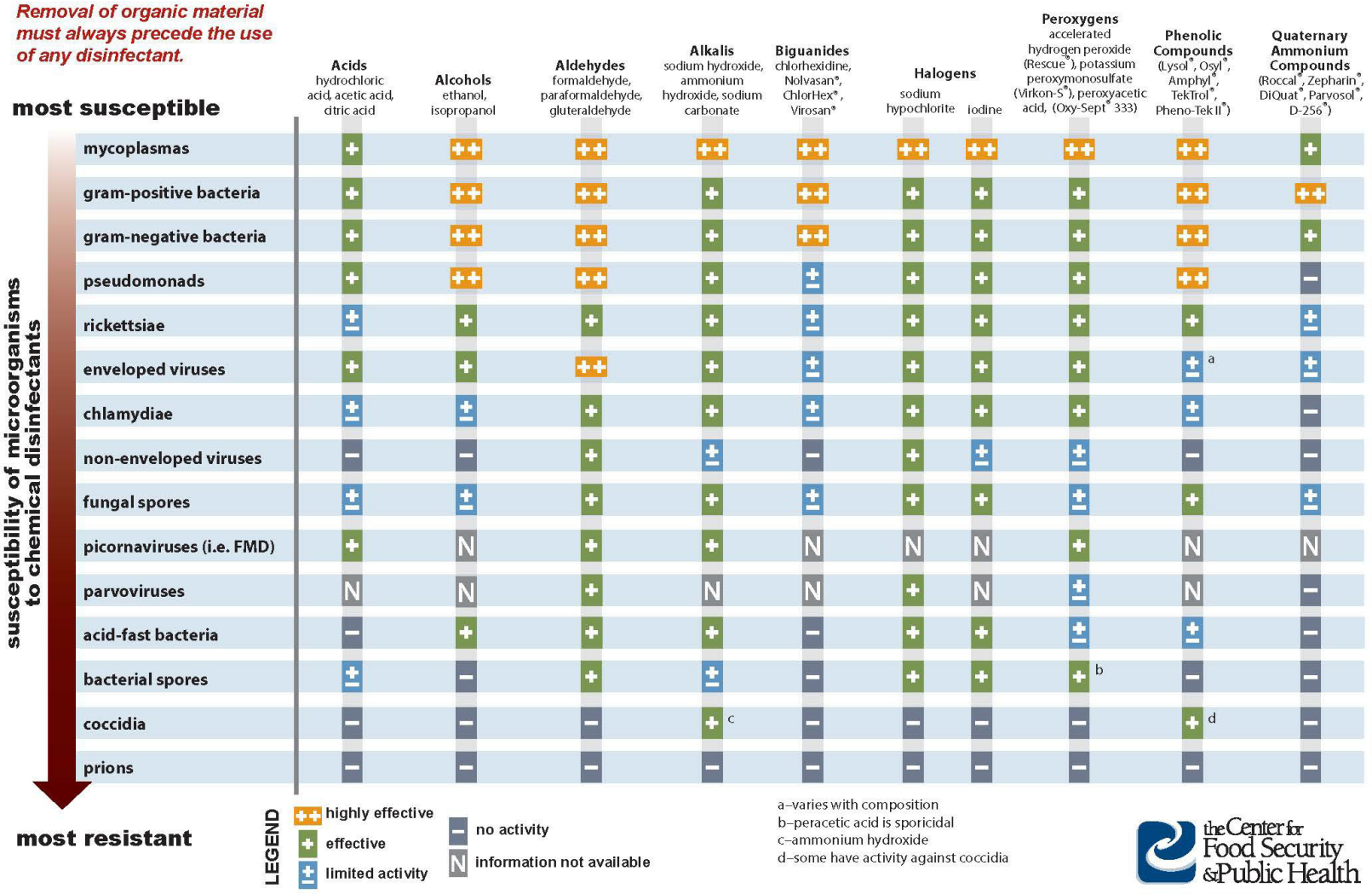
7.3.3 Characteristics of Some Liquid Disinfectants Table

The following tables, prepared by the Iowa State University Center for Food Security and Public Health are provided for reference on properties of various chemical disinfectants.

The Antimicrobial Spectrum of Disinfectants

This table provides general information for selected disinfectant chemical classes. Antimicrobial activity may vary with formulation and concentration. *The use of trade names does not in any way signify endorsement of a particular product. They are provided as examples.*

Removal of organic material must always precede the use of any disinfectant.



a–varies with composition
b–peracetic acid is sporicidal
c–ammonium hydroxide
d–some have activity against coccidia

the Center for Food Security & Public Health

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Quinn PJ, Markey FC et al. (eds). *Veterinary Microbiology and Microbial Disease.* 2nd ed. 2011. West Sussex, UK: Wiley-Blackwell, pp 851-889.

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8 BIOLOGICAL WASTE MANAGEMENT AND SPECIAL WASTE

PURPOSE OF BIOLOGICAL WASTE MANAGEMENT

This section is intended to guide UNT personnel in the safe and legal way to dispose of biohazardous special biological waste. Our program is designed to protect the people who handle, transport, and dispose of your waste; protect the environment; protect the public perception of the university; and minimize UNT's regulatory liability. Attempts to ignore or work around the procedures listed in this manual may place other people and the University at risk. The costs associated with one injury or violation fines can easily exceed annual operational costs.

The biological waste management program does not supersede the requirements for radioactive and/or hazardous chemical waste programs. Radioactive or hazardous chemical wastes shall be disposed of through the radioactive waste stream or the hazardous chemical waste stream respectively. In fact, in mixed waste situations (biological/chemical or biological/ radiological), the waste disposal requirements of the chemical or radiological waste disposal procedures will take precedence over the biological, particularly since biological wastes are more capable of being decontaminated/deactivated prior to placing the waste in the chemical or radiological waste streams.

Environmental Health and Safety is continually working to improve this program. Direct any questions or suggestions to the Risk Management, Environmental Health and Safety Office. Call or email if you have questions about unusual situations or anything not covered in this guide.

DEFINITION OF SPECIAL BIOMEDICAL WASTE

"Biological waste", "biomedical waste", and "biohazardous wastes" are terms commonly applied interchangeably on UNT's campus. "Special Waste" is a regulatory term used by the state of Texas. These materials must be excluded from the general waste stream unless appropriately decontaminated or deactivated per 30 TAC §1.326, 25 TAC §1.136, and 25 TAC §1.135. These include:

1. Waste Cultures and Stocks of Microorganisms or Etiologic Agents Including (special waste):
    a) Cultures and stocks of agents or microorganisms from facilities assigned to Biosafety Levels 1 through 3 (BSL-1, BSL-2, BSL-3). (Note, UNT only has BSL-1 and BSL-2 Labs at this time)
    b) Cultures of specimens from medical and pathological laboratories.
    c) Disposable containers, materials, and supplies that may have been contaminated during the manipulation and transportation of microbial cultures and stocks. This includes culture dishes and devices used to transfer, inoculate, and mix cultures.
    d) Wastes from the production and handling of biological materials (including all tissue culture materials.)
    e) Live and attenuated vaccines.
2. Human Pathological Wastes (special waste)*
    Pathological waste consists of all recognizable human tissues and body parts (except teeth) which are removed during surgery, obstetrical procedures, autopsy, and laboratory procedures. This also includes bulk blood and blood products, exudates secretions, suctionings, and other dialysate; cerebrospinal, synovial, pleural, peritoneal, and pericardial fluids and other bodily fluids; and their respective containers.
3. Waste Human Blood and Blood Products and Their Containers Including (special waste):
    a) Waste human blood and blood products (e.g. blood plasma, platelets, red or white corpuscles, and other derived licensed products such as interferon, etc.)
    b) Items contaminated with human blood or blood products.
    c) Items caked with dried human blood or blood products.
    d) Intravenous bags.
    e) Human tissues and cell cultures

f) Items contaminated with human tissues or cell cultures.

4. Contaminated Sharps Waste (special waste)

   This category includes used hypodermic needles, syringes (with or without the attached needles), Pasteur pipettes, disposable plastic pipettes, scalpel blades, razor blades, blood vials, test tubes, needles with attached tubing, broken plastic culture dishes, unbroken glass culture dishes, and other types of broken and unbroken glassware that was in contact with biological material including microscope slides and coverslips.

5. Unused Sharps Waste

   Unused hypodermic needles, suture needles, syringes, and scalpel blades. This includes those which have never been in contact with any biological materials.

6. Waste Animal Carcasses, Body Parts, and Bedding (special waste)

   All animal carcasses, bedding, and body parts disposal must be approved by the biosafety office. All animal carcass and body parts will be stored in the appropriate freezers and placed in the RedAway waste carts for transport to RedAway and incineration. Any animal bedding, caging or carcasses which have been purposely infected or known to have been exposed to Risk Group 2 agents shall be autoclaved prior to disposal in Biowaste containers.

7. Chemotherapy waste*

   Any disposable material which has come in contact with cytotoxic/antineoplastic agents(agents toxic to cells) and/or antineoplastic agents (agents that inhibit or prevent the growth and spread of tumors or malignant cells) during the preparation, handling, and administration of such agents. Such waste includes, but is not limited to, masks, gloves, gowns, empty IV tubing bags and vials, and other contaminated materials. Liquid waste containers must first be classified as empty which means a quantity that it is not subject to other federal or state waste management regulations prior to being handled as biomedical waste (typically less than 3%).

In general, biological waste at UNT can be divided into five categories:

1. Solid biomedical waste (including sharps)
2. Pathological waste*
3. Liquid biomedical waste
4. Chemotherapy agent wastes**
5. Mixed biological waste (bio/rad or bio/chem.) **

**These wastes must be incinerated and may not be placed in biohazardous waste cardboard cartons unless a special incineration sticker has been applied. For proper disposal information, contact the Biosafety Office IBCprogram@unt.edu or RMS at AskRMS@unt.edu*

***Theses wastes are not disposed of as biohazardous wastes. For proper disposal information, please contact the Biosafety office or RMS.*

SOLID "SPECIAL" BIOMEDICAL WASTE

Following collection from the laboratories and clinics, all solid special biological waste generated by UNT is congregated for transport in a truck for eventual transportation by a professional biomedical waste handling company (as of May 2019, that contractor is RedAway). RedAway will transport the waste containers to their facilities for decontamination and disposal.

Even though the waste is transported away from UNT for decontamination and disposal, UNT is still liable for the environmental repercussions of inappropriate transport or disposal of any waste generated at UNT; therefore, it is crucial that all personnel who may handle biologicals understand the appropriate waste disposal procedures:

- Liquids or soggy materials should NOT be placed inside the RedAway red-bag lined boxes. Although the red bags are of high grade, there is still a potential for these biohazardous wastes to leak from the bags before or during transport. This not only exposes UNT and/or RedAway staff to this material, but transport of uncontained biohazardous waste constitutes a violation of the U.S. Department of Transportation regulations and can result in steep fines.
- No chemicals or radiological materials should be placed in the general biomedical waste stream due to the potential risk posed to the health and safety of both UNT and RedAway handlers, but also to the risks of contaminating the landfill into which this waste is placed.
- Special biohazardous waste, by definition, is hazardous material and must remain secured at all times. Biohazard waste boxes or containers should not be left in unsecured areas (*e.g.,* in hallways or on loading docks) where non-trained personnel or personnel with unknown health status may encounter them. They should be removed from the laboratories and clinics and directly transferred and secured in the biohazardous waste room until pickup by RedAway. Placing this waste in secondary accumulation areas (*e.g.,* closets within buildings) is not permitted. Care should be taken to not allow the boxes to become wet or damaged or exposed to vermin.
- Never remove a leaking biohazard waste box from a laboratory. Notify the laboratory staff and/or the Biosafety Office to enable the staff to safely re-package the wastes prior to removal. If leakage is noticed after removal from the laboratory, contact the Environmental Health and Safety biosafety office immediately, and provide relevant information to identify the laboratory of the waste's origin.
- Because it is significantly more costly to dispose of special biomedical waste than regular trash, items which are not special biological waste or potentially contaminated with biological materials should NOT be placed in the biohazardous waste containers. This includes pipette wrappers, notebook papers, paper towels, which have not been in contact with any biological material. However, if one is unsure whether a particular item is contaminated with biological material, one should default to the biohazardous waste container. Since food items are not permitted in the laboratory, soda cans, candy wrappers, etc. should never be found in biohazard waste containers.
- Biohazardous waste bags/boxes/sharps containers should NOT be used for alternate purposes other than for collection of biohazardous wastes. These are provided by the biohazardous waste contractor and should be used expressly for collection of biohazardous wastes.

8.1.1 Proper Solid Biohazardous Waste Disposal Procedures

- Non-sharps solid biohazardous waste must be collected for final treatment and disposal in a leak-proof container lined with an autoclavable bag of moderate thickness to prevent punctures. The collection container must have a lid or other means of closure, and the container must be labeled with the biohazard symbol regardless of the lab's operating biosafety level.
- Bench-top biohazardous waste containers should meet the same criteria. Bags should be secured closed and transferred to the floor container when full. If the container has no lid, the bag needs to be securely closed and transferred to the floor container at the end of the day.
- Waste generated in a BSC should be collected in a biohazard container inside the BSC whenever procedures permit. Bags should be securely closed and placed in an appropriate leak-proof secondary container for transfer to the designated biohazardous waste pickup location. (Heavy bags should be double-bagged to prevent leakage during the handling process.)
- The UNT EHS/RMS office is responsible for providing the biohazardous "red bag" waste boxes and removing these boxes from the laboratory and clinics. To request additional biohazardous waste boxes, or to request waste pick-ups, please contact RMS AskRMS@unt.edu .
- Biowaste boxes and red bags are delivered in each arriving empty truck from RedAway. Additional Supplies are stored in the biohazardous waste room.

- The UNT EHS RMS office will provide and handle the biohazard "red bag" box. Alternate waste containers need to be provided by the laboratory and placed into one of the authorized RedAway waste containers by the laboratory staff for removal by the EHS Biosafety.

The biohazard "red bag" box

- Only solid waste must be added to this stream. No liquids or soggy materials should be placed in these containers
- No loose sharp items should be placed in these containers which would puncture the red bags
- These containers have a **43 lb. maximum weight** limit.
- The box should be closed and removed before the contents leak or spill over the edge. Do not overfill boxes.
    - Each box should be lined with one or two red biohazard plastic bag, folded over the box flaps,
    - An orange or green UNT waste label should be placed on the box. The label should include the date the waste box was put into service, the PI/Supervisor name, the building and room number where the waste originated, and the contact number of the PI.
    - After filling, while wearing appropriate PPE, **the laboratory staff** should carefully gather the edges of the bag together to avoid production of aerosols, and grasp the bag approximately 10 inches below the edges and twist the bag closed. The twisted bag should be secured with tape or other closure device.
    - After the bags are securely closed, before removal from the laboratory, while wearing PPE (gloves, eye protection, coat/gown), staff should close the top box flaps. EHS will not pick up boxes that are not securely closed.
    - Contact RMS at BIOSAFETY@UNT.EDU for box pickup. **Boxes will not be picked up if the waste label is not filled out and box is securely closed.**

The Sharps Containers:

There are many varieties of sharps containers available. Three are shown below:

Small Sharps Container

Large Sharps Container with Slotted Lid

8 gallon Stackable Sharps Bucket

Each of these containers all comply with OSHA BBP standard requirements dictated for sharps containers. These are:

Closable, puncture-resistant, leakproof on the sides and bottom, labeled and/or color-coded. In addition, to comply with these standards these containers need to be maintained properly within the laboratory.

They should be:

- Easily accessible and located close to work areas where sharp materials are used (place containers near sharps; do not walk across rooms handling sharps to dispose into the containers).
- The containers must be maintained upright.
- These containers need to be replaced routinely. Do not allow these containers to become overfull. As a rule, no sharps container should be allowed to become >2/3 full.

Many sharps containers have handy "Full" arrow marks on the containers to remind users to replace the containers once they have reached ~2/3 full. Sharp items which should be placed in these containers include anything which is capable of puncturing the biohazard waste bags, including hypodermic needles, syringes (with or without the attached needles), Pasteur pipettes, disposable plastic pipettes, scalpel blades, razor blades, blood vials, test tubes, needles with attached tubing, broken plastic culture dishes, unbroken glass culture dishes, and other types of broken and unbroken glassware, including microscope slides and coverslips, that was in contact with biological material.

In addition, hypodermic needles, suture needles, syringes, and scalpel blades which have never been in contact with biological materials should also be placed in these sharps wastes containers, since they pose a risk of injury to any staff member who may come in contact with these. In addition, some of these items (e.g., syringes without needles) may raise a public perception issue should they be placed in the general waste stream. Therefore, these should all be disposed in the sharps biomedical waste containers.

Because these containers have leak-proof sides and bottoms, soggy items and **small amounts** of liquids (e.g., a few ml of blood remaining in a tube) can be disposed in these containers. However, larger volumes of liquids should be handled as liquid waste (described below).

These are typically placed **UPRIGHT** in the red bag/boxes prior to sealing the box as described above.

8.1.2 Non-contaminated glass

Unbroken or non-broken glass which has <u>not</u> been contaminated with *any* hazardous materials should be collected in solid-sided 5-gallon white plastic buckets with fitted lids or closed heavy duty cardboard cartons before disposal in the general waste stream.

8.1.3 Improper Solid Biohazardous Waste Disposal

EHS biosafety only picks up red bag boxes.

EHS biosafety staff will not:

- Lift any biohazard (red) bag from any waste receptacle (lifting only increases the EHS biosafety staff members' potential exposure risk to biohazardous aerosols which may be generated while lifting or materials which may leak from the bag should the bag puncture).
- Combine the contents of multiple waste containers in order to compile one full container for disposal (combining only increases the risk of exposure to biohazardous aerosols, but also risks contaminating floors and surrounding areas).

Laboratory Staff must **not**:

- Leave any waste container unsecured or unprotected outside of the laboratory/clinic.
- Accumulate wastes in any secondary waste collection sites (e.g., closets). The waste should be removed from the laboratory or clinic and taken to the biohazardous waste room. Please note: all sharps containers are **not** intended to be re-usable waste receptacles. Bag liners are not to be used in conjunction with buckets, and they should **always** be used with their fitted plastic lids. Laboratory staff may choose to use alternate intermediary biohazard waste containers provided that these containers are:
    - Clearly demarked as biohazardous waste with color-coded labels (e.g., by using red bags or biohazard stickers).
    - Capable of being decontaminated and are decontaminated often.
    - No sharps are disposed in these intermediary containers (sharps must be directly placed into authorized sharps containers).
    - The waste is transferred to the authorized waste receptacle by the laboratory staff (not the EHS biosafety staff).

A word of caution about the use of lifting biohazard bags: The act of lifting a bag containing biohazardous materials can increase one's risks of exposure to biohazardous materials. First, the act of lifting may generate aerosols; therefore, staff should be fully protected with PPE (at least lab coat, gloves and eye protection), and the bag should be tightly closed before lifting. In addition, any pointed items that may penetrate the bag (*e.g.,* pipettes, micropipette tips) should not be placed in bags that are to be lifted, since they may also increase the exposure risks if the bag were to be punctured during lifting. Care should be taken by any staff member lifting a bag. These should be placed in the red bag/box for eventual disposal.

PATHOLOGICAL WASTES

Any item which is identifiable as a human or animal body part or an animal carcass needs to be disposed as pathological waste. These items must be appropriately labeled for incineration. At UNT, the majority of the pathological waste is generated through animal waste disposal procedures. Therefore, it is critical that all animal carcasses be packaged and labeled according to IBC/IACUC compliant procedures and placed in one of the necropsy freezers. These wastes will be placed in the animal waste carts and placed on the RedAway truck just prior to departure. **Pathological waste streams that are not generated through the animal waste procedures must be disposed of through the UNT biohazardous waste vendor (red bag box) and this waste must be labeled as pathological waste.** Contact EHS (BIOSAFETY@UNT.EDU) if you have any other questions related to pathological waste disposal.

LIQUID BIOLOGICAL WASTES

Liquids which are derived from biological organisms (*e.g.,* blood) or have been exposed to biological materials (e.g., tissue culture media, cell extracts) must be decontaminated prior to disposal. Liquid waste decontamination and disposal methods must be documented in each laboratory's or clinic's Standard Operating Procedures (SOPs).

The sanitary sewer was designed for the disposal of certain liquid wastes. Use of the sanitary sewer reduces the chance for leaks or spills during transport and reduces disposal costs. Whenever possible, decontamination of liquids by autoclaving or use of chemicals which can be disposed in the sewer system (namely, bleach), is highly recommended. Remember to rinse sink with copious water after disposal of decontaminated/deactivated biological materials.

Other chemical disinfectant methods may require subsequent disposal of the wastes through the chemical waste

stream. Similarly, if the biological wastes are contaminated with chemicals or radiological materials, disposal procedures will have to be modified to comply with disposal requirements for these materials (see Section on Mixed Waste).

Caution must be paid to disposal of any material which may clog sewer disposal pipes. This would include large amounts of blood or agar. Disinfection of large amounts of blood may be accomplished by treatment with Isolyzer LTS-Plus®. This chlorine-based granular product solidifies and disinfects the blood or body fluids so they may be placed in general (clear), not biowaste (red) waste bags. Agar should be allowed to solidify prior to disposal in the regular waste stream (if not used in conjunction with biological materials), or placed in solid-sided waste containers, such as the 8-gallon sharps waste bucket (if potentially contaminated with biological materials).

CHEMOTHERAPY WASTES

Chemotherapy agents need to be segregated from the general biohazardous waste. While these agents are often used in clinical therapy, many of these agents may also be used for in vitro and *in vivo* animal research applications (*e.g.,* Actinomycin D, Mitomycin-C, Bleomycin, GM-CSF, Interleukin-2, INF-α, Gleevec) and must be disposed as chemotherapy waste. A list of chemotherapy agents can be found at: http://www.chemocare.com/bio/.

Chemotherapy wastes include: "Any disposable material which has come in contact with cytotoxic/antineoplastic agents (agents toxic to cells) and/or antineoplastic agents (agents that inhibit or prevent the growth and spread of tumors or malignant cells) during the preparation, handling, and administration of such agents. Such waste includes, but is not limited to, masks, gloves, gowns, empty IV tubing bags and vials, and other contaminated materials. The above waste must first be classified as empty, which means such quantity that it is not subject to other federal or state waste management regulations prior to being handled as biomedical waste." (Note: "Empty" is generally defined as containing less than 3% by weight of the total capacity of the container).

Stock solutions of these chemicals and items that are heavily contaminated are disposed of through the Chemical Hazardous Waste Program.

Boxes that include chemotherapy wastes MUST be labeled as such. Contact BIOSAFETY@UNT.EDU for labels.

MIXED WASTE

"Mixed waste" are those wastes which are contaminated with more than one type of hazardous material. For the purposes of this Guide, focus will be on those materials which are contaminated with biologicals and either chemical or radiological materials.

8.1.4 Biological/Chemical Mixed Waste

Many chemicals serve to disinfect biological materials through lysis, dehydration, or protein-crosslinking. Common chemical fixatives (formalin, glutaraldehyde) may serve to decontaminate a biological material, leaving only the chemical waste disposal issues to address. Whenever possible, in biological/chemical mixed waste situations, efforts should be made to determine whether the chemicals have adequately decontaminated the biologicals. If they have not, seek assistance from the Biological and Chemical Safety Offices to determine whether a chemically-compatible decontamination method can be devised which would then allow these items to be disposed as chemical waste. These should be documented in your laboratory SOPs **prior** to initiation of any experiment which would produce the mixed waste.

Items contaminated with ethidium bromide, diaminobenzidine (DAB), phorbol, or phenol-chloroform mixtures should not be mixed with other medical waste. These are already chemically decontaminated and do not autoclave or bleach. These items should be segregated into their own containers and disposed as chemical waste.

Do not dispose of biological items which contain mercury in the biowaste. Biological items which may contain mercury (*e.g.,* extracted teeth which contain mercury amalgams fillings) should first be decontaminated using a broad-spectrum chemical disinfectant (*never* autoclave mercury!) and disposed as mercury waste through the hazardous waste program.

8.1.5 Biological/Radioactive Mixed Waste

All Radioactive wastes are required to be disposed through the Radiation Safety Office. Any biological materials that can be decontaminated with bleach, first, (consult RSO before adding bleach to any mixed waste) should be decontaminated and the pH of the resultant waste adjusted by the addition of non-hazardous buffering agents (sodium bicarbonate, Tris) prior to disposal as radioactive waste.

Radioactive sharps waste should be disposed of in sharps containers to which Radioactive warning labels have been clearly affixed. These should be disposed through the Radiation Safety Office. Animal carcasses, tissue/parts, and excreta containing/contaminated with radioactive materials shall be handled and collected by those with proper radioactive material training and exposure badging (contact the Radiation Safety Office for further information).

REQUISITIONS FOR NEW OR ADDITIONAL BIOHAZARDOUS WASTE CONTAINERS

Extra biohazard waste box-bag units can be obtained from EHS Biosafety at no cost to you. Any alternate required intermediary waste containers (small biohazard bags, sharps containers) must be provided by the PI/laboratory director. If an unusual situation arises which requires disposal of large amounts (>15 gallons) of soggy materials or liquid materials, please contact the Environmental Health and Safety Office for assistance.

REQUISITION FOR BIOHAZARDOUS WASTE PICK-UP

EHS Biosafety is responsible for removal of any full authorized waste containers (red bag boxes). If laboratory staff members notice containers that are full and require removal, please contact the EHS at BIOSAFETY@UNT.EDU.

OTHER BIOHAZARDOUS WASTE

Biohazardous waste that is NOT considered special waste by the state, such as recombinant plants and the associated soil, must be autoclaved at 121°C for 60 minutes prior to disposal in the regular waste stream. Shorter times/temperatures may only be used if researchers have validated procedures showing effectiveness. PIs should keep a record of disinfection and disposal methods as this is a NIH requirement and best practice.

9 RECORDKEEPING

INVENTORY

PIs should also maintain detailed inventory records for all agents or biological materials used and/or maintained in their lab areas. These records should 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. Laboratories with controlled substances or biological toxins have additional inventory recordkeeping requirements.

ADDITIONAL RECORDS

The PI must maintain the following records and be prepared to present these at the annual laboratory inspection:

- Laboratory specific SOPs/biosafety manuals.
- A risk assessment for each project, biological agent, or toxin stored in that room.
- Training Documentation.
- Safety, security, and emergency response plans.
- Safety and security incident reports.
- Annual laboratory self-assessments
- Monthly autoclave testing logs (if applicable)
- Inventory records
- Controlled substances records (if applicable)

10 SAFETY AUDITS

PIs/Laboratory managers should perform annual laboratory self-assessments. Laboratory assessment tools are available online at the biosafety website or can be requested at BIOSAFETY@UNT.EDU and IBCprogram@unt.edu to help laboratories comply with biosafety and biosecurity requirements and make the facility a safer place to work. UNT EH&S will conduct regular (e.g. annual, biannual and/or BSO as part of the IBC review and approval process) inspections of each laboratory to ensure compliance with the procedures and protocols of this manual. Any significant concerns will be reported to the IBC .

The safety audit typically includes an evaluation of the lab space, biological safety cabinet, microbiological techniques, emergency and safety equipment, storage of biohazardous material, general housekeeping, and review of the Laboratory-Specific Biosafety Manual.

RMS/BSO will make every attempt to schedule safety audits with faculty members. However, if the PI 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 by the EH&S. If the safety inspection is in relation to the IBC protocol, the BSO may be the one sending the survey report as required. 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.

11 TRANSFERS, PACKAGING, AND SHIPPING OF BIOLOGICAL MATERIALS

Adopted from the ASU Biosafety Manual https://www.asu.edu/ehs/documents/biosafetymanual.pdf

REGULATIONS (FROM BMBL)

United States Department of Transportation. 49 CFR Parts 171–180, Hazardous Materials Regulations. Applies to the shipment of infectious substances in commercial transportation in, to, or through the United States. Information on these regulations is available at https://www.phmsa.dot.gov/hazmat. United States Postal Service (USPS). 39 CFR Part 20, International Postal Service (International Mail Manual), and Part 111, General Information on Postal Service (Domestic Mail Manual). Regulations on transporting infectious substances through the USPS are codified in Section 601.10.17 of the Domestic Mail Manual and Section 135 of the International Mail Manual. A copy of the Domestic and International Mail Manuals may be obtained from the USPS Postal Explorer website at https://pe.usps.com/DMM300/Index.

Occupational Health and Safety Administration (OSHA). 29 CFR Section 1910.1030, Occupational Exposure to Bloodborne Pathogens. These regulations provide minimal packaging and labeling for blood and body fluid when transported within a laboratory or outside of it. Information may be obtained from your local OSHA office or at https://www.osha.gov.

Technical Instructions for the Safe Transport of Dangerous Goods by Air (Technical Instructions). International Civil Aviation Organization (ICAO). These regulations apply to the shipment of infectious substances by air and are recognized in the United States and by most countries worldwide. A copy of these regulations may be purchased from the ICAO Document Sales Unit on the ICAO website at https://store.icao.int/ or by email to sales@icao.int.

Dangerous Goods Regulations. International Air Transport Association (IATA). Global standards are detailed in this widely recognized publication on requirements for the transport of biological and chemical hazards. They are issued by IATA, an airline association, based on the ICAO Technical Instructions, and followed by most airline carriers. A copy of these regulations may be purchased from IATA at https://www.iata.org/publications/dgr/Pages/index.aspx or by email to custserv@iata.org.

11.1.1 Importation and Transfers

Regulations governing the transfer of biological agents are designed to ensure that possession of these agents is in the best interest of the public and the nation. These regulations require documentation of personnel and facilities, justification of need, and pre-approval of the transfer by a federal authority and are the responsibilities of the PIs. The following regulations apply to this category:

Biological Agent or Vectors of Human Disease Import Permit. 42 CFR Section 71.54. Unless the material meets one of the regulatory exclusions, this regulation requires a permit from the CDC Import Permit Program to import infectious biological agents, infectious substances, and vectors of human disease into the United States. More information is available at the CDC Import Permit Program website at https://www.cdc.gov/cpr/ipp/index.htm.

Transfer of any Select Agents or Toxins requires the intended recipient to be registered with the Select Agent Program and submit an APHIS/CDC Form 2 as required to obtain approval to import the Select Agent or Toxin

prior to each importation event (see 42 CFR Part 73, 9 CFR Part 12, and/or 7 CFR Part 330). UNT does not have labs that are currently equipped to handled these agents.

Importation of Pathogenic Agents of Livestock, Poultry and Other Animal Diseases and Other Materials Derived from Livestock, Poultry or Other Animals. 9 CFR Part 122. Organisms and Vectors. The USDA, APHIS, Veterinary Services (VS) requires that a permit be issued prior to the importation or domestic transfer (interstate movement) of pathogenic disease agents of livestock, poultry, or other animals. Information may be obtained at 301-851-3300 or from the USDA website at https://www.aphis.usda.gov/aphis/ourfocus/animalhealth. Completed permit applications may be submitted electronically at https://www.aphis.usda.gov/permits/learn_epermits.shtml.

Importation of Plant Pests. 7 CFR Part 330. Federal Plant Pest Regulations; General; Plant Pests; Soil, Stone, and Quarry Products; Garbage. This regulation requires a permit to move into or through the United States or by interstate any plant pest or a regulated product, article, or means of conveyance in accordance with this part. Information can be obtained by calling 301-851-2357 or at the USDA APHIS website at https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information.

11.1.2 Transfer of USDA Plant Pests

The movement of Plant Pests is regulated under two distinct and separate regulations: (1) 7 CFR Part 331—Possession, Use, and Transfer of Select Agents and Toxins; and (2) 7 CFR Part 330—Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage. The regulation found at 7 CFR Part 331 requires an approved Transfer Form (APHIS/CDC Form 2) prior to importation, interstate, or intrastate movement of a Select Agent Plant Pest. In addition, under 7 CFR Part 330, the movement of a Plant Pest also requires a PPQ Form 526 permit for movement in, to, or through the United States, or interstate any plant pest or a regulated product, article, or means of conveyance in accordance with this part. Information can be obtained by calling 301-851-2357 or at the Select Agent Program website at https://www.selectagents.gov. PIs must familiarize themselves with these regulations and have appropriate permits and lab spaces to work with these materials. IBC approval is required for any research with plant pests.

Export of Human, Animal, and Plant Pathogens and Related Materials; Department of Commerce (DoC); 15 CFR Parts 730–799. This regulation requires that exporters of a wide variety of etiologic agents of human, plant, and animal diseases, including genetic material, and products that might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoC Bureau of Industry and Security (BIS) at 202-482- 4811 or at the DoC BIS website at https://www.bis.doc.gov. . Additional web resources include:

1. https://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear
2. https://classic.ntis.gov/products/export-regs/

TRANSFERS

Each PI must develop procedures for transferring or shipping from the laboratory. The PI must ensure the following:

- Personnel who package, handle, and ship non-select agents and biohazardous materials (including import and export) are subject to all applicable training..
- Standard operating procedures should be in place for all import and export activities.
- 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).

- 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.
- Decontaminate contaminated or potentially contaminated materials before they are removed from the laboratory area.
- Avoid hand-carrying biohazards when transferring them to other external facilities. If biohazards are to be hand-carried on common carriers, all applicable packaging, transport, and training regulations should be followed.
- Develop and follow a protocol for intra-facility transfer (between laboratories on UNT campuses) of all biological and biohazards. Contact EHS for assistance.
- 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.

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, conveyors). Contents should not leak to the outside of the shipping container even if leakage of the primary container occurs.

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 11.A. Source: Biosafety in Microbiological and Biomedical Laboratories 5th Edition, Appendix C).

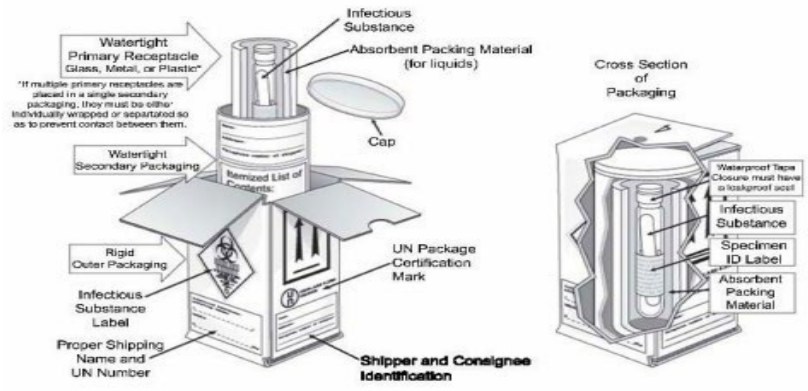

Figure 11.A. Packaging Diagram for Biohazards

Packaging Volumes

11.1.3 Volume not exceeding 50 milliliters (ml)

a. 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.
b. 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.
c. 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.
d. If you package the material with dry ice, see the Packaging with Dry Ice section in this document.

11.1.4 Volume greater than 50 ml

a. Follow requirements for lesser volumes outlined above.
b. 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).
c. 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.

Packaging with Dry Ice

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

Labeling

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

Shipping and Transportation Methods and Requirements

11.1.5 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.

11.1.6 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 11.B and 11.C, respectively.
- Contact the specific carrier's dangerous goods agent prior to shipment for any additional packaging and labeling requirements.

Figure 11.B

Figure 11.C

11.1.7 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.

11.1.8 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.

APPENDIX A. DEFINITIONS

Aerosols: Colloids of liquid or solid particles suspended in gas.

Aerosol, Transmitted: Particles or droplets of biologically active agents or microorganisms that are transmitted in nature by dissemination into the ambient air with subsequent deposition upon receptive areas of the body exposed to the ambient air (mucous membranes, lungs, eye conjunctiva, cut or abraded skin).

Animal Biosafety Level (ABSL): Laboratory practices, techniques, safety equipment and laboratory facilities appropriate for the operations performed and the hazards posed by the particular biohazard material when working in animal research facilities. The NIH and the CDC define four levels of animal biosafety in the U.S. Department of Health and Human Services Publication, Biosafety in Microbiological and Biomedical Laboratories, 2009. This publication recommends animal biosafety levels for work with particular microorganisms.

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).
APHIS: Animal and Plant Health Inspection Services

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, Biohazardous material: Any microorganism (including, but not limited to, bacteria, chlymidia, 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.

Biosafety Cabinet (BSC): Ventilated cabinet, which serves as a primary containment device for operations involving biohazardous materials. The Class II vertical laminar flow BSC is a ventilated cabinet with an air barrier curtain at its open-front sash. This type of cabinet offers product protection, user protection, and environmental protection when properly operated. Exhaust air from the workspace is filtered with a high efficiency particulate air (HEPA)/ultra-low particulate air (ULPA) filter, prior to discharge. The cabinet provides a sterile working environment by also supplying HEPA/ULPA filtered downward airflow within the workspace. Class II BSCs are further classified as type A1, A2, B1, B2, and C1.

Biosafety Level (BSL): Laboratory practices, techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazards posed by the particular biohazard material. The NIH and the CDC define

four levels of biosafety in the U.S. Department of Health and Human Services Publication, Biosafety in Microbiological and Biomedical Laboratories, 2009. This publication recommends biosafety levels for work with particular microorganisms.

Bloodborne Pathogens (BBP): Pathogenic microorganisms that are present in human or non-human primate blood or other potentially infectious materials (OPIM) and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and the human immunodeficiency virus (HIV).

BMBL: Biosafety in Microbiological and Biomedical Laboratories

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

Certification: Procedure by which BSC meets national standards of physical testing which include but is not limited to air balancing, filter integrity, velocity measurements, and electrical grounding.

CFR: Code of Federal Regulations

Containment: Presence or reasonably anticipated presence of microorganisms or other potentially infectious materials in or on a surface.

Contaminated Sharps: Contaminated object or device having rigid corners, edges, or protuberances capable of cutting or piercing skin including, but not limited to all of the following: hypodermic needles, blades, pipettes, and broken glass or sharp-edged plastic items contaminated with microorganisms or other potentially infectious materials.

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.

Decontamination: The reduction of microorganisms on a surface or item to an acceptable level by the use of physical or chemical means so that the organisms are no longer capable of transmitting infectious particles and the surface or item is rendered safe for routine handling, use, or disposal.

Disinfection: Process by which viable microorganisms are reduced to a level which is unlikely to cause disease in healthy people, plants, or animals.

DURC: Dual Use Research of Concern

Engineering Controls: Mechanical equipment, facility characteristics, or other physical controls that isolate or remove the hazards from the workplace.

EPA: Environmental Protection Agency

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

Exposure Incident: Unanticipated, specific eye, mouth, mucous membrane, non-intact skin, inhalation, or parenteral contact with blood, other potentially infectious materials, or microorganisms during the course of an employee's duties.

FDA: U.S. Food and Drug Administration

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.

Greenhouse: The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually (but not necessarily) constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Greenhouse Facility: The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

High Efficiency Particulate Air (HEPA) Filter: Disposable, extended, pleated, dry filter which has rigid casing enclosing the full depth of the pleat. Minimum particulate removal is 99.97% for particles with a diameter of 0.3 μm.

HHS: Health and Human Services

Human Materials – human blood, blood components, blood products, body fluids, tissues, or organs.

Inactivation: Process that destroys the ability of a specific biohazardous agent to self-replicate.

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.

LAI(s): Laboratory-associated infection(s)

Laminar Airflow: Unidirectional airflow through the work area often referred to as turbulence-free airflow.

Other Potentially Infectious Materials (OPIM): Human or non-human primate body fluids: seminal and vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, other body fluids that are visibly contaminated with blood such as saliva or vomit. The definition includes all body fluids, viable research and clinical samples where it is difficult or impossible to determine if the material is contaminated with blood or blood components.
-Any unfixed or viable human tissue or organ from a human or non-human primate (living or dead).

-Cells or tissue culture, organ cultures, and culture medium or other solutions, blood, organs, or other tissues that are not known to not contain human or non-human primate bloodborne pathogens.
-Any viable (unfixed) material within *in vitro* (tubes or flasks) or *in vivo* (animals) experimental systems that have been deliberately infected with bloodborne pathogens from human or non-human primates (including HIV and HBV).

Parenteral: Descriptive of piercing mucous membranes or the skin barrier through events such as needle sticks, human bites, cuts, and abrasions.

Pathogen: Agent containing sufficient genetic information, which upon expression of such information is capable of producing disease in healthy people, plants or animals.

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.

Physical Barrier: Equipment, facility, device, or physical condition that is designed to achieve containment or exclusion of biohazards.

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, i.e., recombinant nucleic acids, 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.

Regulated Waste: Liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; pathological and microbiological wastes containing blood or other potentially infectious materials.

Risk Level: Classification scheme established by federal and world governmental bodies to rate the relative risks associated with exposure to specific biological agents and microorganisms.

Sterilize: Use of a physical or chemical procedure to destroy all microbial life including highly resistant spores.

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.

Universal Precautions: Safe work practice controls for infection control. All human and non-human primate blood, OPIM, and cells/tissue are to be treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

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.

Work Practice Controls: Procedures and practices that reduce the likelihood of exposure by altering the manner in which a task is performed (e.g., prohibiting recapping of needles by a two-handed method).

Zoonotic: Ability of a natural pathogen of an animal species or a genetically engineered natural pathogen of that species to migrate outside of its species of origin and move across a human somatic cell membrane.

Zoonotic Disease: Disease that naturally infects animals that is pathogenic for humans.

APPENDIX B. ACRONYMS

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