Biological Safety Manual

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1. Introduction

This document is designed to serve as the minimum established biosafety and biocontainment standards for the use, storage, and disposal of biological materials including recombinant and synthetic nucleic acids, microorganisms, infectious agents or potentially infectious materials, prions and prion containing materials, and tissues and cells including those isolated from animals or plants. UNT Health Laboratories may utilize this template to create a laboratory specific manual or standard operating procedure; however, all modifications must ensure laboratory safety at or above the level provided by the best practices described in this document.

The containment, safety equipment, personal protective equipment (PPE), and procedures included here provide assurance that biological materials can be safely managed in accordance with the following:

- Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, June 2020.
- The United States (US) Department of Health & Human Services National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, April 2024.
- The US Department of Health & Human Services Centers for Disease Control and Prevention.
- The CDC National Institute for Occupational Safety and Health.
- Public Health Agency of Canada Biological Material Safety Data Sheets.
- UNT Health Policies.
- The determination of the containment level, equipment, and PPE needed, will be defined by the UNT Health Institutional Biosafety Committee (IBC), The Office of Research Compliance (ORC), and The Office of Environmental Health and Safety (EH&S).
- For questions regarding this document, please contact UNT Health EH&S at (817) 735-2253.

2. Applicability

This institutional biosafety manual must be adopted as policy and must be utilized in conjunction with all Biological Hazard Registrations. These documents must be readily accessible to all laboratory personnel.

3. Approval and Implementation

This Biosafety Manual is hereby approved for the University of North Texas Health. This plan shall apply to all UNT Health personnel participating in all scientific and 1nedical research activities at UNT Health facilities or sanctioned activities. The details of this plan are the institutional policies directing the safe use of biological research and materials. This plan is effective immediately and supersedes all previous editions.

Approved Anne-Sophie Brocard, PhD, CBSP(ABSA), CSP, CHMM

Digitally signed by Anne-Sophie Brocard, PhD, CBSP(ABSA), CSP, CHMM
Date: 2025.11.19 16:25:37 -06'00'

Anne-Sophie Brocard, PhD, RBP(ABSA), CBSP(ABSA), ASP, CSP, CHMM
Sr. Director Environmental Health and Safety UNT Health

4. Record of Changes

Change #	Date of Change	Change entered by	Description
1.	10/18/2021	Maya Nair	Add Appendix1 with Forms and SOPs for biosafety operations 1. Biological Safety Cabinet Laboratory Equipment Relocation Installation Form 2. UNT Health Incident Report Form 3. UNT Health IBC SOP For Biohazard Transport 4. Laboratory Safety Survey Guidance Document 2020
2.	10/18/2021	Maya Nair	Update figures and contact information with current information
3.	11/4/2022	Maya Nair	Update figures and contact information with current information
4.	11/4/2022	Maya Nair	UNT Health Biowaste processing updated
5.	3/20/2023	Maya Nair	Added section 16.6 Phlebotomy guidelines for human research participants
6.	11/19/2023	Maya Nair	Update figures and contact information with current information
7.			

5. Contact Information

5.1 EH&S Program Contacts

Subject	Office Name	Telephone	Email
Biosafety Program	Director, Biological Safety	817-735-5431	Maya.nair@unthsc.edu
Biological Hazards and Biological Waste	Assistant Director	817-735-2697	Alan.Corbitt@unthsc.edu
Contacting the IBC	Biosafety Officer	817-735-5431	ibc@unthsc.edu
Safety	Sr. Director	817-735-2253	anne-sophie.brocard@unthsc.edu
Occupational Health	Priority Clinic	817-735-2273	

5.2 Emergency Phone Numbers

Police/Fire Emergency	Police Dispatch	In-house phone: Ext 2600 or 911 Cell phone: 817-735-2600
Emergency Power Outage	Facilities	Ext: 2181 / 817-735-2181
Hazardous Material Release/Spill	Police Dispatch	In-house phone: 2600 Cell phone: 817-735-2600
Hazardous Material Exposure: Skin, Eyes, Ingested, Inhaled, Injected	Occupational Health	Ext. 2273 / 817-735-2273

5.3 Other Important Institutional Phone Numbers

Campus Police/Security Non-Emergency	Ext: 2210 / 817-735-2210
Facilities Non-Emergency	Ext: 2181 / 817-735-2181
Environmental Health and Safety	Ext: 2245 / 817-735-2253
Radiation Safety	Ext: 2243 / 817-735-2243
Department of Laboratory Animal Medicine (DLAM)	Ext: 2017 / 817-735-2017
IACUC	Ext: 2533 / 817-735-2533

5.4 UNT Health Relevant Website links

Report an Ethics Compliant	https://secure.ethicspoint.com/domain/media/en/gui/54789/index.html
First Report of Injury	https://www.unthsc.edu/administrative/wp-content/uploads/sites/23/SORM-Packet_ENG-2.pdf
Student complaints	https://unthsc.qualtrics.com/jfe/form/SV_1Mn0IlToxxTH3QF?Q_JFE=qdg
Waste Pickup Requests	https://www.unthsc.edu/safety/radiological-and-biosafety/chemical-waste- removal-request-form/ https://www.unthsc.edu/safety/radioactive-waste-removal-request-form/ https://www.unthsc.edu/safety/biological-waste-red-box-removal/
UNT Health - IACUC	https://www.unthsc.edu/research/animal-research/iacuc/
UNT Health - IBC	https://www.unthsc.edu/safety/biosafety/
UNT Health CSC	https://www.unthsc.edu/safety/chemical-safety
North Texas Regional IRB	https://www.unthsc.edu/north-texas-regional-irb/

6. Responsibilities

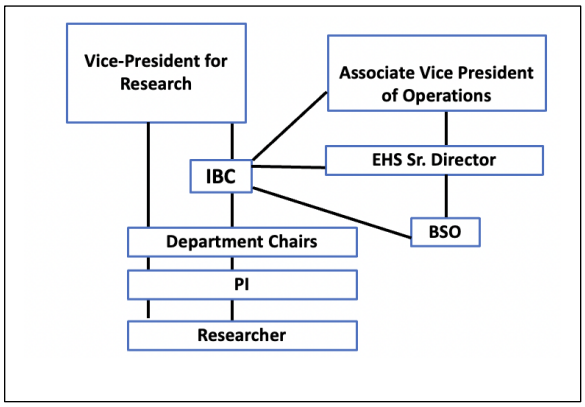
This Manual specifies the minimum criteria to be met with any covered potentially biohazardous materials or activities. Individual PIs and laboratory managers may set more stringent criteria if and when it is considered prudent. This Manual should not be considered final or all-inclusive, however, since all possible situations cannot be foreseen.

Modifications of this Manual will occur on a regular basis in order to meet continuously changing regulations and conditions. It is the responsibility of each individual associated with potentially biohazardous activities to adhere to both the intent of this Manual as well as to its specifics, and to make every reasonable effort to minimize risks to individuals, animals and the environment to the greatest degree possible.

The administrative framework under which potentially biohazardous activities within UNT Health laboratories by UNT Health faculty, staff, students, contractors and visitors will be carried out is described below. This section outlines the basic roles and responsibilities of persons involved at each level of the approval, the monitoring or the supervision of biosafety activities at the University.

Further clarification and interpretation of these roles and responsibilities may be obtained by contacting the Chair of the IBC or the University's BSO.

6.1 Biosafety Program Organizational Structure



6.2 University Responsible Official

The Responsible Official for all laboratory work involving biohazardous materials is the Vice

President for Research.

The University and the Responsible Official recognize their responsibility to monitor and control potentially biohazardous activities conducted within its facilities or by persons associated with the university and thus has established and implemented rules and guidelines for conducting these activities as described in this Biosafety Manual. The Manual outlines the procedures for approval and safe conduct of potentially biohazardous activities and directs compliance with all directives and guidelines pertaining to such activities. The University has established an IBC to meet the requirements specified by the National Institutes of Health Guidelines.

6.3 Vice President for Research

The Vice President for Research (VPR) is the primary oversight official for all research activities occurring on the UNT Health campus. The VPR has the responsibility and authority to perform the following actions relevant to the Biosafety Program including but not limited to:

- Revoke, retract, and/or modify any research activity occurring on the UNT Health campus.
- Monitor all human and non-human research activities occurring on the UNT Health campus.

The VPR, appointed by the President of the University, is the Institutionally Responsible Official for The Institutional Biosafety Committee (IBC). The VPR has the responsibility and authority to perform the following actions relevant to the IBC including but not limited to:

- Appoint IBC members, including the Chair, Vice-Chair, and *Ex Officio* members.
- Retract or modify IBC charters, policies, and procedures.

- Provide discretionary powers to the IBC, Chair, and Vice-Chair.
- Review documentation created, maintained, and/or authorized by the IBC.

6.4 Institutional Biosafety Committee (IBC)

The objectives of the IBC are to protect staff, research subjects, the general public, and the environment from exposure to biological materials generated, stored, used, and managed as waste by UNT HEALTH. The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, current edition (NIH Guidelines), and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories current edition (BMBL), will be utilized along with other applicable regulations and biosafety guidelines to direct IBC requirements and recommendations.

- Institutional Biosafety Committee (IBC) The IBC membership shall be qualified and appointed in accordance with guidelines established by the NIH.
- Membership of the IBC consists of a minimum of five (5) persons, two (2) of which cannot be affiliated with the University except as IBC members and will represent the interests of the surrounding community with respect to health and protection of the environment. The VPR appoint the members of the IBC. IBC members serve a term of 3 years. Members are eligible for reappointment to multiple consecutive terms. The BSO is a mandatory member of the IBC and is eligible to be appointed as its chairperson. The IBC should have a representative from the Safety Office and a representative from the Office of Research Compliance.

The UNT Health IBC has been charged with the responsibility for institutional oversight of the use, storage, and disposal of biological materials including recombinant and synthetic nucleic acids, microorganisms, infectious agents or potentially infectious materials, tissues, and cells including those utilized in animals or plants. The IBC in conjunction with the EH&S will establish processes to:

- Help identify biological materials that are utilized, stored, generated, and disposed at both on-campus and off- campus UNT Health locations.
- Facilitate the registration of identified biological materials.
- Perform risk assessments to ensure the safe use of biological agents.
- Perform risk assessments to ensure safe use of chemical agents.
- Monitor identified personnel, facilities, and laboratories for compliance with established institutional safety policies, manuals, and plans, as well as specific IBC directives.

The IBC operates in accordance with its approved charter (IBC Webpage) and hold monthly meetings on the third Wednesday of each calendar month with exceptions for holidays. The IBC advises the VPR on policy matters concerned with the protection of personnel from biological materials that may be present in laboratory, hospital, or clinical areas. The IBC shall also recommend guidelines relating to procedures and facilities used at UNT HEALTH, including such matters as safety training and

health surveillance.

The IBC will offer its counsel to all UNT Health personnel regarding matters of biological safety. The VPR may ask the IBC to inform the community about developments in the general area of biological safety.

6.5 Biosafety Officer (BSO)

The BSO shall be responsible for:

- Periodically inspecting all laboratories where biohazardous agents, human materials, or recombinant DNA research or other educational activities are being conducted to ensure that laboratory standards are being followed.
- Reporting to the IBC and to VPR any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the Biosafety Officer becomes aware, unless the Biosafety Officer determines that a report has already been filed by the Principal Investigator; developing emergency plans for handling and investigating laboratory accidents involving biohazardous agents, human materials, toxins or recombinant DNA molecules;
- Serving as a liaison between UNT Health and external regulatory agencies concerned with the use of biohazardous agents, human materials, toxins and recombinant DNA molecules.
- Filing an annual update with the NIH.
- Serving as a voting member of the IBC, including eligibility for appointment as Chair.
- Reviewing all funded grants for compliance with applicable sections of this Manual.
- Maintaining a list of organisms present in the agency facilities and where these agents are used and stored.
- Maintaining and updating the Biosafety Manual.
- Working with the Safety Office to provide technical advice on research biosafety and laboratory security procedures to Principal Investigators, laboratory personnel, and the IBC.

6.6 Department Chairpersons

Department Chairpersons bear overall responsibility for the implementation and maintenance of safe practices and procedures in their department. This responsibility includes the assurance that all departmental facilities (e.g. warm rooms, cold rooms) and equipment (e.g. autoclaves, freezers, biosafety cabinets, etc.) are operated and maintained in accordance with all relevant safety manuals and manufacturer's instructions.

6.7 Principal Investigators (PIs)

PIs are faculty members or other UNT Health employees in whose assigned laboratory space where research activities are conducted. Each PI is responsible for full compliance with policies, practices, and procedures set forth by the IBC. This responsibility extends to all aspects of biological safety involving all individuals who enter or work in the PI's laboratory or collaborate in carrying out the PI's research. Although the PI may choose to delegate aspects of their biological activities in their laboratory to other laboratory personnel or faculty, this does not absolve the PI of their ultimate responsibility. The PI remains accountable for all activities occurring in their laboratory. The PI is responsible for assuring the appropriate safety training of employees and for correcting errors and unsafe working conditions. Documentation of training and compliance with appropriate biological safety practices and procedures are essential.

As part of general responsibilities, the PI shall:

- Comply with all federal, state, and/or local regulations, codes, statutes, and/or guidelines.
- Comply with all institutional policies and procedures, as well as adopted guidelines, manuals, and/or standards.
- Register research work involving the use of infectious agents, recombinant or synthetic DNA, or human materials with the IBC. The PI must complete a “Hazard Registration" application that is specific towards the hazard proposed. The application must include details of the nature of the proposed experiments and an assessment of the levels of physical and biological containment required for the experiments. The containment level must be consistent with federal, state, and local regulations, as well as institutional policies, procedures, and adopted standards and/or guidelines.
- Delay the initiation of applicable biological research experiments until such time that said experiments are approved by the IBC.
- Develop and implement written laboratory-specific biosafety standard operating procedures (SOPs) that are consistent with the nature of the current IBC approved Hazard Registration. SOPs must describe specific research activities and copies must be made available in each laboratory facility.
- Ensure that all laboratory personnel understand and comply with IBC approved biological hazard registrations, procedures, and SOPs.
- Ensure that all laboratory personnel, maintenance personnel, and visitors, who may be exposed to biological materials, are informed in advance of their potential exposure risk and of the methods required to minimize that risk.
- Ensure that all maintenance work in, on, or around contaminated equipment is conducted only after that equipment is thoroughly decontaminated by the laboratory staff or PI.
- Ensure that biological materials are properly treated before disposal and that all employees are familiar with the appropriate methods of waste disposal.
- Ensure that infectious agents including select agents are properly accounted for and securely stored to prevent theft, loss, or release.

- Report any significant problems and violations of the policies, practices, and procedures to EH&S as soon as possible.
- Be well versed in standard microbiological techniques.
- Ensure that all research personnel have attended the Institutional Biosafety Training and the Bloodborne Pathogen Training.
- Ensure that all research personnel are proficient in the biosafety techniques and procedures required for their activities.
- Ensure that all research personnel receive appropriate medical surveillance when needed.
- Coordinate with EH&S to develop emergency response plans for handling accidental spills, facility and equipment contamination, and exposure to biological materials.
- Create and foster an environment in the laboratory that encourages open discussion of biosafety issues, problems, and deviations from established procedures.
- Comply with shipping requirements for biological hazards and select agents. EH&S conducts shipping training as required for all institutional personnel. Personnel shipping infectious substances may contact EH&S to assure that all applicable transportation safety regulations have been met prior to shipping microbiological cultures, tissues (human or animal), or body fluids. These materials are often regulated for shipment and must only be shipped by personnel who have been properly trained and authorized by UNT Health to ship such materials.

6.8 UNT Health Researcher

UNT Health researcher shall:

- Complete all initial safety training provided by EH&S prior to handling biological materials.
- Complete all laboratory and project specific training, including but not limited to proficiency, facility, equipment, infectious agent, and biosafety technique training, prior to conducting experiments.
- Follow all standards described in this safety manual unless superseded by an IBC and EH&S approved standard operating procedure or facility operating manual.
- Perform only assigned duties and conduct only institutionally approved research experiments.
- Complete any medical surveillance or health consultation requirements.
- Inform their PI or Occupational Health of any health condition that may increase their exposure risk to infectious agents utilized in their workplace.
- Immediately report to their PI, Occupational Health, and EH&S any spill, injury, or exposure involving biological materials utilized in the workplace.
- Immediately report to their PI and Occupational Health any signs and/or symptoms similar to

an infection caused by microorganisms utilized in the workplace.

- Immediately report to their PI and EH&S any event that may result in the creation of a potential hazard.
- Utilize and maintain any/all equipment in accordance with the manufacturer's instructors. This includes but is not limited to:
    - Autoclave, freezers, pipets, pipet aids, centrifuges, rotors, vacuum traps, biosafety cabinets
- Implement use, cleaning, and maintenance practices to protect facilities and building systems. Examples include but are not limited to:
    - Protecting vacuum lines with filters
    - Cleaning work surface following activities or experiments
    - Properly decontaminating surfaces utilizing disinfectants rated to be microbial against the agent(s) in use.
    - Maintaining properly stocks of laboratory supplies
- Maintain their workspace in efforts to reduce contamination, clutter, and excessive storage.
- Implement practices to secure infectious agents against theft, loss, and release.

6.9 The Office of Environmental Health & Safety (EH&S)

EH&S shall:

- Update this manual on an annual basis.
- Provide safety consultation on operations within laboratory, academic programs, hospital, and clinics areas.
- Provide initial and ongoing institutional safety training including bloodborne pathogens, biosafety, infectious substances shipping, select agent and toxins, and hands on exercises.
- Provide information on regulations that apply to the laboratory and clinical operations.
- Advise on safe methods for new procedures and on the use of new equipment.
- Verify and monitors institutional training records to ensure all pertinent UNT Health Personnel have attended all required initial and ongoing safety training courses.
- Assist the IBC with committee operations as described in the IBC Charter and consults with IBC members on matters of biosafety.
- Implement policy and guidelines approved by the IBC.
- Periodically review hazard registrations to ensure described facilities, equipment, PPE, procedures, and practices are consistent with IBC authorization and institutional policy.
- Ensure that proposed safety policies, manuals, plans, facilities, equipment, and procedures for work with biological materials meet applicable regulatory standards and guideline.
- Evaluate and survey laboratory and clinical facilities to ensure biological hazards are use, stored, and disposed of in accordance with IBC approved safety manuals, procedures, and

hazard registrations, as well as federal and state regulations.

- Investigation of laboratory, hospital, and clinical incidents involving biological material.
- Respond to and remediates large biohazardous spills.
- Identify potential problem areas and suggests to the IBC safety objectives to be achieved.
- Disseminate information on new safety programs and outreach services, as well as revisions to pertinent institutional policies, safety documentations, and federal and state regulations.

7. Registering Infectious Agents, Recombinant/Synthetic DNA, Human Materials

All PIs are required to register their use of applicable biological materials with the Biosafety Program within EH&S. For a list of biological materials that require the submission of a hazard registration, please contact EH&S.

All PIs must submit accurate, current, and complete hazard registrations including providing all pertinent supplemental documents (e.g. plasmid maps, SOPs, etc.). Additionally, PIs must inform EH&S of following:

- The purchase or acquisition of new infectious agents.
- Changes to project or experiment scope.
- Changes in project locations including areas where biological materials are used, stored, and disposed.
- Addition or deletion of employees to a project.
- Providing biological materials to another investigator on or off campus.
- Arranging for visiting researchers to work in your laboratory.
- If minors will be working in their respective laboratories.
    - Please refer to UNT HEALTH's policy on minors on campus.

Hazard Registrations must be received by the EH&S at least 10 business days before the next IBC meeting. If not received by this deadline, the registration may be deferred to the next monthly meeting. Additionally, the submission of a hazard registration does not authorize use of applicable biological materials and PIs are not authorized to use these materials until their hazard registration has been approved by the IBC and a formal approval letter from the IBC has been received.

Any PI who fails to register the use and/or storage of applicable biological materials with the Biosafety Program will be reported to the Biosafety officer. PIs who fail to register these materials following a request from the Biosafety officer will be immediately reported to the Sr. Director of EH&S, the IBC Chair, the Department Chair and the VPR. The IBC Chair and/or Vice Chair have the authority to temporarily suspend all approved hazard registrations at which time the IBC will submit a suspension letter to the PI. A copy of this letter will be sent to the VPR, PI's department Chair, the EHS Director, the Director of Office of Research Compliance. The IACUC and IRB committees will be notified of the suspension if the IBC protocol is linked to these committees. Any official suspension of work from the IBC will require an official notification to the NHI-OSP.

To obtain or regain IBC authorization to use applicable biological material, the PI will be required to submit all pertinent hazard registrations and all applicable supplemental documentation to EH&S. This information will be provided to the IBC, at the next scheduled meeting, for evaluation. The IBC may, at their discretion, authorize/re- authorize the PI's project, authorize the PI's project under specific/defined conditions, place the PI's hazard registration(s) under continued suspension, or terminate the PI's registration(s). Additionally, the Sr. Director of EH&S and the IBC Chair may recommend to the Department Chair, the VPR appropriate action if an investigation reveals significant violations.

7.1 Research Involving Vertebrate Animals

All research experiments involving research vertebrate animals must be conducted in accordance with the UNT Health IACUC approved protocol. Animal research that involves biological materials must be registered with the EH&S and authorized by the IBC. Additionally, all procedures, locations, and biological materials described in a submitted hazard registration must be consistent with the information described in all associated animal protocols.

Once approved, the IBC may require the PI and/or their designees to attend a work-start meeting with members of EH&S and DLAM. Work-start meetings ensure that the biological materials, animal use locations, required equipment, and project specific operating procedures are discussed, understood, and implemented.

7.2 Research Involving Invertebrate Animals

PIs must inform EH&S of the use of research involving invertebrate animals including but not limited to insects and gastropods. Additionally, if these organisms have been genetically modified, a recombinant DNA registration may be required.

7.3 Research Involving Plants

PIs must inform EH&S of the use of research involving plants and plant products. Additionally, if these organisms have been genetically modified, a recombinant DNA registration may be required.

7.4 Human Specimens

PIs using specimens and materials acquired from human subjects (whether they be obtained on-site UNT Health or off-site at another institution) must have a current and approved IRB protocol describing the acquisition, use, and storage of this material. The PI must list all applicable IRB protocol numbers in submitted human material registrations.

7.5 Amendment of an Existing Registration

As defined by the IBC approval, faculty must inform the Biosafety Program of any changes to their approved project objectives, hazard, process, use and storage locations, and/or personnel. PIs may amend their existing registration or submit a new registration describing any changes or alterations to existing research projects. The Biosafety Program will review the submission and determine if the amendment requires IBC evaluation or if the original risk assessment covers the proposed changes.

7.6 Expiration of a Hazard Registration

Prior to the expiration of an existing registration, PIs will be required to resubmit an updated registration for full IBC review. Registration approvals expire as follows:

- Human and Animal Pathogen Registration: 3 years from the date indicated on the approval letter unless otherwise noted.
- Recombinant DNA Registration: 3 years from the date indicated on the approval letter unless otherwise noted.
- Human Material Registration: 3 years from the date indicated on the approval letter unless otherwise noted.

7.7 Update to regulations

EH&S Biosafety in consultation with the IBC will notify PIs of regulatory changes that affects their research protocols. PIs may be required to update of provide supplemental information to the IBC to comply with regulatory changes.

8. Risk Assessment and Risk Management

Responsibility for biosafety exists at all levels and is shared throughout the UNT HEALTH. The UNT Health Administration acknowledges the institution's role in providing a safe workplace and has given the IBC, as well as EH&S the authority to administer the campus biosafety program. The IBC establishes policies, procedures, manuals, and guidance documents for the safe use of biohazards and for compliance with all applicable regulations. As an administrative agent for the IBC, EH&S disseminates pertinent information; consults with faculty, staff, students, and visitors and surveys laboratories to ensure institutional safety standards are implemented. The researchers, clinicians, and technicians who perform work with biological materials are perhaps the most important component of the biosafety program, as they must incorporate biosafety requirements and safety precautions into all facets of their work.

The PI is ultimately responsible for safety within the laboratory. 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). A risk assessment/risk

management matrix is shown below (Table 1) to illustrate key elements of the process. Relevant sections providing additional details are indicated within the matrix. Information on the routes of exposure is included at the end of this section.

The five Ps 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 Ps in each facet of laboratory work. If properly conducted, a risk assessment can help minimize exposure to biological materials; prevent laboratory acquired infections; and reduce the risk of agent transmission from the laboratory or clinical area.

Table 1: Risk Assessment and Management Matrix

	Risk Assessment	**Risk Management**
Pathogen	▪ Agent classification ▪ Risk group ▪ Routes of infection ▪ Infectious disease process ▪ Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors	▪ Registration • Biosafety Office • IBC • Texas Administrative Code (TAC) • USDA – Restricted Agents • CDC – Select Agents • NIH – Recombinant DNA • FDA/NIH - Human Gene Therapy ▪ Agent substitution to a less hazardous agent
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.	▪ Written set of SOPs with safety practices incorporated ▪ Adherence to basic biosafety principles ▪ Label labs, areas, and equipment housing BL2 or higher agents ▪ Conduct lab surveys to review practice sand containment equipment ▪ Use trial experiments with non-infectious material to test new procedures/equipment

Personnel	▪ Host immunity • Neoplastic disease • Infection (HIV) • Immunosuppressive therapy • Age, race, sex, pregnancy • Surgery (splenectomy, gastrectomy) • Diabetes, Lupus ▪ Immunization ▪ Post-exposure prophylaxis ▪ Attitude toward safety ▪ Comfort ▪ Experience in the field ▪ 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 Employee Health ▪ Investigation/review of incidents/spills, etc. to prevent future occurrence ▪ Protocol training
Protective Equipment	▪ Protection (containment) for: • Aerosols – (respirable size particles) <10µm • Droplets/splatter • Sharps • Contamination • Spills	▪ PPE: • Respirators – High Efficiency Particulate Air (HEPA) Cartridge, N-99, N-95, etc. • Face (eye, nose, mouth) protection – mask and safety glasses, or chin length face shield • Solid front gown or lab coat • Gloves ▪ Biosafety Cabinet (BSC) ▪ Centrifuge safety buckets/rotors ▪ Safe Sharps and Sharps Containers
Place – Laboratory Facility	▪ BSL requirements ▪ HVAC and exhaust systems ▪ Aerosol risk ▪ Restricted access	▪ Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open ▪ Labels ▪ Containment laboratory with directional airflow

8.1 Risk Group Categorization of a Hazardous Agent

The principal hazardous characteristics of an agent are: 1) its capability to infect and cause disease in a susceptible human or animal host, 2) its virulence as measured by the severity of disease, and 3) the availability of preventive measures and effective treatments for the disease. The World Health Organization (WHO) has recommended an agent risk group classification and the NIH Guidelines establish a comparable classification. Both entities have assigned human etiological agents into four risk groups on the basis of the above criteria. The descriptions of the WHO and NIH risk group classifications correlate with but do not equate to biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level.

Risk Group Categorization:

- Risk Group 1: Minimal hazard to humans, not known to cause disease in healthy adults or agricultural plants and animals.
    - Note that these organisms may cause disease in immunocompromised personnel.
- Risk Group 2: Agents associated with disease which is rarely serious or there is treatment and/or prophylaxis available, generally oral or inoculation hazards.
    - Note that disease severity may be higher in individuals with compromised immune systems or who have health conditions that may promote infection.
- Risk Group 3: High individual risk, associated with serious disease which may or may not have treatment, generally aerosol transmission in the laboratory setting.
- Risk Group 4: Serious or lethal disease for which there is usually no therapeutic intervention, and exotic viruses.

9. Routes of Exposure of a Hazardous Agent

In order for biological agents to cause disease, they must first enter or invade the body in sufficient numbers. Routes of entry include oral, respiratory, parenteral, mucous membrane, and animal contacts (bites, scratches). Once inside the body, biohazards must meet other requirements to cause disease; they must colonize and establish in body cells, tissues and/or organs, overcome the body's natural defense mechanisms and mutate or adapt to body changes.

When evaluating an infectious agent, the risk assessment process must account for the factors that 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).

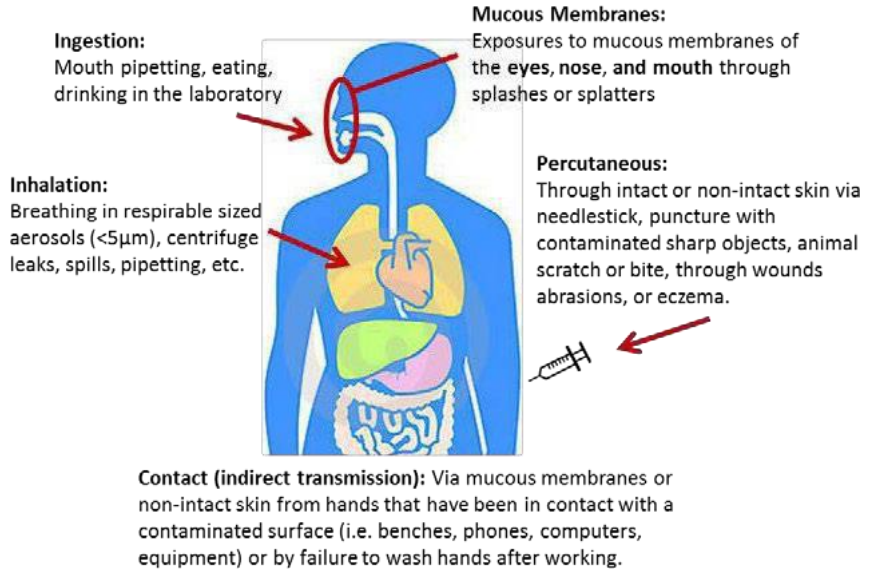


Figure 1. Main routes of entry for infectious agents.

To reduce the risk of exposure in the laboratory, always adhere to these basic biosafety principles:

- Keep all laboratory doors closed and labels doors with hazard warning signs as appropriate for the biological material in use.
- Do not eat, drink, or smoke in the laboratory.
- Wear appropriate PPE when conducting any/all laboratory and clinical procedures.
- Remove all PPE prior to leaving the laboratory.
- Decontaminate gloves with appropriate disinfectant often and PPE when compromised or contaminated.
- Ensure all reusable laboratory coats are periodically laundered.
- Always wash hands after removed gloves and before leaving the work area.
- Add street clothes definition
- Never mouth pipette, always use mechanical pipettors.
- Use extreme caution when working with sharps.
- Contain aerosols by using appropriate equipment (i.e. biosafety cabinet, aerosol proof rotors).
- Decontaminate work surfaces and equipment at the completion of each procedure and decontaminate and clean work surfaces and equipment at the end of the work period.
- Use and maintain equipment according to the manufacturer's instructions.

Table 2: Protective measures to minimize transmission to infectious agents in the laboratory

Route of Exposure	Protective Measures
Mucous Membranes	Achieve face protection by: • working in a BSC or behind a protective shield • following good microbiological practices • following good hygiene practices including hand washing • wearing appropriate PPE (e.g. safety glasses and surgical mask or a full-face shield)
Inhalation	Avoid exposure to aerosols by: • working in a BSC • using sealed rotors or canisters when centrifuging • following good microbiological practices • wearing appropriate PPE (e.g. respirators)
Ingestion	Prevent exposure via ingestion by: • never eating, drinking or smoking in the laboratory • always using mechanical pipettors • following good microbiological practices • following good hygiene practices including hand washing • wearing appropriate PPE (e.g. laboratory coat, gloves, and safety glasses)
Percutaneous	Prevent percutaneous injuries by: • substituting plastic for glass • using extreme caution with sharps • discarding sharps immediately into a rigid leak-proof sharp container • properly restraining animals or anesthetize animals prior to procedures • covering non-intact skin with waterproof bandages and wearing double gloves • wearing appropriate PPE (lab coat, gloves, safety glasses, cut resistant gloves, and sleeves)
Contact (indirect exposure)	Prevent indirect exposure by: • decontaminate and clean used work surfaces • following good microbiological practices • washing hands when finished working or gloves have been compromised • not touching the face or hair with gloves or non-gloved hands (good personal hygiene) • not handling or using personal mobile electronics (e.g. cell phones) in the laboratory • not applying cosmetics within the laboratory wearing appropriate PPE (e.g. laboratory coat, gloves, and safety glasses)

9.1 Procedural hazards

Workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. Protection depends on the conscientious and proficient use of good microbiological practices, the correct use of safety equipment, and the use of appropriate PPE. Equipment can be defined as instruments, machines, and/or structures that are designed and/or engineered to confine hazardous material for the purpose of manipulation under a controlled environment. When assessing the hazards associated with the use of common laboratory equipment, laboratory staff must consider how biological material will act when subject to the functions of that equipment.

10. Bio-Containment

As defined by Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th edition, bio-containment refers to the safe methods for managing infectious material in the lab environment where they are being handled and maintained. The purpose of the different levels of bio-containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. Once a complete risk assessment has been made, the appropriate bio-containment needs to be selected to ensure safe work conditions. The bio- containment required for laboratory operations (including laboratory biosafety level, PPE, and practices) will be determined by EH&S and the IBC following a review of the laboratory, equipment, procedures, training, and the hazards utilized.

10.1 Biosafety Levels

The CDC and NIH have established Biosafety levels (BSLs) for work with biohazardous materials in their publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 6th edition. This publication provides combinations of microbiological practices, laboratory facilities, and safety equipment as well as their recommended use in four levels of laboratory operations. Also included in the BMBL is a parallel set of BSLs for research involving small laboratory animals. Additionally, the BMBL and the American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene have established containment levels for research involving the use of insects.

Tissue culture rooms are defined as BSL2. Below is a summary of practices, equipment and facility requirements for agents assigned to BSLs 1–4. UNT Health does not have BSL3 or BSL4 laboratories. Additional information on BSLs may be found in the BMBL 6th edition.

10.2 Biosafety Level 1

BSL1 is suitable for work involving Risk Group 1 agents that are well-characterized agents not known to consistently cause disease in immunocompetent adult humans or agricultural plants and animals, and present minimal potential hazard to laboratory personnel and the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

10.3 Biosafety Level 2

BSL2 builds upon BSL1. BSL2 is suitable for work involving Risk group 2 and certain risk group 3 agents that pose moderate hazards to personnel and the environment. BSL2 laboratories are usually clinical, diagnostic, teaching, research, or production. It differs from BSL1 in that 1) laboratory personnel have specific training on the containment, manipulation, cultivation, and disposal of pathogenic agents and are supervised by scientists competent in handling these agents; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in Biosafety Cabinets (BSC) or other physical containment equipment.

10.4 Biosafety Level 2 Enhance

BSL2E builds upon the classic BSL2 environment. While BSL2E uses the same basic layout and equipment as a BSL2 it uses procedures and Standard Operating Procedures (SOP)s more typically associated with a BSL3 lab, such as the use of front closing gowns and respiratory protection

10.5 Biosafety Level 3

BSL3 builds upon BSL2. BSL3 is applicable to risk group 3 agents where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease following exposure. Laboratory personnel have specific training on the containment, manipulation, cultivation, and disposal of high-risk pathogenic agents, and must be supervised by scientists competent in handling these agents. All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate PPE. A BSL3 laboratory has special engineering and design features. Currently UNT Health does not have any BSL3 facilities.

10.6 Biosafety Level 4

BSL4 builds upon BSL3 and is used to handle Risk Group 4 agents. Currently, UNT Health does not have facilities that support BSL4 containment. For more information on BSL4 facilities, containment, equipment, and practices, please refer to the BMBL 6th edition.

11. Specific regulatory requirements

11.1 Select Agents and Toxins

The use and storage of non-excluded select agents and select agent toxins are regulated by the Centers for Disease Control Division of Select Agents and Toxin (DSAT). Institutions that use and store these agents and toxins must register with DSAT. DSAT, and requires registered institutions to implement enhanced training, security measures, containment, equipment, and procedures. Additionally, DSAT may adopted the guidelines described the BMBL 6th edition as policy which will require institutions to enforce these measures in registered facilities including applications specific to equipment, procedures, and personnel. UNT Health is not currently a registered entity for the storage and use of Select agent and Select Agent toxins. It is the responsibility of the PI that store and handle exempt quantities of Select Agent Toxin to NEVER exceed the permissible limit and to notify EHS of the toxins present. A full list of agent and toxins can be found, Select Agents and Toxins List | Federal Select Agent Program

11.2 NIH Guidelines

The use of recombinant or synthetic nucleic acid molecules, genetically altered microbes and animals, and the use of recombinant DNA in human patient is regulated by the NIH Office of Science Policy (NIH-OSP). In accordance with their oversight function, any institution that receives NIH funding is required to comply with the NIH Guidelines. These guidelines define the strict containment of microorganisms based on risk group classification. As written, these standards may not provide a containment level of recombinant attenuated or avirulent strains of specific microorganisms created in the laboratory. Therefore, the containment level established for the listed genus and species must be adhered to unless a containment downgrade is authorized by the NIH OSP. EHS will assist in the process to obtain approval for downgrade of proposed recombinant work. If using material from a colleague that has been downgraded by NIH_OSP a new application must be obtained for each PI using the material. Contact EHS for assistance

11.3 Department of Defense (DOD), CDC Etiological Agent Division, Unites States Department of Agriculture (USDA), and Food and Drug Administration (FDA)

The listed above agencies may require the implementation of additional biosafety and animal containment levels specific to microorganisms and experimental deigns covered under their regulatory

authority. Please check with EH&S if you have any questions regarding these regulatory agencies.

12. Biosafety Training

Once a risk assessment of the work to be done has been performed, it is important to ensure that all personnel have been provided with accurate information about the possible risks, as well as all appropriate training. These measures are necessary in order for personnel to perform their jobs safely and in compliance with all applicable regulations. A well-designed intra- laboratory training program facilitates safe work practices, increases technical proficiency, and motivates employees to adhere to establish procedures.

Objectives of the UNT HEALTH's Biosafety Training https://www.unthsc.edu/safety/biosafety/biosafety-training/

- Provide information on biosafety, the identification and containment of biological hazards, and universal precautions, as well as the proper selection and use of laboratory and clinical safety equipment.
- Provide information and updates on new safety techniques and protocols.
- Demonstrate safe work techniques.
- Provide instruction on emergency response procedures.
- Convey information on important regulations (such as the transport of dangerous goods).
- Motivates personnel to work safely.

Laboratory personnel must receive initial training on potential hazards associated with their work, necessary precautions to prevent exposures, and exposure evaluation procedures. New employee training is mandatory regardless of the employee's perceived or proven experience. Personnel must also receive applicable annual updates (e.g. Bloodborne pathogen training) and additional training as necessary for procedural or policy changes, or as required by regulation. Classroom and online training will be provided by the Biosafety Program. Extensive on-the-job training will be provided by PIs, expert collaborators, and/or staff with applicable expertise.

Mandatory Biosafety Program training classes relevant to biosafety are summarized in Table 3.

Table 3: Biosafety Program Training Courses

Course	Who Must Take the Course?	Delivery Method	Frequency
Biosafety training (includes Biosafety and NIH Guidelines)	Faculty, Clinicians, Fellows, Postdoctoral fellows, Students, Staff, Temps, Interns, Volunteers, Visiting Scientists	Online	Upon Hire and every 3years

Biosafety Refresher	Faculty, Clinicians, Fellows, Postdoctoral fellows, Students, Staff, Temps, Interns, Volunteers, Visiting Scientists	Classroom Scheduled	As needed
Bloodborne Pathogen Refresher	Every individual working with or exposed to human blood, body fluids, or other potentially infectious materials	Online	Annually
Specialized Courses	Courses are designed as needed to capture individuals who have a need- to-know not provided by the training requirements above. Examples: EH&S staff, Physical Plant staff, Housekeeping staff, EH&S Emergency Responders, Police Officers, and Security Guards.	Classroom and On-site	As needed
Medical Waste	Any individual who packages or transports medical waste. Any individual who signs a medical waste manifest.	On-site/online	annually
Autoclave	Any individual who utilizes autoclaves to treat biological waste.	On-site	As needed
Exposure and/or Incident Remediation	Faculty, Clinicians, Fellows, Postdoctoral fellows, Students, Staff, Temps, Interns, Volunteers, Visiting Scientists, Auditors/Inspectors, Physical Plant, Police/Security	On-site	Post incident

The Biosafety Program provides a variety of additional training courses that are designed to provide hands-on training covering Biosafety Level 2 and 2E operations, the proper use of the biosafety cabinet, centrifuges, and other laboratory equipment. For more information, send an email to the Biosafety Program at safety@unthsc.edu

13. Standard Laboratory Practices

13.1 Standard Microbiological Practices - The Common Sense of Laboratory Safety

Standard Microbiological Practices refer to the basic safe laboratory protocols for working with biological materials. In general, the objectives of good microbiological practice are to prevent contamination of laboratory workers, the environment, and prevent contamination of the experiment/samples. Basic good microbiological practices include, but are not limited to:

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

- Eating, drinking, chewing gum, smoking, handling contact lenses, applying cosmetics (including lip balm), and storing food for human consumption is not permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- To allow gloves to fit properly and for good infection control, fingernails should be no longer than 0.25 inch beyond the end of the finger.
- Laboratory surfaces must be designed for standard laboratory applications including handling and manipulating biological materials and standard laboratory chemicals (e.g. ethanol, acids, bases, and various solid and liquid hazardous and nonhazardous chemicals).
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Perform all experimental procedures to minimize the creation of splashes and/or aerosols. If the procedures or agent manipulation inherently will generate aerosols, perform the procedure within a biosafety cabinet.
- Utilizing sterile technique while handling biological materials, samples, and cultures.
- Properly treating (i.e. chemical neutralization and/or autoclave) waste products produced during the handling and manipulation of biological materials.
- Sharps, such as needles, scalpels, pipettes, and broken glassware, must be handled and disposed of properly and safely in order to prevent accidental needle sticks and cuts. Precautions, including those listed below, must always be taken with sharp items. These include:
    - Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - Used disposable needles and syringes must be carefully placed in conveniently located puncture- resistant containers used for sharps disposal.
    - Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.

13.2 Special Practices-Biosafety Level 2 and Above

- All personnel entering the laboratory must be advised of the potential hazards, meet specific entry/exit requirements, and wear appropriate clothing and required personnel protective equipment (e.g. gloves, lab coat, and safety glasses).
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory. When appropriate and at the direction of a licensed health care practitioner, baseline serum sampling may be advised.
- Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory surfaces (e.g. benches) and equipment (e.g. centrifuges) must be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
    - Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained to work with infectious material.
    - Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to post exposure procedures described in this Biosafety Manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained (e.g. First Report of Injury).
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

14. Personal Protective Equipment

The direct, often hands on use of biological, chemical, and physical hazards in UNT Health laboratories requires that UNT Health establish minimal workplace standards for attire and for the use of Personnel Protective Equipment (PPE) (protective clothing and safety apparatus/equipment). Although not considered the first line (i.e. facility design, equipment, and procedures) of hazard control, the correct selection and use of PPE will prevent exposure to biohazardous and infectious material. The extent and kind of safety clothing and equipment to be selected for any particular activity depends upon the laboratory facility, the research operations, and levels of risk associated with the research (Table 4).

The Institutional Biosafety Committee has determined that this manual shall establish the minimum PPE required for working in laboratories as well as provide guidance on the selection and use of PPE.

14.1 Minimum Standard for Laboratory Operations

According to this manual lab personnel *must* wear clothing that is appropriate for the workplace. Personnel should ensure that pants or skirts cover the legs down to the ankles; shirts are composed of cotton rather than synthetic fabrics; and shoes cover the complete foot up to the ankle.

As per this manual, laboratory personnel must wear, at minimum, a laboratory coat, eye protection, and gloves when engaged in any research activities on laboratory work surfaces (e.g. lab bench, fume hood, tissue culture hood, microscope station, etc.). Research activities may include but are not limited to laboratory experiments, preparing laboratory reagents, handling chemicals, processing clinical or research laboratory samples and specimens, and handling, manipulating, and cultivating microorganism or cell culture. Additional PPE (e.g. respirators, cryo- gloves, etc.) maybe required based on the workplace, the hazard, and/or how the hazard is manipulated (e.g., aerosol production, etc.).

When not engaged in the above activities (i.e. reading a research article, checking emails at provided desks, or entering and exiting the laboratory) the above PPE is not required. PIs are responsible for ensuring their staff members have access to and are wearing the appropriate PPE.

14.2 Lab Coats

Both reusable and disposable laboratory coats are provided by the PI to all the researchers working in the lab. Whichever is used, it must be durable, designed to provide protection and be compatible with the methods of decontamination employed.
Lab coats serve 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 different fabrics and be easily disseminated.

Important points to remember:

- Lab coats are not 100% water repellant.
- Lab coats can be disposable or reusable
- Lab coats should have long sleeves, go down the legs and may have cuffs sleeves depending on the model.
- Lab coats worn within the laboratory or clinical area should not be worn outside the facility to the library, cafeteria, or other places accessible to the public.
- Lab coats should be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.
- All lab coats 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.

- We recommend not take lab coats home to launder.
- Change lab coat must be changed out whenever it is compromised, soiled or torn.
- Wear appropriate lab coat sizes.
- Wash hands whenever lab coat is removed.

14.3 Gloves

Glove selection and use procedures must be based on an appropriate risk assessment. This risk assessment must consider the disinfectants used to decontaminate PPE and work surfaces as these chemicals may damage or penetrate/permeate through gloves. As most research procedures involves the direct handling of biological materials, it is anticipated that the hands and wrist could become contaminated and serve as a primary factor in the transmission of biological materials and infectious agents from the laboratory. Therefore, the proper use of gloves can be considered a basic precept of preventing infectious agent transmission.

Important points to remember:

- Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists.
- Check gloves for visible tears before use.
- 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.
- Do not reuse disposable gloves. Discard contaminated gloves in a biohazard bag immediately after use.
- Gloves shall be removed and hands washed before exiting the laboratory.
- All material must be properly packaged in an appropriate transport container , when transporting materials outside of the laboratory areas. Alternatively utilize a cart to transport the material within an appropriate secondary container.
- Gloves must not be worn outside of the laboratory
- Gloves need to be changed during long procedures to ensure proper integrity is maintained; gloves must be changed when damaged or contaminated.
- Gloves will not prevent needle sticks or other puncture injuries.
- Double gloves should be used when handling hazardous material
- Double glove or use thicker rubber when cleaning biohazardous spills.
- Latex gloves generally are suitable for providing protection from biological hazards, but personnel should be aware of the existence of latex allergy in a portion of the population.it is recommended to use Nitrile gloves

- Report to the PI and EHS any sensitivity or allergies to gloves as soon as the first symptoms are present.

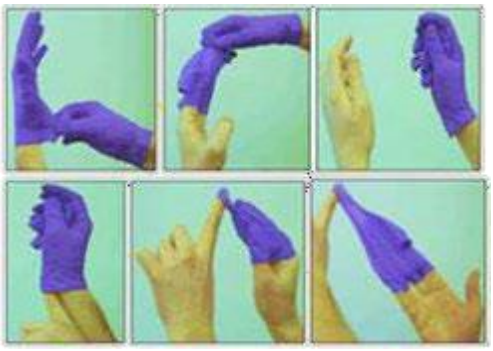


Figure 2. Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

Proper usage and removal of personal protective equipment (PPE) is a critical component of biosafety and occupational health. Gloves, gowns, masks, and other PPE form the first line of defense against hazardous materials, infectious agents, and chemicals. However, improper glove removal can compromise protection and increase the risk of contamination. To address this, UNT Health Environmental Health and Safety (EHS) developed a training initiative highlighting correct glove removal techniques. In collaboration with the Compliance Department and Academic Innovation, EHS produced two instructional videos demonstrating safe glove removal methods.

Please use the following link the watch the videos.

- https://unthsc.mediasite.com/Mediasite/Play/d6fd3d5dee58472894885ef37f102c901d. – Part A
- https://unthsc.mediasite.com/Mediasite/Play/3335e98ed2a54b0191d26da84d9c9b841d. -Part B

14.4 Basic Hand Washing (soap and water):

a. Use soap and water when:

- After removing gloves
- Before leaving the lab
- Hands are visibly soiled (dirty).
- Hands are visibly contaminated with blood or body fluids.
- Before and after eating.
- After using the restroom, sneezing, or coughing.

14.5 Shoes

Shoes worn in the laboratory must be closed-toe and cover the foot up to the ankle. Laboratory and clinical staff should be aware that cloth shoes such as athletic shoes and dress shoes, although closed at the toe, may be absorbent or vented allowing the penetration of hazardous materials and exposure of the skin. Sandals or flip- flops are not allowed. Additionally, based on laboratory, clean room, or animal facility entry requirements, shoes cover or Tyvek may be placed over shoes to reduce the potential for facility contamination and/or to prevent the contamination of personal clothing.

14.6 Face and Eye Protection

Protection of the face and mucosal membranes (i.e. eyes, nares, oral-pharyngeal tissue) 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 into the mouth. A variety of face shields, head covers/hoods, protective goggles, and safety glasses should be made available to all laboratory personnel. The selection of appropriate face/eye protection is dependent upon the work to be conducted and the overall facial area requiring protection.

Important points to remember:

- All eye protection must be ANSI Z87.1 compliant
- 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 operating the autoclave. Impact resistant face shields will protect the user's face against splatters of hot liquids or broken glass fragments.
- Safety glasses do not protect the eyes from aerosol exposure or from multiple direction splashes and sprays. Tight fitting goggles should be worn.
- Face shields protect the face and the neck from flying particles and sprays of hazardous material.
- Shields should cover the entire face and be easily removed in the event of an exposure.
- It is recommended that contact lenses not be worn when working around chemicals, fumes, dust particles, and other hazardous materials. When contact lenses are worn, eye protection is mandatory.
- Prescription glasses are not an alternative to safety glasses. Prescription safety glasses can be purchased \.
- Safety glasses, face shield, and other eye and face protection PPE must be decontaminated after use and properly stored and readily available for use. If heavily contaminated, this PPE can be disposed of as biological waste.

- Although this manual primarily requires the use of safety glasses or face shields to protect against exposure to biological materials, laboratory personnel must consider exposure to chemical (e.g. disinfectants) and physical hazards (e.g. U.V. lights) that will be used during the manipulation of biological materials.

14.7 Respiratory Protection

Protection of the respiratory system is important because infectious organisms can readily enter the human body through inhalation. In recognition of this risk factor, UNT Health has created a respiratory protection program and a respiratory protection manual. The respiratory protection program is managed by EH&S and The Health Pavilion (HP) The respiratory protection plan describes known respiratory hazards, potential exposure and health risks, and the operations of the respiratory protection program. Refer to this document for a complete description of respiratory hazards, respiratory protective gear, and the medical and physical requirements covering the use of respirators.

Engineering controls, such as the use of BSCs, should always be considered as a first line of control against exposure to airborne hazards. The use of respirators must be considered when feasible engineering controls have failed to remove the airborne hazards to safe levels.

14.8 Additional Protective Apparel

- PPE can be designed to cover either the entire body/clothing (jumpsuit – whole body) or specific areas of the body/clothing (show covers - feet, aprons – torso). The following represents common manufactured PPE utilized at UNT HEALTH:
    - Breathable with minimal moisture resistance PPE – Disposable polypropylene jumpsuits, and gowns, scrubs, shoe covers, and hair nets.
        - Generally worn in ABSL1 animal facilities and is designed to provide a simple barrier between a person's clothing, skin, and/or hair and hazards present in the work environment. Since this PPE is disposable, the ability to shed this clothing in the lab reduces the potential for biological materials, allergens, and other related contaminants to be inadvertently transmitted from the facility.
    - Breathable moisture resistant – Solid front gowns composed of microporous fabrics.
        - Generally worn in areas where there is a high risk of exposure to blood, fluids, and pathogens. These areas include enhanced BSL2 facilities, and surgical rooms.
    - Non-breathable moisture impervious – Tyvek and Tychem jumpsuits, aprons, smocks, sleeve covers, shoe covers, and head covers, as well as rubber aprons.
        - Fluid impervious materials that provide excellent protection for activities that have high exposure risks and/or for biological and chemical agents that have high health consequences.

- PPE can be designed for specialized functions or to protect against a specific type of hazard. The following are examples of this type of PPE:
    - Kevlar gloves and sleeves are cut resistant and will help guard against slices, scratches or cuts, but will not prevent direct puncture or needle stick 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.
- Other use factors to consider:
    - Elastic cuffs present at the wrist and ankle may move when limbs or flexed and bent. Depending on the operations with the workplace and the hazards present, taping the suit/apron wrist cuff to the gloves and/or the ankle cuff to boots may be necessary.
    - The use of biosafety cabinets requires personnel to extend their arms into the cabinet, which exposes this area of the body or clothing worn over the arms to the hazards utilized in the cabinet. The use of Tyvek sleeves may be warranted if the hazard in use is considered highly infectious and/or high transmissible.
    - Workplace activities as well as spills can contaminate the floor with liquid and solid hazards that can become trapped in the crevices of shoes. Once this occurs, these hazards can be tracked from the spill area leading to large scale contamination. In these environments, the use of Tyvek shoe or boot covers is warranted. These covers must be shed and discarded into the appropriate waste container before exiting the workplace or hazardous spill area.

Table 4: Summary of Biosafety Level and PPE Requirements When Engaged in Research Activities

PPE	BSL1	BSL2	BSL2E
Gloves	At least a single layer of gloves is required.	At least a single layer of gloves is required. Double gloves may be required during specific high-risk laboratory operations.	Double gloves required.
Lab Coat	All laboratory staff must be provided with and are required to wear a laboratory coat.	All laboratory staff must be provided a laboratory coat. It is recommended that a separate laboratory coat be worn in BSL2 laboratory.	Solid front back fastening gown with tight fitting cuffs or Tyvek jumpsuits must be worn to protect street clothing and skin from contact with infectious agents.
Face Protection	Safety glasses are required when working with liquids on the open bench. Mask with visors, face	Safety glasses are required. Mask with visors, face shield, and safety goggles may be required based on laboratory operations and the hazards	eye protection is required

	shield, and safety goggles may be required based on laboratory operations and the hazards present or utilized	present or utilized	
Respiratory Protection (All those who may wear a respirator must be enrolled in the UNT Health Respiratory Protection Program.)			All those required to wear an N 95 or PAPR respirator need to have an annual fit test on file with the PCC. Annual training instead of Fit testing is required for PAPR users.
Other PPE		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 material, such as organ harvesting or large spill response and clean up.	

15. Signage and Labeling

15.1 Laboratory Entrance Doors

All laboratory entry doors must have a sign similar to what we have shown in figure 3. This door sign is provided by EHS. This door sign must have the following information posted:

- The hazards present in the laboratory. If the laboratory stores and/or utilizes infectious agents the doors sign must incorporate the universal biohazard symbol.
- The door sign may list the biocontainment level if required.
- A primary, secondary, and tertiary contact person:
    - name of supervisor or PI
    - lab manager
    - senior technician
- Telephone numbers.

- Required personal protective equipment.
- Vaccine requirement if needed
- Required procedures for entering and exiting the laboratory.
- Agent specific information should be posted

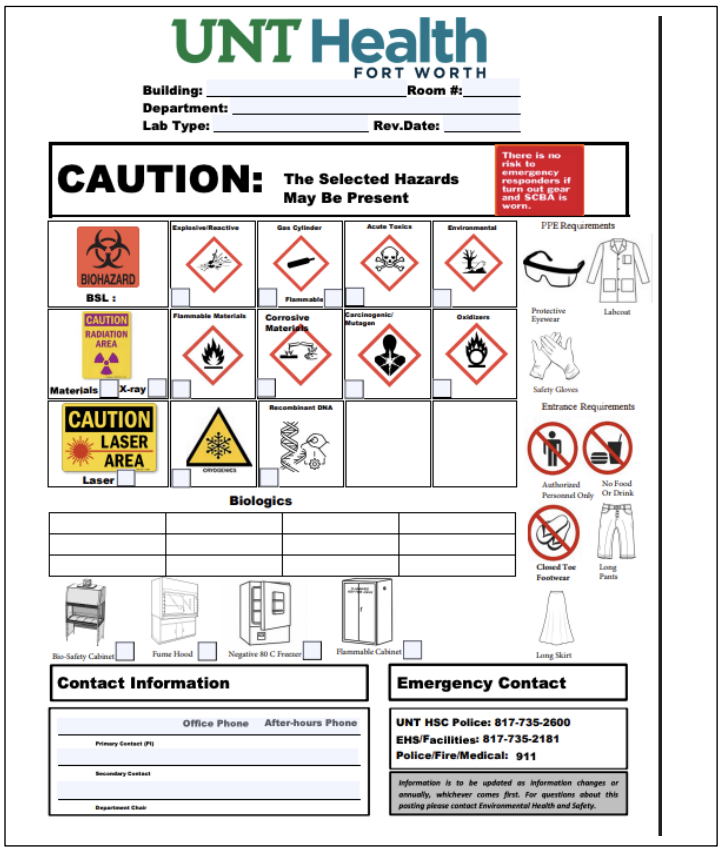


Figure 3. Entry Door Sign

The entry doors of BSL-2 laboratory areas (tissue/cell culture rooms) must have a Biohazard Door Sign indicating the biohazard materials used at that location (Figure3)

Laboratory doors must not have additional signage, or posters without EHS approval.

Windows present on laboratory doors must remain unobstructed. Experiments involving animals or that need light controlled environment may have window covers but these must be removed when experiments are not in process.

15.2 Equipment

All equipment used to manipulate, cultivate, and store infectious agents, such as BSCs, incubators, centrifuges, refrigerators, freezers, etc. *must* be labeled with a biohazard symbol sticker.

Figure 4. Equipment Labeling

15.3 Animal Rooms Hazard Door Sign

Rooms housing animal exposed to infections agents must have a hazard door sign posted as shown in figure 5 during the entire exposure period or as defined by EH&S and DLAM. Door signs are prepared by the PI in consultation with BSO.

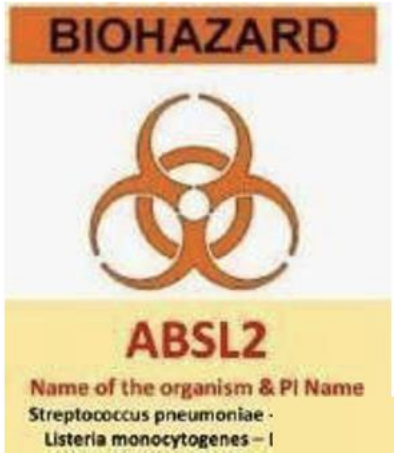


Figure 5. Door Sign for Research Involving Animals and Pathogen

16. Safety Practices for the Different Biosafety Levels

Prevention is an important element to biohazard control, and it is recommended that anyone working in a laboratory read this section carefully.

16.1 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.

Supervisors and PIs play an important institutional role in establishing acceptable laboratory behaviors. For this role to be effective, supervisors and PIs must understand the importance of attitudes and human factors (noise) in the development and transmission of both appropriate and inappropriate behaviors. This can be accomplished through mentorships and management. Mentorship facilitates hazard and risk communication by integrating personnel experience and expertise into training and hands-on exercises. Good management skills allow the supervisor or PI to intervene when inappropriate behaviors are identified.

16.2 Prevention

Prevention is achieved through proper risk management to include hazard identification, assessment, evaluation, control, communication, review, surveillance, post incident investigation, and root cause analysis. As attitude and human factors impact our personal perception of risks, this risk management strategy must include methods to establish structured processes (e.g. training, procedures, facilities, and equipment) and expectations (e.g. appropriate behaviors - adherence to safety protocols, good laboratory practice, and proper hygiene).

This can be accomplished with continued training and hazard awareness in-services provided by EH&S, Research Compliance, and the PI. To ensure these initiatives are effective trainers, supervisors, and staff must be aware of the problems with perceived mastery and its impact on hazard complacency. New training and awareness impact our perception of risks which typically raises the internal concepts of safety and wellbeing.

When the use of hazardous materials becomes routine, staff can become complacent regarding the hazards posed by these substances.

It is not until a near miss or incident occurs that staff realizes the impact of this complacency. If this realization event is appropriately fostered by the employee, EH&S, Research Compliance, and the PI, it will lead to sustainable behavioral changes. Examples include numerous eye injuries that have occurred on our campus. Personnel who received these injuries chose not to wear eye protection, even though they were trained to wear this PPE and it was available. Following the injury, these staff

members typically wear eye protection until complacency returns and the cycle begins again (Figure 6).

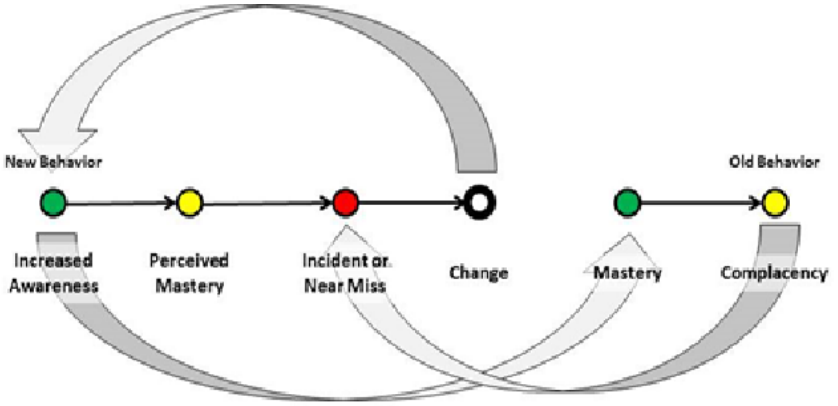


Figure 6. Change and Train – Utilize Lessons Learned

Prevention provides an effective means to identifying this cycle with the goal of preventing reoccurrence. This requires a team approach that must include buy in from the supervisor or PI and their staff. Simple activities such as taking 15 minutes during laboratory meetings to discuss hazard awareness and safety helps facilitate continued hazard awareness and provides a forum to discuss issues, near misses, and concerns.

The following observations may help supervisors and PIs recognize activities that may lead to laboratory accidents:

- Inflexibility of work habits, that tends to preclude last minute modification when an accident situation is recognized.
- Working at an abnormal rate of speed.
- Intentional violations of laboratory safety standards are a frequent cause of accidents.
- Failure to wear provided personal protective equipment.
- Failure to use available safety equipment (fume hood or biosafety cabinet) when conducting experiments or procedures that generate hazardous vapors, droplets, or aerosols.
- Working when one is very tired.
- Utilizing cluttered worksurfaces.

16.3 Operational Standards for UNT Health Laboratories

This section explores the standard practices, equipment, and procedures required for research that involves the use of biological materials. Similar to physical bio-containment features, laboratory procedures incorporate a hierarchy of controls that are based on the risk of the hazards being utilized. As described above in the Biosafety Level Section, these controls grow in complexity as the exposure and health risks associated with the biological materials increase. Additionally, the controls established for standard wet labs (Biosafety Level 1) are seemingly incorporated into higher level laboratory processes. Thus, if something is required at Biosafety Level 1 it can be expected to be required at Biosafety Level 2 and above.

16.3.1 Biosafety Level 1 (BSL1) – Figure 7

- Keep laboratory door closed.
- Wear laboratory coats, gloves, and eye protection.
- Do not reuse disposable PPE.
- Decontaminate reusable PPE.
- Use procedures that minimize aerosol formation.
- Do not smoke, eat, drink or store food in laboratories.
- Do not mouth pipette, use mechanical pipetting devices.
- Critically evaluate the use and need for hypodermic needles.
- Implement sharps protection procedures.
- Receiving training on and properly use the fume hood/BSC to contain hazardous vapors, aerosols, and particulates.
- Wash hands after completing experimental procedures, following removing PPE, and before leaving laboratory.
- Change PPE when soiled or compromised.
- Decontaminate work surfaces daily and immediately after a spill.
- Liquid culture waste and unused/unwanted culture stocks must be treated with bleach to a final concentration of 10% v/v. Allow at least 30 minutes for the bleach to inactivate viable material prior to discharging this material to the sanitary drain.
- Decontaminate other non-disposable contaminated materials before washing or reuse.
- For off-site decontamination, package contaminated materials in closed, durable, leak-proof containers.
- Protect vacuum spigots with properly established vacuum traps and with vacuguard filters. The membrane in these filters needs to be composed of hydrophobic material

like polytetrafluoroethylene (PTFE) 0.45µm in-line disk filter. Filters must be changed yearly or when contaminated or liquid has touched the membrane.

- Maintain spill kit appropriate for the biological, chemical, and radiological hazards used and/or stored within the laboratory.
- Report spills, accidents, near misses and disease symptoms related to laboratory acquired infection to the PI and EHS.
- Keep animals and plants not used in experiments out of the laboratory.
- Keep areas neat and clean.
- Control insect and rodent infestations.
- Do not wear PPE outside of the laboratory.

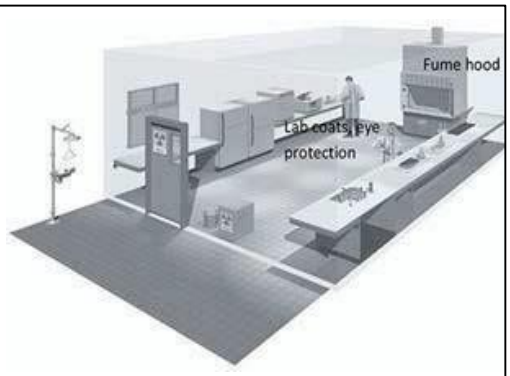


Figure 7. Biosafety Level 1 laboratory.

16.3.2 Biosafety Level 2 (BSL2) – Figure 8

- Allow only persons informed of the research hazards and authorized by the PI to enter BSL2 areas.
- Following any/all medical surveillance requirements established by EH&S and Research Compliance.
- Wear additional PPE (e.g. Tyvek sleeves, face shield) based on the hazard and/or process.
- Do not wear PPE outside of the BSL2laboratory.
- Post a universal biohazard label on equipment where infectious agents are stored, manipulated or cultivated and on waste containers.
- Liquid culture and stocks containing infectious agents must be autoclave sterilized prior to disposal.
- Substitute plastic for glass where feasible.
- Use biosafety cabinets to contain aerosol-producing equipment/procedures.

Note: Tissue culture laboratories are commonly found within UNT Health research buildings and according to institutional standards, tissue culture laboratories are operated as biosafety level 2 facilities.

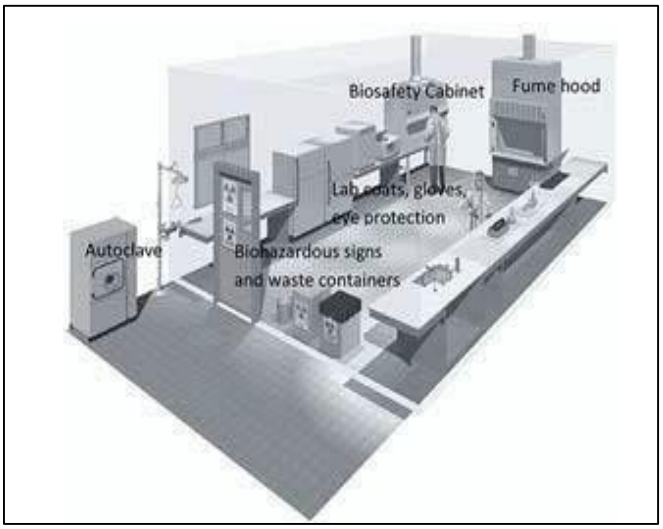


Figure 8. Biosafety Level 2 Facility

16.3.3 Biosafety Level 2 Enhanced (BSL2E)

BSL2E is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BSL2 procedures. Generally, BSL3 practices are mandated in a space designed for BSL2E work. It is preferred that the BSL2E laboratories be self-contained with all equipment required for the experiment located within the laboratory. A biohazard door sign listing the agent in use, emergency contact, and entry requirements is posted on the door while BSL2E work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment and all surfaces have been decontaminated, the sign is removed and the laboratory is returned to standard BSL2 or BSL1 use if the work is not routine.

Summary of Recommended Biosafety Levels for Infectious Agents*

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy adults	Standard Microbiological Practices	None required PPE: laboratory coats, gloves, face protection as needed	Open bench top, sink required
2	Associated with human and animal, plant disease, hazards are autoinoculation, ingestion, mucous membrane exposure	BSL1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions; • Biosafety manual defining any needed waste 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: laboratory coats, gloves, face protection as needed	BSL1 plus: Autoclave available Directional airflow and self-closing doors

*Adapted from the CDC Office of Health and Safety

17. Safety Considerations When Using Engineering Controls and Equipment

17.1 Biosafety Cabinets (BSC)

BSCs, when used properly, provide a clean work environment for research or patient care activities. BSCs function to provide personnel, product, and environmental protection by isolating and containing biohazardous materials inside a cabinet. As designed, BSCs are considered primary containment barriers for the handling of infectious materials. Based on this classification, the IBC will require open vessels or containers housing virulent risk group 2 pathogens, all risk group 3 pathogens, viral vectors, human cell lines, and human tissues and unfixed animal tissues to be handled within a Biosafety Cabinet. This will include but is not limited to the following processes:

- Inoculating culture media with stocks of infectious agents such as plating, seed cultures, overnight cultures, generating freezer stocks, etc.).
- Prepping culture material for further processing such as centrifugation, spectroscopy, microscopy, etc.

BSCs function by drawing ambient air inward into a sealed cabinet through grills located at the front of the work surface. This inward draw pulls air under the primary work surface through a plenum structure. The type of BSC will impact how air is circulated in the cabinet and whether the cabinet returns air to the room or exhaust air through attached ductwork to the outside. Air within the cabinet

is HEPA filtered before being directed over the BSC work surface and must pass through a HEPA filter before leaving the cabinet.

The number of intricate parts and the functionality of the cabinet air flow systems, as well as the efficacy of the cabinet's HEPA filters require BSCs to be periodically maintained by certified technicians. Based on the need to ensure these cabinets properly function, the IBC has adopted the standards defined in NSF/ANSI 49 and by the NIH, CDC, and OSHA specific to the maintenance and certification of BSC. By adopting these standards, the IBC will require the following:

- Biosafety cabinets must be certified by a NSF accredited technician when installed, following movement or repair, and at least on an annual basis. EH&S has identified local technicians who are NSF accredited. Please refer to the BSC program for more information.
- Biosafety cabinets must be properly decontaminated prior to repairs, movement, sale, or disposal. Acceptable decontamination methods include chlorine dioxide, vaporized hydrogen peroxide, and formaldehyde gas. EH&S has identified local technicians who have the equipment and expertise to conduct BSC decontaminations.

During laboratory surveys, EH&S will evaluate the status of laboratory BSCs, as well as horizontal and vertical laminar flow benches. During this evaluation, EH&S surveyors will determine if the cabinet certification is current and whether the cabinet is being operated in accordance with this manual.

The containment provided by BSCs depends upon the behavior of the operator, the maintenance of the unit, the cabinet's location in relation to other facility features (i.e. doors, supply diffusers, exhaust ports), and the placement of items on the cabinet's work surface. Users must receive hands on training from their PI or EH&S prior to using a BSC. The following sections provide additional information on the proper use and maintenance of BSCs.

Basic Startup Procedures for Class II BSC:

- If used, turn off UV light; turn on fluorescent light and blower.
- Check certification date and airflow.
- Disinfect all interior surfaces with suitable disinfectant.
- Decontaminate all items placed in the biosafety cabinet and only place items necessary for the experiment on the cabinet's surface.
- Do not obstruct the front or back grills.
- Wait 2-3 minutes for contaminants to purge from work area.
- Avoid multiple entries and exits from the BSC during a single procedure.

- After procedure, allow cabinet to run 2-3 minutes before removing materials.
- Surface decontaminate all martial prior to removal from the BSC.
- Wipe down all work surfaces with suitable disinfectant.
- Turn off fluorescent light and blower if desired.

Many BSCs are equipped with germicidal ultraviolet (UV) lamps. UV lights should never be used as the primary or sole means of decontamination of surfaces and equipment. Time of exposure, distance, presence of dust or debris and UV lamp intensity affect the germicidal effect of the UV lamp. 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 and tested to monitor the loss of intensity. UV lamps may damage eyes, skin, and laboratory equipment. UV lamps should be turned off while the room is occupied. EH&S discourages the use of UV lamps due to the potential damage resulting from UV lamp use.

- **DO NOT PERMANENTLY STORE** pipette tips, pens, racks, etc. inside the BSC. Unnecessary items block BSC airflow and increase the likelihood of spills and accidents. Further, these items may become contaminated by splashes, aerosols, or spills. Therefore, limit the number of items in the BSC.
- **DO NOT BLOCK THE AIR FLOW OF THE BSC** by placing objects on or near the front or back ventilation grates. Any items that divert or restrict air flow will compromise the protective biosafety barrier.
- **The use of Bunsen burners** inside BSCs is not authorized as this can create hazardous conditions which may cause serious fire. Open flames used in the BSC will also disrupt the airflow decreasing the effectiveness of the BSC to protect the users and samples. Alternative technologies such as electric incinerators, pilot less burners, or touch-plate micro burners is encouraged. Single use equipment is also recommended when possible.

Important points to remember:

- Ensure your laboratory coat and gloves cover all available skin (i.e. hand, wrist, and forearm). If needed wear disposable Tyvek sleeves.
- Prepare for the experiment/process:
    - Ensure that disinfectant spray bottles (both inside the BSC and in room) are adequately filled.
    - Ensure that laboratory supplies and equipment (e.g. pipettors, pipettes, micropipettes, micropipette tips, pipette trays, Kimwipes, markers, etc.) are available.
    - Ensure that a cart is available for transport of live cultures, disposal of waste, etc. This cart should be kept adjacent to the BSC to expedite work.
    - Prep a BSC biological waste container

        - Example: Place a 24”x12” autoclave bag into a 4liter plastic beaker.
    - Prep a pipet tray with disinfectant (2-5% diluted bleach)
- Prepare the work BSC worksurface:
    - Place the prepped waste container and pipet tray into the BSC.
- Working in the BSC:
    - Keep materials at least 4 inches inside work area.
    - Once inside the cabinet, all movements must be slow and methodical.
    - Work should proceed from clean to contaminated areas.
- If needed, place liquid waste container and/or aspirator flask inside a secondary containment (plastic bin) on the floor adjacent to the BSC. Fill containers with appropriate volume of disinfectant to obtain a final concentration of bleach of 10%. Visually inspect the vacuum aspirator flask and verify that the plastic tube extends below the sidearm level. Verify that the stopper is secure and sealed with either Parafilm or silicone. Attach a VacuGuard 0.2µm filter in-line with the vacuum tubing before attaching the tubing to vacuum inlet on the BSC.
- Waste disposal/disinfection of BSC:
    - Secure top of bagged waste with a piece of autoclave tape and spray the surface of the waste bag with disinfectant. After the appropriate disinfectant time, small waste bag may be removed from the BSC.
    - Once removed from the BSC, small waste bags are to be placed in large biohazardous waste bags within waste containers.
    - Liquid waste containers should be cleaned daily to prevent accumulation and tubing contamination.
    - Surface disinfect (either spray or wipe) re-usable items (e.g. pipettors, vortexer, etc.) before removing from BSC.

17.2 Fume Hoods

A fume hood is a ventilated enclosure designed to contain and exhaust chemical fumes, vapors, mists and particulate matter generated within its interior. Fume hoods should not be used with infectious/biohazardous material.

17.3 Centrifuges: Procedures for Centrifugation

All centrifugations shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors (biosafety lids). Most centrifuge manufacturers have developed aerosol proof rotors, sealed centrifuge tubes, and other related devices to increase the safety associated with centrifuging biohazardous materials/agents.

If a small centrifuge is used and centrifuge safety cups are not available, the centrifuge should be operated in the BSC. Each person operating a centrifuge should be trained on proper operating procedures.

Important points to consider:

- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the BSC. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- 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.
- Load and unload samples in BSC when possible.
- Place all tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors.
- Ensure that safety buckets and rotors are properly balanced per manufacturing recommendations.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket.
- Never exceed safe rotor speed.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- When working with ultra-centrifuges ensure that staff know where the circuit breaker is located to shut down the centrifuge in case of emergencies.
- Wait five 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 safety buckets or rotors in a BSC.

17.4 Incubator Shakers

Proper use of the shaking incubator by all personnel is crucial in preventing spills during liquid culture growth. Important points to remember:

- Only approved Standard Flask should be used in the shaker. All caps should be secure before use in shaker. HEPA filter caps are available to ensure proper air exchange if needed.
- Each flask or tube may only be used with its appropriate size holder attached to the platform in the shaker.
    - Styrofoam, paper towels, and other foreign materials are not to be used in an attempt to make the flask “fit” into an overly large clamp.
- All holders must be attached tightly by at least 2 or more screws to the platform at all times.
- When placing items into shaker, make sure the holder is attached properly, check that the cap is secure, and test that flask/tube is seated firmly into its holder. Please do not move other flasks without express consent of their owner.

- Label your flask appropriately.
- Check on the samples at least twice a day to ensure no spills have occurred.
- When retrieving cultures from the shaker, look for signs of a spill (missing caps, overturned flasks, puddles of liquid, etc.). If any signs are observed, proceed immediately with Emergency Procedures Section for spill remediation.

17.5 Vacuum Line Traps and Filters

Vacuum line traps and filters prevent suction of infectious and non-infectious materials into the vacuum lines. The membrane in these filters needs to be composed of hydrophobic material like polytetrafluoroethylene (PTFE) 0.45µm in-line disk filter.

Important points to remember:

- If vacuum trap is to be used in a BSC and has to be placed on the floor, use secondary containment in case of spills.
- Add full strength bleach to chemical trap flasks. Allow the aspirated fluids to complete the dilution to a final concentration of 10%. (For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard).
- Vacuum line VacuGuard 0.45µm filter must be used and shall be examined and replaced yearly, if clogged or if liquid makes contact with the filter. Used filters shall be discarded in the medical waste stream.

17.6 Syringes and Needles

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 when used for parenteral injections:

- Use the syringe and needle in a BSC only 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.

- Wear 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 an absorbent (cotton) 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.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pledget 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 a sharps container. DO NOT bend, shear, recap or otherwise manipulate the needle.
- DO NOT discard syringes into a red bucket or biohazard bag.

17.7 Pipette Aids and Pipettes

Mouth pipetting is prohibited, always use some type of pipetting aid when pipetting infectious materials. Preferably, all activities should be confined to a BSC as pipetting can cause aerosolization of material.

Important points to remember:

Infectious or toxic materials should never be forcefully expelled from a pipette.

- 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.
- Discharge from a pipette should be as close as possible to the fluid 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.
- 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 BSC. Removing contaminated pipettes from the BSC 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 BSC.

  • After suitable contact time, excess disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together. Use a clean pan with fresh disinfectant when you start a new experiment.

17.8 Blenders, Mixers, Sonicators, and Cell Disruption Equipment

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, ultrasonic cell disintegrators, and shakers. Adequate decontamination is essential after the use sonic cleaning due to possible aerosol generation.

When operating "open system" (the container with the sample needs to be left opened during the experiment) cell disruption equipment the procedure must be conducted in a BSC or other approved containment device.

Important points to remember:

- Certain sonicator might require the use of hearing protection.
- Operate blending, cell disruption, and grinding equipment in a BSC.
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leak proof 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 best done within a BSC.

17.9 Lyophilizing

The process of using a laboratory scale lyophilizer presents a number of unique hazards. These hazards include but are not limited to extreme pressure changes, a potential for glassware to explode or implode, and the possibility of aerosols creation. Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a biological safety cabinet (BSC).

The use of a lyophilizer requires labs to have an IBC approved protocol. All staff must have documented training on the unit to be used.

The vacuum pump exhaust should be filtered to remove any hazardous agents. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. Handling of cultures should be minimized, and vapor traps should be used wherever possible. To ensure that there will be no glass breakage, only use glassware that has been designed for the lyophilizer. Also ensure that the glassware is free of any visible defect (cracks, chips, or scratches), no matter how seemingly minor. Any glassware that is defective in this way must not be used under any circumstances.

17.10 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 or infected tissues, microtome/cryostat users are required to attend Bloodborne Pathogens training. Fixatives take time to penetrate tissue, ensure that tissues are stored in fixative and that the fixative is replaced sufficiently to ensure complete tissue penetration and inactivation of biological material. Freezing and drying do not inactivate pathogens, so, the pathogens that may be present in the tissue should be considered capable of causing infection. Additional precautions need to be in place when working with unfix infectious tissues.

Important points to consider:

- 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, 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.

17.11 Warm Room- Currently we do not have any warm room.

Warm Rooms (37ºC) for bacterial, viral, fungal, and parasite incubation and/or shaking cultivation can be high risk location for exposure to these agents. If these areas are not properly maintained, cleaned, and monitored.

The following are designed to ensure warm rooms are maintained to safe and clean expectations:

- The departments must assign a Warm Room oversight person who has the authority to oversee these departmental areas and if necessary, apply corrective actions to staff who misuse these areas or elicit disciplinary actions from management.
- Proper Storage:
    - No Cardboard should be stored in warm rooms.
    - No food or drink should be ever stored in warm rooms.
    - Un-inoculated Media, solid and/or liquid, stored in warm rooms should be dated and removed after 3 months or at listed expiration date.
- Equipment:
    - All equipment that utilizes liquids should be monitored for leaks daily.
    - All equipment should be monitored for mold growth.

- Equipment must be cleaned after each use.

- Proper use:
  - All culture vessels must be appropriately labeled so that the contents can be determined (staff member's identification, hazard inside vessel).
  - All culture vessels must have their lids secured to prevent leakage of contents. If aeration is needed HEPA filter lids must be used.
  - It is recommended that flasks, tubes, or containers be filled to no more than 35% capacity when shaking is required.

Spill Response

Spills of Risk Group 2 cultures of volumes less than 100mls or any volume of Risk Group 1 Agents accidentally spilled inside the warm room. Aerosolization must always be considered, and staff should be aware of the risk of respiratory exposure. Immediately post a spill sign.

- Aerosolization must be considered and staff should be aware of the risk of respiratory exposure. Based on the type of spill, if aerosolization is expected immediately contact EH&S (ext. 2245).
- Immediately post spill sign.
- Spill must be immediately reported to the supervisor ,Warm Room oversight person and EHS.
- Spilled material must be immediately absorbed with absorbent material.
- Liberally coat the absorbent material with a 10% (1-10 concentrated) bleach solution.
- Allow the material to incubate/disinfect for no less than 30minutes.
- Collect all spill material in an autoclave bag and autoclave immediately.
- Reassess area for missed material: under mats, equipment, shakers, plates, flask, etc. and disinfect as described above.
- All material present in the area must be wiped down with appropriate disinfectant.
- Discard cleaning material into a biohazard bag and autoclave.
- A sign must be posted describing the agent, the spill size, and any equipment exposed to the material.
- Complete an incident report form and submit to EHS.

Spills of Risk Group 2cultures of volume larger than 100mls:

- Immediately post spill sign.
- Spill must be immediately reported to the supervisor or Warm Room oversight person.
- Department must contact EH&S.
- Warm room must be deactivated and cleaned according to EH&S's recommendations.

17.12 Cold Rooms and Walk in Freezer

Cold Rooms are grammatically sealed rooms utilized to keep materials, supplies, media, and equipment at temperature below room ambient conditions. The process of cooling the air creates conditions that a prime growth factor for sporulating and mildew classes of mold/fungus which cause eventual experimental contamination and personnel exposure. These conditions are typically acerbated by bad habits and habitual misuse of these areas.

The following are designed to ensure cold rooms are maintained to safe and clean expectations:

- The departments should assign a Cold Room oversight person who has the authority to oversee these departmental areas and if necessary, apply corrective actions to staff that misuse these areas or elicit disciplinary actions from management.
- ABSOLUTELY NO DRY ICE OR COMPRESSED GAS STORAGE IS ALLOWED.
- No cardboard should be stored in cold rooms.
- No food or drink should be ever stored in cold rooms.
- Media, solid and/or liquid, stored in cold rooms should be dated and removed after 3 months at listed expiration date or is contaminated.
- All equipment that utilizes liquids should be monitored for leaks daily.
- All equipment should be monitored for mold growth.
- Equipment must be cleaned after each use.
- Ice or other baths utilized to store equipment, culture, etc. must be within secondary containment.
- Cold rooms should be disinfected and cleaned on a schedule as determined by use and departmental oversight.

Spills of Risk Group 2-3 cultures of volumes less than 100mls or any volume of Risk Group 1 Agents:

- Aerosolization must be considered and staff should be aware of the risk of respiratory exposure.
- Must be immediately reported to the supervisor, Cold Room oversight person and EHS.
- Spilled material must be immediately absorbed with absorbent material.
- Liberally coat the absorbent material with a 10% (1-10 concentrated) bleach solution.
- Allow the material to incubate/disinfect for no less than 30 minutes.
- Collect all spill material in an autoclave bag and autoclave immediately.
- Reassess area for missed material: under mats, equipment, shakers, plates, flask, etc. and disinfect as described above.
- Discard cleaning material into a biohazard bag and autoclave.
- A sign must be posted for at least 10 business days describing the agent, the spill size, and any equipment exposed to the material.

Spills of Risk Group 2-3 cultures of volume larger than 100mls:

- Aerosolization must be considered and staff should be aware of the risk of respiratory exposure. Based on the type of spill, if aerosolization is expected immediately contact EH&S (ext. 2245).
- Immediately post spill sign.
- Spill must be immediately reported to the supervisor or Cold Room oversight person.
- Department must contact EH&S.
- Cold room must be deactivated and cleaned according to EH&S's recommendations.

17.13 Miscellaneous Equipment

17.13.1 Water baths

Water baths and Warburg baths should contain a disinfectant to prevent growth of algae. For cold water baths, 70% propylene glycol is recommended. Sodium azide (a shock sensitive chemical) should not be used as a bacteriostatic.

17.13.2 Cold storage

All tubes stored in freezers and fridges should be stored in cryo approved tubes and placed in boxes not in tube racks for storage.

Samples stored in Liquid nitrogen (LN_2) need to be placed in the vapor phase only to prevent tube breakage. Ensure that the tube is rated for very low-grade temperatures and are properly secured in boxes or canes to prevent losing samples in the LN tanks. When samples are removed from LN_2 they need to be allowed to warm up slowly to prevent breakage.

All bottles stored in refrigerators must be kept upright at all times.

Deep freeze, liquid nitrogen, and dry ice chests as well as refrigerators should maintained organized. Freezers should be deiced on a regular basis to prevent ice accumulation on the door seal and shelves. All storage units should be cleaned out and decontaminated periodically to remove expired and unused supplies.

All material stored in refrigerators, LN_2 or deep freezers must be in the appropriate cryo-safe container and must be properly labeled. Security measures should be commensurate with the hazards.

17.13.3 Membrane filters

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 proved their sterility.

18. Safety Considerations During Experimental Procedures

Research specific to the study of bacterial, viral, fungal, and protozoan human pathogens typically involves some form of cultivation and/or propagation of these agents to yields much higher than normally found in nature. To ensure these agents are properly contained during this cultivation and any subsequent experimental manipulation, it is crucial that lab staff follow good microbial technique. In conjunction with these techniques, the use of safety equipment is absolutely necessary to minimize exposure to the utilized agents.

18.1 Culture Plates, Tubes and Bottles

Particular care is required when opening plates, tubes, or bottles containing, for this operation may release infectious material.

Due to the potential of aerosolization when handling samples, all cultures of infectious or hazardous material must be open and manipulated in a BSC wearing gloves and a long-sleeved lab coat.

It is common to concentrate organism, vectors, biotoxins, and human materials to ensure that a large dose is administered. In this respect the final concentration may exceed the infectious dose of a human and in turn the dose could have the potential of overwhelming normal immune protection.

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 potential of aerosolization of samples and potentially will damage the agent. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is re-suspended, 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 spattering 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. 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. Disposable inoculating loops are available commercially; they can be discarded into biohazard waste containers. Only use smooth agar plates and slants to streak cultures to minimize the potential for aerosols.

To prevent contamination of samples and environment, ensure plates are properly dried before storing them in the refrigerator. Allow plates to warm up to room temperature and dry before inoculation.

Always open inoculated agar plates in the BSC.

The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. if the flask or centrifuge tube is not held in an upright position during incubation or procedures this will create the risk of infected media to be located in the lid of the container.

When visible liquid is present in the tube lid due to samples being mixed by inversion or too vigorously shaken during incubation, they need to be quickly centrifuged to pull the liquid away from the lid and prevent exposure to the hands and environment.

If dry media is visible near the rim or neck of the container care must be taken when opening the container to minimize disrupting the infectious material.

Lyophilized material has a high potential of aerosolizing when being manipulated, opening the container and resuspending the sample must be done very slowing and with deliberate movements.

18.2 Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created. Opening of ampoules should be done in BSCs. When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. To accomplish this, work in a BSC and wear gloves.

When possible, use safety tool to prevent hands from being close to the glass when it breaks or getting cut on metal lids.

Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted gauze. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then wrap the ampoule in disinfected wetted gauze and snap it open.

The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling and withdraw the contents into a fresh container.

Examples of tube opener.

Thermo Scientific™ Manual Crimpers, Decrimpers and Decrimping Pliers

Millipore Sigma Ampule breaker/collar

18.3 Tissue Culture Work Involving Infectious Agents

All routine culturing of infected cells should be performed in a designated and appropriately labeled BSC. Before bringing infected cells into the BSC, make adequate aliquots of any media, buffers, or other reagents. Remove the stock bottle reagents from the BSC before bringing any cells or cultures into the BSC. Any reagents that were inside the BSC while agent was present will be considered 'inoculated.' All 'inoculated' reagents will either be used in the present experiment or chemically disinfected and discarded into a liquid waste container. 'Inoculated' reagents will never be returned

to 4°C refrigerator.

Important points to consider:

- When possible, use designated and appropriately labeled incubators for infected tissue culture.
- The volume per flask must be determined so that horizontal placement during incubation does not cause media to reach to top of the neck or leakage.
- When outside of the BSC, all flasks and plates should be secured in designated plastic containers with sealable lids.
- When possible, all flasks and plates should be surface decontaminated before being placed inside the incubators.
- If a vacuum flask is used for tissue culture spent media removal:
    - Vacuum flask not contained within the BSC must be in secondary containment.
    - Vacuum tubing must be monitored for contamination. If contaminated, the tube should be autoclaved prior to disposal.
    - Flask should contain enough concentration of bleach (10% final volume) to inactivate any agents present in the waste media.
    - Flask should be emptied daily and restocked with fresh disinfectant prior to next use.
- It is required that a VacuGuard 0.45µm filter be attached in line with the Vacuum Spigot to prevent house line contamination. The membrane in these filters needs to be composed of hydrophobic material like polytetrafluoroethylene (PTFE) 0.45µm in-line disk filter. Change yearly or when contaminated or blocked.
- All waste generated during tissue culture activities must be treated as biohazardous and autoclaved prior to disposal.

18.4 Microscopy work with infectious agents

The movement of tissue culture plates and/or flask for the purpose of microscopic examination can serve as a potential exposure point if care is not taken in their transport from the BCS or Incubator to the microscope.

Important points to consider:

- Move slow and methodical.
- Ensure no one is in the path from the culture origin to the microscope.
- Flasks and plates should be transported on the tray, in a pan or on a cart.
- Demonstrate care in how you set the vessel onto work surfaces including the microscope platform.
- Keep an eye on the edge of the platform field to prevent the plate or flask tipping over the edge of the microscope platform.

- Immediately return all culture vessels to the BSC or incubator after the examination is completed.

18.5 Phlebotomy guidelines for Human participants

Whenever blood is drawn from people on the UNT Health campus as research subjects the following minimum standards must be applied. Please refer to the SOP. EHS SOP

19. Safety Precautions During Work with Animals

The use of biological agents/materials, biotoxins, in animals requires additional risk analysis and assessment for which the IBC and IACUC have oversight. Once a viable agent enters a susceptible host, this host may serve as a reservoir and/or vehicle for transmission. In this regard, users must consider how the nature of the animal host changes the dynamics of exposure. With this understood we can design methods to prevent and/or minimize exposure, injury, and/or illness.

Important points to consider when working with animals:

- Animal bites, scratches, or other forms of animal induced trauma.
- This includes type of administration, injections, and/or inoculation: needle, aerosols, topical etc.

- Fecal, Urine, saliva, bodily fluids, tissue otherwise obtained from the animal must be considered hazardous.

- Contaminated animal dander, bedding, and food dust.

- Contaminated water.

In addition, infectious agent, biotoxins injected/administered and/or human material xenographed into animals can be a source of exposure to employees. These materials possess unique hazards in that:

- Infectious agents/biotoxins act in a dose dependent manner and may not be shed by the animal model. However, animal inoculation usually involves needles which by themselves pose a significant hazard. Also, most if not all of these are extremely toxic with low lethal doses for humans and exposure may lead to death.

- Human material is similar to infectious agents/biotoxins in that it is likely not shed post administration or injection, however, these materials do actively replicate and to may create tumor masses in the animal model. There have been documented cases of cancerous material/cells becoming infectious and causing similar cancer prognosis following exposure.

The inoculation, injection, and/or administration of biological agents into animals creates a significant hazard that in itself deserves special mention and/or discussion. Specifically, the following are examples of increased risk factors:

- Aerobiology research serves to study an important route of exposure in that it mimics the routes associated with natural spread and/or it may serve to replicate what might happen in a potential bioterrorism attack. This method of delivery is usually achieved through devices designed to

aerosol agents for either full body/deep lung exposure (aerosol chambers) and/or oral-nasal/pharyngeal exposure. These devices must require significant risk analysis and must be housed and utilized under stringent containment parameters. In this respect these types of experiments are highly scrutinized by the IBC and the IACUC.

- Some experimental parameters require delivery through food and/or water. In this respect the food and water and bedding must be considered hazardous and handled appropriately.

- Topical administration or intradermal inoculations of biological agents possesses a risk when handling the animal due to the potential of direct contact with the administered material.

The recent advances in the genetic alteration of animals has seen a sharp increase and has become common practice. The risk involving work with transgenic animals is typically low in comparison to other hazards. Animals, specifically mice, have been genetically altered to become susceptible to human disease and/or biotoxins. This creates an un- natural host that now has the potential to become a carrier of and potential spread human pathogens. Multiple regulations have been enacted to monitor these transgenic models; therefore, institutions must ensure that these animals are secured and are required to have procedures in place to prevent escape and/or loss of the animals.

19.1 Animal work areas

Laboratory animal facilities are a special type of laboratory. As a general principle, the BSL (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories.

The co-application of BSLs and Animal Biosafety Levels (ABSLs) are determined by a protocol driven risk assessment. These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., *Guide for the Care and Use of Laboratory Animals* 1 and *Laboratory Animal Welfare Regulations* 2) and that appropriate species have been selected for animal experiments.

These four combinations, designated ABSL 1-4, 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 BSLs 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.

19.1.1 ABSL1

ABSL1 is suitable for work 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. ABSL1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

19.1.2 ABSL2

ABSL2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL1. ABSL2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL2 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be conducted in BSCs or by use of other physical containment equipment.

Appropriate PPE must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

19.1.3 ABSL3 – Currently we do not have any ABSL3 facility

19.2 Department of Laboratory Animal Medicine (DLAM)

Biological agents and material may be used in animals only as described under an active animal protocol and IBC registration. The DLAM supervisor for the area in which, the animals to be used, will be or are currently housed *must* be contacted at least two weeks before initiation of these experiments to ensure proper training of DLAM staff that will be handling your animals and/or their wastes during any treatment or wash-out period.

Administration and handling of the agent/material and infected animals, as well as the required animal

husbandry must be performed as determined by committee review and approval of the proposed research. This approval will define the type of containment, equipment and practices required for animal inoculation, as well as animal husbandry. Staff must don DLAM facility- specific PPE prior to initiating animal applications.

The Biosafety Program and DLAM must approve any transport of infected and/or contaminated live animals from the DLAM facility.

When animals are manipulated outside of a DLAM facility, all staff must be made aware of proper PEE to wear when the animals are present and be notified of the potential presence of allergens.

19.2.1 Transgenic Animals

ABSL1 containment and standard DLAM conditions are sufficient for breeding, rearing, and housing transgenic animals. All carcasses must be bagged in carcass bags and, unless approved for long term storage, must be immediately returned to DLAM and placed in the DLAM provided incineration containers.

19.2.2 Human Cells, Risk Group 1 Organisms

The IBC requires all animal manipulation involving administration or injection of these agents to occur within a BSC, fume hood, or EH&S and DLAM approved area. However, the IBC has determined this risk of exposure to material/agents shed from an animal following injection is minimal. Thus, after inoculation the animals can return to the ABSL1 housing and be handled under standard DLAM conditions. All carcasses, post application (i.e. necropsy or euthanasia), must be bagged in carcass bags and, unless approved for long term storage, must be immediately returned to DLAM and placed in the DLAM provided incineration containers.

19.2.3 Viral Vectors

All animal inoculations must be conducted in a fume hood, BSC, or by an EH&S/DLAM-approved method and designated area. The cages must be clearly labeled with the name of the hazard, the date and time of inoculation, and an DLAM provided biohazard hazard sticker must be placed on the cage card. After inoculation, the animals must be placed in cages, according to IACUC defined housing requirements, and be returned to an DLAM designated housing area.

Animal caging systems available for animals exposed to these agents depend on the DLAM facility and its capabilities. Experiments involving rodents and/or small mammals must be conducted in an DLAM, as well as EH&S approved caging systems and/or location. The type of

caging system utilized and the method to process the animal's cages post inoculation will be detailed in a work start or pre- continuation meeting between the PI and/or their designee and EH&S/DLAM.

Following inoculation with these agents, animals should be placed in a clean cage. A cage change must be completed no sooner than 72hrs post inoculation regardless of animal species or DLAM housing location. If the animals are to be inoculated again this process must be repeated at that time. The required cage change must occur within a certified BSC or fume hood. The animals are to be placed into a clean caging system, as defined by the housing location, and be henceforth maintained under standard animal husbandry. At this time, the animals are considered hazard free and the biohazard sticker can be removed or defaced.

The IBC requires bedding exposed to these agents to be inactivated by autoclave sterilization prior to dumping or be incinerated. These options will be discussed during the work start or pre-continuation meeting discussed above. All carcasses, post application (i.e. necropsy or euthanasia), must be bagged in carcass bags and, unless approved for long term storage, must be immediately returned to DLAM and placed in the DLAM provided incineration containers.

19.2.4 Risk Group 2-3 Pathogens

Animal work involving Risk Group 2 and/or 3 pathogens must be conducted under ABSL2 conditions unless otherwise specified. The IBC requires all animal inoculations must be conducted in a fume hood, BSC, or by an EH&S/DLAM-approved method and designated area. All cages housing infected animals must be labeled with a biohazard sticker, the date and time of inoculation, as well as the hazard identification. After inoculation, the animals must be placed in cages, according to IACUC defined housing requirements, and be returned to an DLAM designated housing area.

Animal manipulations and cage changes must occur within a BSC or fume hood. All dirty/contaminated animal cages must be bagged for sterilization in DLAM provided autoclave bags. Bagged cages must be left at and/or transported to DLAM designated waste collection areas at which time the cages will be autoclaved by DLAM technicians. All carcasses, post application (i.e. necropsy or euthanasia), must be bagged in carcass bags and, unless approved for long term storage, must be immediately returned to DLAM and placed in the DLAM provided incineration containers.

19.3 Medium and Large Animals

Some experimental objectives require the injection, administration, or inoculation of medium to large animals with biohazardous materials/agents. If these materials/agents have the potential to be shed by these animals, a more intensive risk assessment must be conducted to determine how and where these animals must be housed. Most animals of this size (i.e. rabbit up to a cow) cannot be housed in a caging system that allows for manipulation under a primary barrier. In these cases, the methods of

containment focus on the facility rather than a specific piece of equipment (i.e., BSC).

Project utilizing medium sized mammals (e.g. rabbits and chinchillas) can occur in rooms containing housing equipment suitable to their size. If special ventilation equipment is not available, then facilities will need to rely on proper use of PPE and respiratory protection. In addition, disposal equipment such as HEPA filtered dump stations may be required. SOPs for these housing locations must be developed to define entry and exit procedures, the type and proper donning and doffing of PPE, and the required decontamination and waste disposal activities.

Facilities utilized for dogs, pigs, and/or other animals that may be housed in pens, must be examined for their ability to contain any form of material shed form the animal. Along with the requirements listed for medium sized mammals, methods should be developed to define how the waste is removed and the room is decontaminated. Also, user must assess the potential for animals such as dogs, cats, etc. to potential carry diseases that can infect humans. Rabies and Q fever is one agent that must be considered during the assessment process when designing facilities to house these animals.

Experiments involving the exposure of ruminants and/or farm animals to biohazardous materials/agents require all of the stipulations listed above, but due to the animal size and the amount of waste they produce, and the type of facility utilized is extremely important. In addition, certain endemic diseases can be carried by farm animals to which human contact with or inhalation of their secretions could lead to serious exposure.

19.4 Use of Radioactive Materials

All the research work involving usage of radioactive material in animals MUST be approved by UNT Health Radiation Safety Committee and IACUC. If co-administration of any radioactive materials is part of the protocol, contaminated bedding and animal carcasses **must** be handled as radioactive waste as described under separate guidelines for use of radioactivity in animals.

19.5 Exemptions

Only the IBC and the IACUC may grant exemption to the above-described housing and husbandry procedures. An exemption must be scientifically justified and/or corroborated by scientific literature. Request for exemptions must be submitted to the IBC through the Biosafety Program and to IACUC through the IACUC manager.

20. Hazard Transportation

20.1 Off campus transport

Hazards to be transferred off campus must follow Department of Transportation Code of Federal Regulations Title 49 and the International Air Transport Association. For more information, please contact EH&S.

Only specially trained personnel are permitted to ship Dangerous Goods. Training should include IATA class 6.2 (Cat A, Cat B), class 9, dry ice, dry shipper

An MTA must be in place between research group prior to shipping any material to a collaborator contact Research Compliance Department for more instruction. Permits from CDC, USDA, Wildlife and Fishery may be required to be obtained for the shipper and/or recipient for domestic and international shipment.

Export Control and International regulations must be followed prior to any international shipment. For export and import control information, please contact Research Compliance department.

Failure to comply with ALL shipping regulation can lead to significant finds and legal actions against an individual and the institution.

20.2 Intra-campus transport

Hazards to be transferred from the laboratory to other areas of the campus must be contained, labeled, packaged, and transported in accordance with the IBC SOP's.

Transport of Biohazards on Campus (between labs or buildings):

- Must have three leak-proof containers, including the following:
    - A sealed primary container.
    - A sealed secondary container.
    - Absorbent (paper towels) between the primary and secondary containers suitable for the volume transported.
    - A biohazard sticker on the outside of the secondary container.
    - Utilize plastic whenever possible, avoid glass. If glass primary containers must be used, place containers in a sealed rigid plastic container with absorbent and padding to cushion the vials during transport.
    - A sealed tertiary container not made from porous materials (e.g. plastic sealed container, coolers) so it can be easily disinfected.
    - A biohazard sticker and the name and phone number of the PI on the outside of the container.
    - Do not use gloves when handling the tertiary container.

20.3 Intra-lab transport

Care must be taken in the transport of large volumes of liquid culture from the area of cultivate to the area of manipulation. Best practice requires that a cart be used for two or more flask of volumes over 250mls.

Refer to your approved IBC protocol for information on required procedures for transportation.

21. Decontamination, Disinfection, and Sterilization

Sanitization- is the process of reducing the presence of microorganisms on surfaces. E.g. Healthcare providers sanitize a site prior to vaccination.

Disinfection- It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure total inactivation of all pathogens and therefore lacks the margin of safety achieved by sterilization procedures.

Decontamination-Decontamination renders an area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission). The primary objective of decontamination is to reduce the level of microbial contamination so that infection transmission is eliminated.

Sterilization-A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores.

21.1 Liquid Solutions

In general, the liquid 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.

Liquid decontaminants can be categorized as halogens, acids and alkaline, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines.. Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel making up use- concentrations from stock solutions must be informed of the potential hazards and trained in the safe procedures to follow and appropriate PPE to use as well as the toxicity associated with ocular, skin and respiratory exposure.

All solutions used in the laboratory for disinfection, decontamination, sterilization needs to be approved by EHS and listed on IBC protocols.

Many of these solutions cannot be mixed with each other as they may generate toxic gases. E.g.

ammonium compound and bleach should never be mixed. As well many of these solutions cannot be autoclave as they could explode or generate toxic gases.

Bleach- is the most used solution for decontamination and inactivation of the majority of microorganism. Household bleach should be diluted at the time of use to a final ratio of 10% by volume or 1/10. A contact time of 30 minutes is generally employed.

Quaternary Ammonium Compounds--These cationic detergents are strongly surface-active and are effective against lipid containing viruses. These compounds are nontoxic, odorless, stable, non-staining, non-corrosive to metals, and inexpensive.

Alcohol--Ethyl or isopropyl alcohol at a concentration of 70-85% by weight is often used; however, both lose effectiveness at concentrations below 50% and above 90%. A contact time of ten minutes is generally employed. Due to the high evaporation rate of alcohols, repeated applications may be required to achieve the required ten-minute contact time for decontamination. Isopropyl alcohol is generally more effective against vegetative bacteria; ethyl alcohol is a more efficient with viruses. These solutions are not used for sanitization and not decontamination. They are very useful to remove residue of chlorine after the use of bleach or quaternary solutions.

21.2 Decontamination Using Vapors and Gases

A variety of vapors and gases possess decontamination properties. The most useful of these are), Vaporized Hydrogen Peroxide (VHP) and Chlorine Dioxide (CD). Vapor and gas decontaminants are primarily useful in decontaminating BSCs and associated air handling systems and air filters; bulky or stationary equipment that are difficult to treat by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; rooms, buildings and associated air-handling systems.

Time, humidity and temperature required for proper effectiveness must be properly calculated and monitored.

When these methods are used equipment and rooms must be sealed to ensure that no vapor or gas can be released in other areas and possibly be harmful to personnel present.

Vapor and gas decontamination can only be performed by trained professionals and is usually performed by third party vendors. EHS must approve the use of these techniques.

21.3 Sterilization Using 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 (please refer to the Waste Management section for more information).

- Incineration is another use of heat for decontamination. Incineration serves as an efficient means of disposal for human and animal pathological wastes.

21.4 Selecting Chemical Disinfectants

No single chemical disinfectant or method will be effective or practical for all situations in which decontamination is required. 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 medium is the organism suspended
- What is the highest concentration of organisms anticipated to been countered?
- 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?

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 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.

21.5 Decontamination Procedures

- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
    - Work areas should be solid surfaces that can be readily cleaned and/or covered with polyethylene backed absorbent coverings. Bench coverings should be assumed to be contaminated after use and disposed of as described in the waste disposal section.
    - Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    - Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- Disinfectants:
    - The proposed working strength of a disinfectant should kill 10^7 cfu / ml of the infectious agent within a 15 min contact time.
    - Each disinfectant will be prepared in dedicated containers in accordance with the manufacturer's recommendations, and its working concentration marked on the container.

22. Waste Management

Proper management of Hazardous Waste is important in order to protect human health and the environment. All laboratory personnel must know how to properly dispose of waste to avoid exposure to hazardous materials, prevent possible injury, and protect the environment. Laboratory personnel must have access to methods/equipment for decontaminating all laboratory waste generated (e.g., autoclave, chemical disinfection, incineration, etc.).

22.1 Containers

Containers must be appropriate for the contents and interchangeable use of containers is not permitted (i.e., broken glass box used to hold a biohazard bag). All containers must be leak- proof; be properly labeled; and maintain their integrity if thermal or chemical treatment is used. Containers of biohazardous material should be kept closed when not in use.

- *Sharps*. Place in a commercially purchased sharps container. These must be provided by each laboratory. Never attempt to retrieve items from a sharps container. Do not place sharps in plastic bags or other non- sharps containers.
    - *When full close and tape the container lid and place the container in a Medical Waste box provided by EH&S*. No not place other waste bags in the same box.
- *Broken glassware*. non-contaminated glassware must be disposed of in either a broken glass

container or sturdy cardboard box. The box must be line with a clear bag. Seal securely and clearly label "BROKENGLASS".

- *Solid biohazardous, human specimens waste*.
    - If autoclaving biohazardous waste for pre-treatment, only use orange autoclave bags. Place bags in a secondary heavy-duty plastic container clearly labelled with a biohazard sticker.
        - Once autoclaved place bags in Medical waste box provided by EH&S
    - Medical waste and waste generated from Human specimens – Use only red bags and leak proof Medical waste boxes are provided by EH&S
- *Liquids.* Liquid waste should be placed in leak-proof containers able to withstand thermal or chemical treatment. Please place these containers in secondary containment and clearly labelled for content(s).

22.2 Bio-waste Separation

Some experimental procedures might involve a mixture of biological and chemical hazards. Chemical waste must be separated from biological waste prior to sterilization and eventual disposal. This includes, but is not limited to:

- Decanting formalin off of fixed samples prior to disposing of the tissue samples in a Medical Waste Box. These tissues should not be autoclaved at any time due to the potential vaporization of the formalin absorbed into the tissue. The formalin must be decanted into a chemical waste container.
- Minimizing the amount of bleach that is present in materials to be autoclaved. In most cases bleached material, after at least a 30-minute incubation, can be poured down the sanitary drain. Bleach produces corrosive vapors which will damage the stainless-steel components of the autoclave.
- If Biological material is used in the presence of radioactive isotopes, contact EHS to determine the correct segregation and neutralization procedure.
- Many chemicals cannot be autoclaved safely, when chemicals are used with biologicals and cannot be safely autoclaved contact EHS for further instructions.

22.2.1 Prescription Pharmaceutical waste

- **Prescription Pharmaceutical waste from experiments**

Prescription medications used in the course of an experiment must be placed in a clear bag and dropped off at the collection drop box located at UNT Health Police department.

- **Personal Prescription Pharmaceutical waste**

UNT Health has a program to collect and properly disposed of personal prescription medication. Expired and unused prescription drugs should be packed in a zip lock bag and taken to the drug collection drop box located at UNT Health police department.

Figure 11. Drug Collection Dropbox

22.2.2 Training

The *Autoclave Use and Safety Training Program* is available through EH&S. The laboratory PI/supervisor is responsible for ensuring their autoclave operators are appropriately trained on departmental autoclaves.

22.3 Autoclaves

Autoclaves use high pressure and high temperature steam for sterilization, posing physical and thermal hazards that can cause serious to life threatening injuries. The following PPE is required when using the autoclave:

- Eye protection/Face shield.
- Buttoned lab coat.
- Clothing that covers exposed skin.
- Closed-toed shoes.
- Heat and liquid resistant gloves.

22.4 Performance Verification and Monitoring of Autoclaves

According to the Texas Administrative Code, the decontamination process utilized to treat biological waste must be validated. For this purpose, responsible Departments must keep copies of all maintenance and repair records for all autoclave equipment. In addition, personnel that use autoclaves to inactivate biohazardous waste must:

- Log the approximate weight of every waste load treated.
- Ensure that the autoclave parameters are met for each load.
- Perform a weekly biological indicator test to ensure that the autoclave equipment is functioning correctly.
- Follow proper procedures when the equipment fails.

22.5 Medical Waste

Autoclaving is a good method to ensure proper inactivation of hazardous waste through steam sterilization. However, autoclaving human tissue, mouse carcasses, or other large-scale bodily fluids can lead to disturbing odors. In addition, it is against the law to dispose of bulk human material (e.g. a finger, eye, or biopsy section) through the municipal waste stream EH&S provides Medical Waste boxes for these types of materials. Items that **must** be disposed of in a Red Medical Waste box include, but are not limited to:

- All contaminated biological material
- Bulk human material.
- Animal carcasses.
- Blood or bodily fluid collection tubes.
- Any item contaminated with blood or bodily fluids.

The following items **must never** be found in a Medical Waste box:

- Free sharps of any kind, unless they are sealed a ridged sharps container that has a biohazard label.
- Large volumes of liquid (e.g., urine).
- Pharmaceutical waste.

When 75% full, medical waste bags must be tied shut by laboratory personnel, placed in the provided medical waste box, and the box must be properly closed and labeled prior to onsite storage. Laboratory personnel should promptly submit a medical waste pick up request following the generation of a full medical waste box. Medical Waste Boxes are collected by a contracted vendor and incinerated. If any chemicals are found in these boxes, UNT Health is in violation of our contract and the institution can be legally liable for these incidents. At no time should medical waste be stored in public hallways. When Medical Waste Boxes are full complete the online pick-up request form to have the container removed and replaced. https://www.unthsc.edu/safety/biological-waste-red-box-removal/

22.6 Disposal of Recombinant DNA Materials

Depending upon the type of recombinant or synthetic material (DNA, animals, microorganisms, etc.), autoclave treatment, chemical treatment, or incineration may be employed for inactivation prior to disposal. There are no exceptions to this policy without prior notification and approval by the IBC.

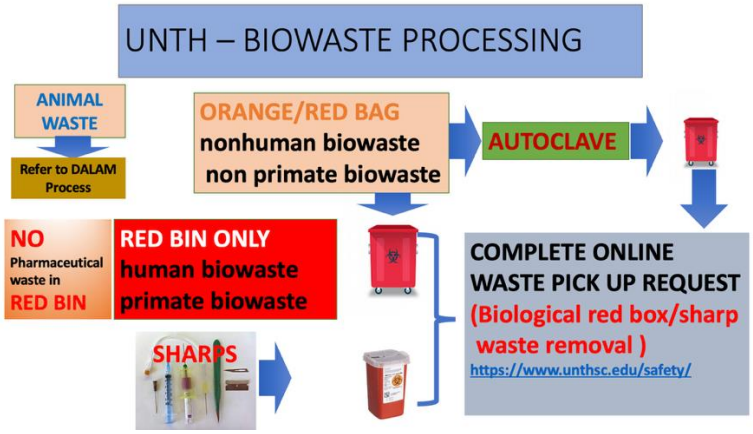


Figure 12 - Summary of biowaste management process at UNT Health

23. Special Considerations

23.1 Medical Restrictions

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, staff must inform their treating physician of the biological and chemicals handled. It is recommended that staff self-report to their PI or supervisor and EHS Sr. Director. EH&S is also available for questions regarding the potential harm from the biological agents present within your work environment. Whenever necessary, the Biosafety Program will review work procedures in the lab to ensure that potential exposure is minimized. Consideration for reassignment to other tasks that don't involve exposure to the reproductive hazard should be given. Also, Principal Investigators actively working with reproductive hazards explain the risk assessment at time of hire.

23.2 Reproductive Biological Hazards

Reproductive biological hazards can affect both females and males of child barring age. These agents 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 Choriomeningitisvirus
- Toxoplasma gondii (Toxoplasmosis)
- Listeria monocytogenes
- Varicella-zoster virus (chicken pox)
- Zika

23.3 Other Restrictions

Restrictions or recommendations will be made on an individual basis after discussion with the EH&S and/or the employee's personal medical doctor. It is recommended that staff self-disclose medical conditions that could place and employee in an increase susceptibility exposure or infections.

- Examples of conditions that might warrant special precautions are, immunosuppressive conditions and drug therapy that suppress the immune system. Therefore, if you are suffering from any of the above conditions, you must inform your personal physician of the hazards present in the laboratory.

- Open wounds in exposed area such as arms, hands, neck and face. Open wounds can be unhealed scraps, cuts, tattoos and pricings. Wounds should be kept covered with waterproof bandages and kept dry at all time. Frequent replacement of badges might be needed to maintain the cleanness and dryness of the site. It is recommended that staff self-report and consult with their PI and EHS on the safety of working with hazardous material pending complete healing of the wound. Contact EHS for questions and recommendations.

- 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 and EHS of any severe skin conditions or wounds. Avoid working with BSL2, BSL2E or other potentially infectious materials if non-intact skin cannot be adequately covered.

- Mobility impairment, following surgeries or injuries staff may be required to use medical boots, crutches, cans or other mobility devises. These devises might present hazards for the staff and colleagues when used in a laboratory environment. Staff that are facing these mobility restrictions need to contact EHS for assistance in performing a work job analysis.

23.4 Immunizations

Immunization may be recommended for personnel engaged in particular research activities, such as rabies, rubella and measles. Vaccines not commonly available will be obtained, whenever possible.. Information on recommended vaccination will be indicated in the IBC protocol. Please contact EH&S for more information.

24. Emergency Response

UNT Health requires that research-related incidents be reported immediately to EH&S. Such incidents include research-related accidents, exposures, illnesses, injuries and fatalities as well as any inadvertent release or improper disposal of hazardous material/agents, recombinant or synthetic DNA, and/or animals.

24.1 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. An employee who sustains a known or potential exposure incident must remove gloves and treat the affected area immediately by following the appropriate exposure incident response below.

Emergency procedures in the event of personnel exposure

- Scene assessment: determine affected surfaces and condition of PPE. It is critical that all contaminated PPE must be removed.
- If inhalation and/or ingestion exposure is suspected contact PI and seek an immediate medical consultation from urgent care or ED.
- If ocular and/or mucosal exposure is suspected, rinse the area for no less than 15 minutes, and seek an immediate medical consultation from urgent care or ED.
- If inoculated through puncture, laceration, and/or previous skin injury immediately wash the area with copious amounts of water using an antibacterial soap, avoid scrubbing the area. Seek a medical consultation from Occupational Health Department. All events require immediately notification of the direct supervisor/PI and EH&S.
- Incidents should be reported to Biosafety officer. UNT Health incident report form and the full response procedure are available on the EHS webpage.

Emergency procedures in the event of a spill or release not involving personnel exposure

- Scene assessment: determine affected surfaces and condition of PPE. It is critical that all

contaminated PPE must be removed prior to continued spill remediation.

- Spills that occur inside the BSC:
    - Spills less than 100mls only involving the work surface. Place absorbent material around and on top of the liquid allowing for absorption of all free liquid. Coat the absorbent material with the appropriate disinfectant. Using tongs and/or gloves, place the absorbent material into a biohazard bag. Reapply disinfectant to the area and affected equipment and repeat absorption and disposal. All material must be autoclaved. Gloves must be discarded and new pair reapplied prior to starting other applications.
    - Spills greater than 100mls and/or involves the grills or plenum spaces. Contact EH&S to discuss and implement the appropriate decontamination process.
- Spills occurring outside of primary containment equipment (e.g., floor, incubator, centrifuge):
    - *Minor spills*: Small scale spills (<10mls) that are rapidly absorbed by protective linings or diaper paper.
        - Assess spill area: affected surfaces, equipment, PPE, and exposed areas of your body.
        - Alert people in immediate area of spill.
        - Remove any contaminated PPE and don new PPE prior to cleanup procedures.
        - Cover the spill with absorbent paper. First place absorbent material around the spill to prevent further spread, second place absorbent material on top of the spill.
        - Soak absorbent material with a working solution of the appropriate disinfectant (10% bleach solution made fresh) and allow contact with spill for at least 15 minutes).
        - Decontaminate all equipment with appropriate disinfectant or as described by the manufacturer.
        - Wearing disposable gloves, remove the absorbent material and place in a biohazard bag. Autoclave all spill generated waste.
        - Clean spill area with detergent and water, followed by 70% ethanol.
    - *Major spills*: Large scale spills, spills/leaks in high-speed centrifuges, incubators, pressurized equipment and any high impact spill or aerosol generating event.
        - Assess spill area including: affected surfaces, equipment, PPE, and exposed areas of your body.
        - Alert people in the immediate area of spill, your immediate supervisor/PI, and EH&S by phone at (817) 735-2245.
        - Remove any contaminated PPE and don new PPE prior to cleanup procedures.
        - Leave area for 30 minutes to allow aerosols to settle.
        - Cover the spill with absorbent paper. First place absorbent material around the spill to prevent further spread, second place absorbent material on top of the

spill.

- Decontaminate all equipment with appropriate disinfectant or as described by the manufacturer.
- Using tongs or gloves remove the absorbent materials and discard into biohazard bags, boxes, or containers.
- Reapply disinfectant to area, absorb and discard into biohazard bags, boxes, or containers.
- Request disposal through EH&S or sterilize via autoclave.
- Contact Occupational Health Department to receive a medical consultation regarding the potential exposure.

24.2 Aerosols

Aerosols can be easily generated in the following situations:

- Opened centrifuges in cases of failure or bottle/rotor leakage.
- Opened shaking incubators in cases of container leakage, breakage, or failure.
- High risk experiments involving aerosolization chambers or intox units.

The following actions should be implemented for all spills outside of containment:

- Hold your breath and immediately leave room. Remove PPE carefully. When removing PPE make sure to turn the exposed areas inward. Wash hands well with soap and water. Post spill sign on lab entry; lab should be evacuated for at least 30 minutes. PI must clear lab for re-entry. For extensive BSL2 contamination (i.e. centrifuge incident) or incidents involving BSL2E agents, EH&S must be notified and will assume responsibility, in conjunction with the PI, to clear the laboratory for re-entry.

24.3 Spills Involving biological material

All incidents involving infectious materials are to be immediately reported to the BSO and the Safety Office. Please see Appendix A of this manual for an incident report form. Such incidents may include spills or releases of materials or agents, escape of infected animals, rupture of plastic bags of infectious/medical waste, other loss of containment, or equipment failure. The BSO will direct or oversee cleanup, capture of animals, protection of personnel, packaging and disposal (after sterilization if possible) of residues and/or make arrangements for temporary storage and subsequent treatment of equipment, wastes and/or the area.

Any emergency incident requiring immediate assistance from UNT Health PD or EH&S, or from non- campus agencies such as the Fort Worth Fire Department, is to be reported immediately to UNT

Health PD (817-735-2600).

Reports should provide the dispatcher with the following information:

- Where and what type of incident has occurred.
- Assistance needed, if not obvious, such as firefighters for a fire.
- Whether the incident involves any injured or trapped persons.
- What actions have been taken since the incident began: i.e., building evacuation has been initiated, etc.
- Identity of caller, location from which they are calling and who and where someone will meet response personnel upon their arrival.
- Wear gloves, eye protection, and a lab coat.
- Absorb blood with paper towels and place in a biohazard bag. Collect any sharp objects with forceps or other mechanical device and place in a sharps container.
- Cover the area with paper towels and pour the site with a 10% bleach solution (made fresh), leave the lab and allow contact for 30minutes.
- Wipe the area down.
- Discard all disposable materials used to decontaminate the spill and any contaminated PPE into a biohazard bag.

24.4 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. Refer to the written SOP in the laboratory for proper decontamination to be followed. Use procedures that protect you from the radiochemical while you disinfect the biological material. Before any clean up, consider the type of radioisotope, characteristics of the microorganism, and the volume of the spill. Immediately contact the Radiation Safety Office (817-735-2243) and PI to report the incident. Complete the incident report form and submit to EHS.

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door and post a warning sign.
- As close to the exit, remove contaminated clothing, turning exposed areas inward, 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, for 30min.

- If mucosal membranes have been exposed rise with running water for 30min.
- Proceed to the closest medical facility.

24.5 Hygiene Practices Following Cleanup

- Staff must be cognizant of their PPE following the cleanup to ensure no contaminated material and/or fluid is present that could be aerosolized.
- Staff must completely decontaminate their PPE prior to removal.
- All disposable PPE should be placed in biohazard bags for disposal.
- Staff **must** thoroughly wash their hands, wrist, and forearms following PPE removal.

24.6 Investigation of Laboratory Accidents

EH&S, in cooperation with the PI and their 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, the Biosafety Program, in consultation with Occupational Health 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 the incident to Occupational Health, EH&S, and Campus Police. Evaluation of near misses can lead to alternative work practices and implementation of engineering controls to minimize future incidents.

24.7 Infectious Material Incidents Including rDNA and Infected Animals

If an infectious organism or one containing recombinant DNA molecules were to acquire the capacity to infect and cause disease in humans, the first evidence of this potential may be demonstrated as a laboratory-acquired infection. For this reason, the Principal Investigator or laboratory manager must investigate any serious, unusual, or extended illness of a laboratory worker or any accident that involves inoculation of infectious organisms or those containing rDNA molecules through the skin, by ingestion, or probable inhalation. A finding that an infection is associated with such work or research will provide sufficient warning for evaluation of hazards and initiation of additional precautions to protect the general public, if necessary, in addition to other workers.

Prompt reporting of all accidents involving overt releases of or exposures to microorganisms is essential. The laboratory worker involved with such an occurrence should notify the Principal Investigator or laboratory manager (or another person in authority in their absence) immediately. The

PI or manager should determine the immediate response to be taken. This response may include immediately requesting the support of medical personnel to help monitor individuals for possible infection or disease. The investigation of all accidents associated with research involving biohazardous agent (e.g. toxins, microorganisms, human materials) or rDNA should also include a review of techniques, procedures and types and uses of equipment that may have been involved in the accident. The investigation should also establish the circumstances leading to the accident. In addition, the investigation report, by the BSO to the IBC, should provide recommendations for preventing future similar occurrences.

APPENDIX 1
Forms and SOPs for Biosafety Operations

The following forms are available on UNT Health Biosafety webpage
https://www.unthsc.edu/safety/safety-office/biological- safety/

- Biological Safety Cabinet Laboratory Equipment Relocation Installation Form
- UNT Health Incident Report Form
- UNT Health IBC SOP for Biohazard Transport
- Laboratory Safety Survey Guidance Document 2020

APPENDIX 2 Infection Control Process

2.1- Infection Prevention and Control Plan
2.2- Institutional Biosafety Program
2.3- Occupational Safety
2.4- TB Exposure Control
2.5- Waiting Room Infection Control
2.6- Hand Hygiene
2.7- Plan to Manage the Influx of Potentially Infectious Patients
2.8- Cleaning of Blood or Body Fluids
2.9- Disposal of Biohazardous/Infectious Waste

2.1. Infection Prevention and Control Plan

Procedures and Responsibilities.

1. The Goals of the Infection Prevention and Control Plan are to:
    - Establish and operate a practical system for proactively preventing, identifying, reporting, and evaluating infection in the clinics, educational areas, and research facilities.
    - Initiate proper measures to limit unprotected exposure to pathogens throughout the organization or further spread from identified sources of contagion.
    - Enhance hand hygiene and Universal Precautions.
    - Review patient outcomes as related to Infection Prevention and Control.
    - Minimize the risk of transmitting infections associated with procedures and with the use of medical equipment and medical services.
    - Communicate to the medical staff and all UNT Health clinic employees regarding potential infection prevention and control problems and suggest improvements.
    - Serve as a resource for education.

        Responsible Party: All staff and faculty responsible for Infection Control and the Infection Control Committee.

2. The Strategies of Infection Prevention and Control include the following:

    - Employment of an individual(s) with appropriate infection control and prevention knowledge to manage the program.
    - Incorporate appropriate regulatory and accreditation requirements into the organizational processes.
    - Referencing and resourcing guidelines from relevant organizations regarding current ambulatory care infection control practices (CDC, APIC).
    - Participate in effective risk management and performance improvement activities designed to improve patient care, education, and research, encouraging adherence to sound principles and organizational policy.
    - Provide Infection Prevention and Control education regarding regulations, guidelines, risk management concerns and performance improvement initiatives.
    - Conduct surveillance/monitoring/reporting of infection control practices in clinical areas.
    - Annual TB testing program for appropriate clinical, education, and research staff.

- Employee Immunization Program.

  Responsible Party: All staff and faculty responsible for Infection Control and the Infection Control Committee.

2.2 Institutional Biosafety Program

Procedures and Responsibilities.

a. Establish and implement biosafety policies (rules and guidelines) for the control of potentially biohazardous activities conducted within its facilities or by persons associated with UNT HEALTH.
b. Establish an Institutional Biosafety Committee (IBC).

   Responsible Party: Institutional Biosafety Committee, Executive Vice President of Research, Biosafety Officer, and the Principal Investigators.
c. Ensure training IBC members regarding the IBC's standard operating procedures.
d. Ensure that necessary biosafety training of supervisory personnel as required by the UNT HEALTH Biosafety Manual.

   Responsible Party: Biosafety officer (BSO), PI or laboratory manager

   - PI is directly and primarily responsible for the safety of operations under their control? He/she is also ultimately responsible for full compliance with the UNT HEALTH Biosafety Manual and other applicable directives (state and federal law and NIH and USDA "Guidelines", etc.) during the conduct of activities involving potentially biohazardous materials.
   - IBC and BSO is responsible for reviewing application for projects involving biohazard materials.

References and Cross-references**.**

- UNT Health Biosafety Manual
- CDC/ Biosafety in Microbiological and Biomedical Laboratories - 6thEdition
- NIH Guidelines for recombinant DNA Research

2.3 Occupational Safety

Procedures and Responsibilities.

a. Maintaining an acceptable level of safety is a shared responsibly of all who enter upon agency owned or leased land, and use agency owned and leased buildings, clinics, and vehicles.
b. EH&S Sr. Director maintains the document known as the UNT Health Biosafety Manual and makes it available on the EH&S webpage
c. EH&S Sr. Director provides primary input regarding needed changes and additions to the UNT Health Biosafety Manual to address institutional safety concerns.
d. Deans of each school and Vice President for Operations review proposed substantive changes to the UNT Health Biosafety Manual and provides input before changes are finalized.
e. EH&S Sr. Director with input from those listed in above as necessary approves changes to the UNT Health Biosafety Manual.
f. EH&S staff and compliance department and PI provides training opportunities to foster understanding and compliance.
g. Individuals who are present on agency owned or leased land, and to all individuals who enter agency owned and leased buildings and vehicles are responsible for compliance with state and federal laws, regulations and guidelines contained in this manual.

h. EH&S staff monitors compliance with this policy.

References and Cross-references.

NFPA Life Safety Code, as adopted by the Texas State Fire Marshal, Texas Department of Insurance as shown in Texas Administrative Code Title 28, Part 1, Chapter 34, subpart C rule §34.303 http://tinyurl.com/4lcykf

Risk Management for Texas State Agencies as revised by the Texas State Office of Risk Management as authorized by The Texas Workers' Compensation Act, Texas Labor Code, Section 412.011(b)(3) http://tinyurl.com/4mckz9

2.4 TB Exposure Control

Procedures and Responsibilities.

a. The prevalence rate and number of tuberculosis cases (locally and regionally) will be assessed for the facility by the Occupational Health Program and leadership annually to define organizational risk.
b. At New Employee Orientation, employees should be educated regarding the signs, symptoms, and transmission of TB. Additionally, clinical personnel should receive appropriate TB-specific education from their supervisor.
c. N-95 masks shall be available for employees in each clinic for use when in direct contact with suspected TB patients (CDC, 2005).
d. Clinic receptionist staff should immediately notify the nurse if they observe patients in the lobby/waiting room exhibiting suspicious respiratory symptoms. Patients suspected or known to have active TB should be given a surgical mask, shown how to wear it, and placed in an examination room immediately with door closed. All patients should be asked to cover their mouth and nose with tissues when coughing/sneezing and dispose of used tissues in a lined trash receptacle.
e. The number of persons entering the exam room of a suspected TB patient should be minimal. The door should be kept closed at all times. The staff should wear a N- 95 mask for protection against M. tuberculosis which should meet the standard performance criteria for respiratory protection as defined by CDC.
f. If the suspected TB patient is to be admitted, arrangements should be made to transfer the patient to an appropriate room as soon as possible to decrease the length of exposure in the clinic setting. Patients with known active TB who need to be seen in the clinic should have appointments scheduled to minimize exposing other patients.
g. If employee exposure is suspected, the nurse or clinic physician should notify Occupational Health Program administrator and proceed to initiate appropriate post-exposure protocols.
h. Annual TB testing should be conducted for all faculty and staff with direct patient care. Responsible Party: Clinical Department Manager/Supervisor
i. For TB exposure, the following steps will be implemented: Employees with previous negative TB tests:
    - All previous negative reactor employees should have a baseline TB skin test done as soon as possible after an exposure has occurred.
    - Eight to ten weeks after the exposure, these employees should be retested and assessed for conversion.
    - If an employee begins showing symptoms of active TB, the occupational health department should be notified.

    Employees with previous positive TB tests:
    - TB Questionnaire will be done as soon as possible after exposure.

- Eight to ten weeks after a TB exposure, employees should complete a follow-up TB Questionnaire.
- If the employee begins showing symptoms of active TB or notes positive symptoms on the TB Questionnaire, the occupational health department should be notified.

j. All patients with confirmed TB will be reported to the local Health Department promptly. The physician treating the patient, or an employee designated by the physician will be responsible for notifying the Health Department in accordance with State Law.
k. If clinic waiting room patients/visitors have been exposed to a suspected or active TB patient, the clinic should notify the Infection Control Officer who will consult with the treating physician to develop a plan of notification/action according to the history and exam of the suspected or active TB patient.
l. The treating physician or designee will be responsible for notifying the affiliated hospital if the suspected or active TB patient will be admitted from the ambulatory clinical site.

Responsible Party: All staff and faculty responsible for Infection Control.

References and Cross-references.

Centers for Disease Control and Prevention, 2005.

2.5 Waiting Room Infection Control

Procedures and Responsibilities.

Responsible Party: All staff and faculty responsible for infection control in their areas.

a. Visual identification of any of the following individual symptoms or concerns should alert staff to implement appropriate interventions and promptly notify nurse/medical assistant staff:
    a) Draining Rash
    b) Persistent Cough
    c) Head or Body Lice
    d) Draining open sores and/or wounds
    e) Any other potentially infectious fluids that may present

b. Known or suspected infectious individuals should be placed in an exam room with door closed. (i.e.: MRSA, TB).
c. If there is delay in taking an individual to an exam room, front desk staff may ask patient to wear a mask and offer tissues.
d. Immuno-compromised individuals should be placed in an exam room as soon as possible.

2.6 Hand Hygiene

Procedures and Responsibilities.

1. Basic Hand Washing (soap and water):

a. When soap and water should be used:
    - Hands are visibly soiled (dirty).
    - Hands are visibly contaminated with blood or body fluids.
    - Before and after eating.
    - After using the restroom, sneezing, or coughing.
b. How to wash hands effectively with soap and water.

- Wet hands first with warm (avoid HOT) water.
- Apply 1 – 3 ml (about the size of a quarter) of soap to hands.
- Rub hands together for 15 seconds.
- Cover all surfaces of the hand and fingers with soap using friction.
- Rinse hands with water and dry thoroughly.
- Use paper towel after drying hands to turn off the faucet and open door.

c. When alcohol based hand rubs should be used:

- Alcohol based hand wash should be available in every patient care area of each clinic and used whenever soap and water is impractical.
- Before having direct contact with a patient.
- After having direct contact with a patient's skin.
- After having direct contact with body fluids, wounds or broken skin.
- After touching equipment or furniture near the patient.
- After removing gloves.

d. How to effectively use alcohol based hand rub:

- Apply 1 - 3 ml (about the size of a quarter) of alcohol rub to the palm of the hand and rub together.
- Coverall surfaces of your hands and fingers including areas around/under fingernails.
- Rub hands together until alcohol dries (15-20 seconds). Make sure hands are completely dry prior to putting on gloves.
- Wash hands with soap and water when you feel a "build up" of emollients on your hands, typically after 5-10 applications.

Responsible Party: All staff, students, and faculty responsible for patient care, education and research. References and Cross-references.
CDC, Morbidity and Mortality Weekly Report, October 25, 2002, Vol 51, No. RR 16

2.7 Plan to Manage the Influx of Potentially Infectious Patients

Procedures and Responsibilities.
Responsible Party: All staff and faculty responsible for infection control

1. Patients exposed to biological/chemical agents requiring immediate decontamination will be referred to affiliated hospitals, and ambulatory clinics will provide follow up treatment of patients as coordinated with affiliated hospitals.
2. UNT Health leadership will establish initial and ongoing contact with the following organizations as appropriate to determine the specific nature and extent of the infectious issue:
    - Community Hospitals
    - Health Department
    - Texas Department of State Health Services/Emergency Management System
    - Centers for Disease Control and Prevention

3. Based on information and recommendations from these organizations, the scope and depth of UNT HEALTH's planned response will be determined and appropriate measures will be implemented including:
    - Choosing a location at which infectious patients will be received and treated
    - Relocation of non-infectious patients from areas anticipated to receive incoming infectious patients. If necessary, a clinic should be cleared of non-infectious patients and designated as the admission unit for patients presenting to the organizations with an infectious process.
    - Designation of physicians and staff who will see and treat infectious patients.
    - Determination of supplies and equipment needed.
4. Consideration should be given to the impact of the infectious process on the community where the patient will be discharged to determine if additional precautions or services are warranted.
5. The need to maintain appropriate infection control precautions will be paramount during this type of emergency. Staff should be informed of the following by organizational leadership before assuming responsibility for providing care during the emergency:
    - The specific nature of the infectious process.
    - The mode of transmission
    - The clinical manifestation
    - What precautions need to be implemented to prevent cross-contamination.
    - The procedure for use and disposal of appropriate protective equipment.
6. Daily/Weekly debriefings shall be conducted by leadership as appropriate until the situation is resolved.

2.8 Cleaning of Blood- or Body Fluids-Soiled Areas

Procedures and Responsibilities.
Responsible Party: All staff and faculty responsible for infection control

1. Areas soiled with blood should be cleaned immediately using the following steps:
    - Put on disposable gloves.
    - Soak up the spill with disposable towels.
    - Place drippable or saturated material with blood/body fluids into a red bag.
    - Clean area with EPA approved cleaner or bleach/water solution (1 part bleach to 10 parts water) – allow cleaner to remain on surface 1-3 minutes, dry with paper towels.
    - Place all disposable towels, etc., into red bag.
    - Remove gloves and place into red bag.
    - Place secure red bag in designated area/container.

- Wash hands with soap and water.

2.9 Disposal of Biohazardous/Infectious Waste

Procedures and Responsibilities.
Responsible Party: All staff and faculty responsible for Infection Control and Waste Disposal.

1. All Biohazardous/Infectious Waste must be handled in the following way to prevent the transmission of infectious disease.
    - Place vaccine vials in a clear plastic bag and call for Pick Up.
    - Place all needles/Sharps in approved puncture resistant sharps container and place in biohazardous Pick Up area.
    - Place all other waste in designated biohazardous containers with lids for Pick Up.
2. Unless saturated with blood or body fluids or known to be infectious, waste items that fall outside the definition of "Biohazardous/Infectious" do not require special disposal and can be placed in the regular trash containers.
3. If a healthcare provider believes that an item represents a real and substantial risk of exposure to infectious disease to themselves, a patient, or an employee, the item should be placed in the biohazardous container, even if the item does not meet the definition of "Biohazardous/Infectious."
4. Once the above items are set aside for Pick Up, UNT HEALTH's outside vendor will collect, transport, treat and dispose of the items in accordance with all applicable regulations.

5. Definitions.
   Bulk. "Bulk" means a containerized, aggregate volume of 100 mL or greater.
   Bulk human blood, bulk human blood products, and bulk human body fluids. "Bulk human blood, bulk human blood products, and bulk human body fluids" means all free- flowing waste: human blood, serum, plasma, and other blood components and body fluids, including disposable items saturated with blood or body fluids.
   Contaminated. "Contaminated" means the presence or the reasonably anticipated presence of blood or other body fluids.
   Pick Up. "Pick Up" means the items will be collected, transported, treated and disposed of by UNT HEALTH's outside vendor in accordance with applicable regulations.
   Saturated. "Saturated" is defined as thoroughly wet such that liquid or fluid flows freely from an item or surface without compression.
   Biohazardous/Infectious Waste from health care-related facilities. "Biohazardous/Infectious" means solid waste which if improperly treated or handled may serve to transmit an infectious disease(s) and which is comprised of the following:
   Animal waste
   Microbiological waste (includes discarded live and attenuated vaccines);

Pathological waste, including:

Human materials removed during surgery, labor and delivery, or biopsy, Laboratory specimens of blood and tissue after completion of laboratory examination, and Anatomical remains;

Sharps (any object that can penetrate the skin) including: Hypodermic needles and syringes with attached needles,

Contaminated scalpel blades, razor blades, disposable surgery scissors, and intravenous stylets and rigid inducers, and contaminated glass pipettes, broken glass, specimen tubes, blood culture bottles and microscope slides

References

Texas Administrative Code: Title 25 Health Services, Part 1, Chapter 1, Rules 1.131 – 1.137