HSC Biological Safety Manual

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. HSC 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, 5th Edition, December 2009.
- The United States (US) Department of Health & Human Services National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, September 2013.
- 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.
- HSC Policies.

The determination of the containment level, equipment, and PPE needed, will be defined by the HSC 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 HSC EH&S at (817) 735-2245.

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.

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Approval and Implementation

This Biological Safety Plan is hereby approved for the University of North Texas Health Science Center. This plan shall apply to all HSC personnel participating in all scientific and medical research activities at HSC 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: _____ **Date:** 9/23/20

Brian Gladue, PhD

Vice-President for Research and Innovation

University of North Texas Health Science Center

Contact Information

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	IBC Coordinator/ Biosafety Officer	817-735-5431	ibc@unthsc.edu
Safety	Director	817-735-2245	Christopher.erickson@unthsc.edu
Occupational Health	Occupational Health	817-735-2273	

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

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 / 817-735-2245
Radiation Safety	Ext: 5431 / 817-735-5431
Department of Laboratory Animal Medicine (DLAM)	Ext: 2017 / 817-735-2017
IACUC	Ext: 2533 / 817-735-2533

HSC 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/WC_Employee_Forms.pdf
Student complaints	https://unthsc.qualtrics.com/jfe/form/SV_1Mn0IlToxxTH3QF?Q_JFE=qdg
Waste Pickup Requests	https://forms.unthsc.edu/view.php?id=240571
UNTHSC - IACUC	https://www.unthsc.edu/research/animal-research/iacuc/

UNTHSC - IBC	https://www.unthsc.edu/safety/biosafety/
North Texas Regional IRB	https://www.unthsc.edu/north-texas-regional-irb/

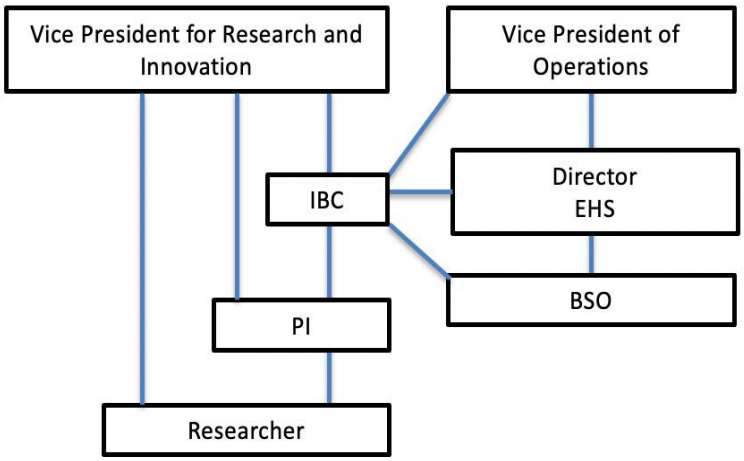
Responsibilities

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 ifand when it is considered prudent. This Manual should not to be considered final or all-inclusive, however, since all possible situations can never 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 UNTHSC laboratories by UNTHSC 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.

Biosafety Program Organizational Structure



University Responsible Official

The Responsible Official for all laboratory work involving biohazardous materials is the Vice President of Research and Innovation. 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.

Vice President for Research and Innovation

The Vice President for Research and Innovation (VPRI) is the primary oversight official for all research activities occurring on the HSC campus. The VPRI 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 HSC campus.
- Monitor all human and non-human research activities occurring on the HSC campus.

The VPRI, appointed by the President of the University, is the Institutionally Responsible Official for The Institutional Biosafety Committee (IBC). The VPRI 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.

Vice President of Operations

The Vice President of Operations (VPO) is the primary oversight official for EH&S. The VPO has the responsibility and authority to perform the following actions relevant to the Biosafety Program including but not limited to:

- Review the EH&S Program.
- Review any documentation created, maintained, or authorized by the IBC.

Institutional Biosafety Committee (IBC)

- 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 VPRI appoint the members of the IBC. IBC members serve a term of 3 years. Membership appointments will be arranged in a staggered fashion, with approximately 1/3 of the positions expiring each year. 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.

- Collectively the committee's members shall have the necessary experience and expertise in all areas necessary to carry out risk assessment of all activities involving potentially biohazardous agents within the University's facilities or by persons associated with the University. This shall include a working knowledge of recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health and the environment; potentially biohazardous organisms; the risks of various activities involving the use of human-derived materials. The IBC shall include at least one scientist with expertise in animal pathogen containment principles. The IBC is encouraged to use consultants who are knowledgeable of institutional policies; applicable laws; occupational health and safety standards; environmental protection regulations; standards of professional conduct and practices; and of community attitudes.
- The current IBC membership shall be submitted to the NIH and, as appropriate, to other contract, grant or media authorities.
- No member of the IBC may be involved (except to provide information) in the review or approval of any project in which he/she has been, or expects to become, engaged or in which he/she has a direct financial interest.
- IBC meetings are to be open to the public whenever feasible and consistent with the protection of privacy and proprietary interests.
- Minutes of the IBC meetings (including closed meetings) and documents submitted to or received from funding agencies are to be made available to the public in accordance with NIH Guidelines and the Texas Public Information Act. If comments are received from members of the public, the press or other governmental agencies on the IBC actions, the IBC will forward a copy of both comments and the IBC's response to the OBA.
- The IBC, through its Chair and the BSO, shall keep the VPRI informed of developments and practices regarding the use of potentially biohazardous materials and, upon request, provide an overall safety, health and environmental review of the University's activities involving potentially biohazardous materials.

The HSC 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. Additional to biological hazards, the IBC also evaluates chemical hazards from the submitted protocols as HSC does not have an independent Chemical Safety Committee. 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 HSC locations.
- Facilitate the registration of identified biological materials.

- Perform risk assessments to ensure the safe use of biologicalagents.
- 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 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 HSC. *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.

The IBC operates in accordance with its approved charter and hold monthly meetings on the third Wednesday of each calendar month with exceptions for holidays. The IBC advises the VPRI and VPO 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 HSC, including such matters as safety training and health surveillance.

The IBC will offer its counsel to all HSC personnel regarding matters of biological safety. The VPRI and VPO may ask the IBC to inform the community about developments in the general area of biological safety.

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 beingfollowed;
- reporting to the IBC and to VPRI 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 UNTHSC and external regulatory agencies concerned with the use of biohazardous agents, human materials, toxins and recombinant DNAmolecules;
- filing an annual update with the NIH;
- serving as a voting member of the IBC, including eligibility for appointment asChair;
- reviewing all funded grants for compliance with applicable sections of thisManual;
- 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, andtheIBC.

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. The Chairperson may choose to share this responsibility with a departmental biosafety committee and/ or a unit director.

Principal Investigators (PIs)

PIs are faculty members or other HSC 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 his/her biological activities in his/her laboratory to other laboratory personnel or faculty, this does not absolve the PI of his/her ultimate responsibility. The PI remains accountable for all activities occurring in his/her 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/orguidelines.
- 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 laboratoryfacility.
- 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 HSC to ship such materials.

HSC Researcher

HSC 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 to 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 microcidal against the agent(s) in use.
  - Maintaining properly stocks of laboratory supplies
- Maintain their work space in efforts to reduce contamination, clutter, and excessive storage.
- Implement practices to secure infectious agents against theft, loss, and release.

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

- Updates this manual on an annual basis.
- Provides safety consultation on operations within laboratory, academic programs, hospital, and clinics areas.
- Provides initial and ongoing institutional safety training including bloodborne pathogens, biosafety, infectious substances shipping, select agent and toxins, and hands on exercises.
- Provides information on regulations that apply to the laboratory and clinical operations.
- Advises on safe methods for new procedures and on the use of new equipment.
- Verifies and monitors institutional training records to ensure all pertinent HSC 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.
- Implements policy and guidelines approved by the IBC.
- Periodically reviews hazard registrations to ensure described facilities, equipment, PPE, procedures, and practices are consistent with IBC authorization and institutional policy.
- Ensures that proposed safety policies, manuals, plans, facilities, equipment, and procedures for work with biological materials meet applicable regulatory standards and guideline.
- Evaluates and surveys laboratory, hospital, 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.
- Responds to and remediates large biohazardous spills.
- Identifies potential problem areas and suggests to the IBC safety objectives to be achieved.
- Disseminates information on new safety programs and outreach services, as well as revisions to pertinent institutional policies, safety documentations, and federal and state regulations.

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 BioSafety.

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 yourlaboratory.
- If minors will be working in their respective laboratories.
- Please refer to HSC'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 his/her hazard registration has been approved by the IBC and a formal approval letter from the Biosafety Program has been received. The approval letter is provided by the EH&S at the behest of theIBC.

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 over the Biosafety Program will be immediately reported to the Director of EH&S, the IBC Chair and the VPRI. 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 VPRI, PI's department Chair, the Director of I Director of Office of Research Compliance.

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 Director of EH&S and the IBC Chair may recommend to both the VPRI and VPO appropriate action if an investigation reveals significant violations.

Research Vertebrates

All research experiments involving research vertebrates must be conducted in accordance with the HSC 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 his/her designees to attend a work-start meeting with members of EH&S and the DLAM. Work-start meetings ensure that the biological materials, animal use locations, required equipment, and project specific operating procedures are discussed, understood, and implemented.

Research Invertebrates

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

Human Specimens

PIs using specimens and materials acquired from human subjects (whether they be obtained on-site HSC 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.

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.

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: Annual renewal is required from the date indicated on the IRB approval letter unless otherwise noted.

Risk Assessment and Risk Management

Responsibility for biosafety exists at all levels and is shared throughout the HSC. The HSC 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 P's of risk assessment and risk management are:

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

Consider the five P's in each facet of laboratory work. 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 ▪ 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
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 practices and 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 ▪ Serum banking ▪ Attitude toward safety ▪ Comfort ▪ Open wounds, non-intact skin, eczema, dermatitis	▪ Safety training ▪ Prior work experience with biohazards ▪ Demonstrated proficiency with techniques ▪ Prompt reporting of all exposure incidents, near misses, as well as signs and symptoms of related disease to PI and Employee Health ▪ Investigation/review of incidents/spills, etc. to prevent future occurrence

Protective Equipment	Protection (containment) for: ▪ Aerosols – (respirable size particles)<10µm ▪ Droplets/splatter ▪ Sharps	▪ 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 faceshield - Solid front gown or lab coat - Gloves ▪ Biosafety Cabinet (BSC) ▪ Centrifuge safety buckets/rotors ▪ Safe Sharps and Sharps Containers
Place – Laboratory facility	▪ Risk group/BSL requirements ▪ HVAC and exhaust systems ▪ Aerosol risk ▪ Restricted access	▪ Basic lab – door, sink, surfaces easilycleaned, eyewash, screens on windows that open ▪ Labels ▪ Containment laboratory with directional airflow

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.
  - Note that these organisms may cause disease in immunocompromisedpersonnel.
- Risk Group 2: Agents associated with disease which is rarely serious or there is treatment 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 hazard.
- Risk Group 4: Serious or lethal disease for which there is not usually a therapeutic intervention, generally dangerous and exotic viruses.

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).

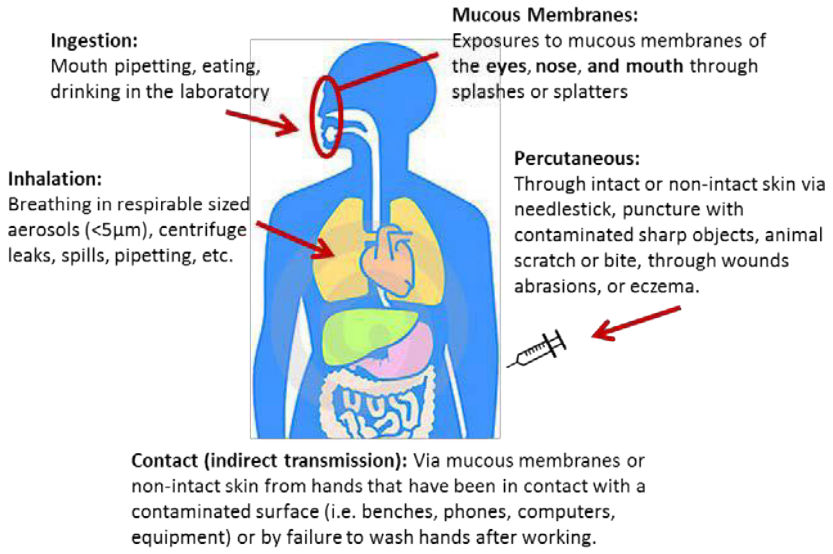


Figure 1. Main routes of entry for infectious agents.

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 clinicalprocedures.
- 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.
- 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'sinstructions.

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 protectiveshield ▪ 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-proofsharpscontainer ▪ 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 personalhygiene) ▪ 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)

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.

Bio-containment

As defined by Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th 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 potential 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 will be determined by EH&S and the IBC following a review of the laboratory, equipment, procedures, training, and the hazards utilized.

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) 5th 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.

Most laboratories on campus are BSL1. Tissue culture rooms are defined as BSL2. Below is a summary of practices, equipment and facility requirements for agents assigned to BSLs 1–4. Additional information on BSLs may be found in the BMBL 5th edition.

Biosafety Level 1-- BSL1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. 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.

Biosafety Level 2-- BSL2 builds upon BSL1. BSL2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. 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.

Biosafety Level 2+ -- BSL2+ builds upon the classic BSL2 environment. While BSL2+ uses the same basic layout and equipment as a BSL2 it uses procedures and SOGs more typically associated with a BSL3 lab space.

Biosafety Level 3-- BSL3 builds upon BSL1 and BSL2. BSL3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease 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 anddesign

features. Currently HSC does not have any BSL3 facilities.

Biosafety Level 4-- BSL4 builds upon BSL1, BSL2, and BSL3. Currently, HSC does not have facilities that support BSL4 containment. For more information on BSL4 facilities, containment, equipment, and practices, please refer to the BMBL 5th edition.

Select Agents and Toxins

The use and storage of non-excluded select agents are regulated by the Centers for Disease Control Division of Select Agents and Toxin (DSAT). Institutions that use and store these agents must register with DSAT. DSAT, at its discretion, may require registered institutions to implement enhanced training, security measures, containment, equipment, and procedures. Additionally, DSAT may adopted the guidelines described the BMBL 5th edition as policy which will require institutions to enforce these measures in registered facilities including applications specific to equipment, procedures, and personnel.

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. 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 attenuated or a-virulent strains of specific microorganisms. Therefore, the containment level established for the listed genus and species must be adhered to unless a containment downgrade is authorized by the NIH Office of Science Policy. An example is *Mycobacterium tuberculosis*. Although attenuated strains of this organism exist, they must be contained at BSL3 unless a containment waiver is granted by the NIH Office of Science Policy.

Department of Defense, CDC etiological agent division, Unites States Department of Agriculture, and FDA

The listed agencies may require the implementation of additional biosafety and animal containment levels specific to microorganisms covered under their regulatory authority. Please check with EH&S if you have any questions regarding these regulatory agencies.

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 HSC's Biosafety Training Program:

- 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 andprotocols.
- Demonstrate safe work techniques.
- Provide instruction on emergency response procedures.
- Convey information on important regulations (such as the transport of dangerousgoods).
- 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 applicableexpertise.

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
Complete Laboratory Safety (includes Biosafety and Bloodborne and NIH Guidelines)	Faculty, Clinicians, Fellows, Postdoctoral fellows, Students, Staff, Temps, Interns, Volunteers, Visiting Scientists	Online	Upon Hire or Job Advancement
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	As needed
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 2+ operations, the proper use of the biosafety cabinet, centrifuges, and other laboratory equipment. For more information, send an email to the Biosafety Program at ibc@unthsc.edu.

Standard Laboratory Practices

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 thispurpose.
- 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 nonhazardouschemicals).
- 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.

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 infectiousmaterial.
    - 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).
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containmentdevices.

Personal Protective Equipment

The direct, often hands on use of biological, chemical, and physical hazards in HSC laboratories requires that HSC 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.

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 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. eye protection, respirators, cryo- gloves, etc.) may be 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.

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% leak-proof.
- 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 thepublic.
- 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. Please follow the instruction from the manufacturer prior to autoclave
- We recommend not take lab coats home to launder.
- Change lab coat as soon as feasible or whenever it is compromised, soiled or torn.
- Wear appropriate lab coat sizes.
- Wash hands whenever lab coat isremoved.

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.
- Use the one glove method (See Figure 2), or an appropriate secondary container, when transporting materials outside of the laboratory areas. Alternatively utilize a cart to transport the material within an appropriate secondary container.

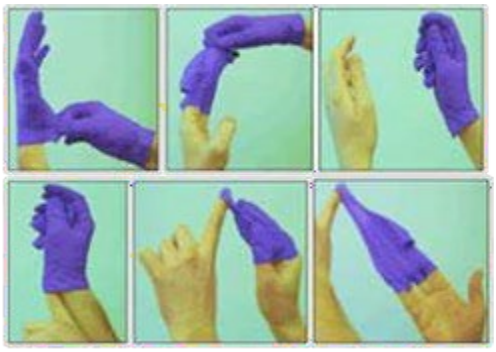


Figure 2. Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

- Gloves are not 100% leak-proof; change gloves periodically and when soiled. This fact reemphasizes the need to wash hands after removing gloves.
- Gloves will not prevent needle sticks or other puncture injuries.
- Double glove or use thicker rubber when cleaning biohazardousspills.
- 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.

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 covers or Tyvek may be placed over shoes to reduce the potential for facility contamination and/or to prevent the contamination of personal clothing.

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 lenses should be made available to all laboratory personnel. The selection of appropriate face/eye protection is dependent upon compatibility with the work and the overall facial area requiring protection.

Important points to remember:

- 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 can be worn, however, personnel comfort should be considered.
- Face shields and hoods protect the face and the neck from flying particles and sprays of hazardous material; however, they may not provide basic eye protection against impacting objects.
- 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.
- 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.

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, HSC has created a respiratory protection program and a respiratory protection manual. The respiratory protection program is managed by EH&S and Occupational Health. The respiratory protection manual 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.

Additional Protective Apparel

Note: The barrier provided by PPE is based on the composition of material or material(s) used to produce of this clothing; how the material is stitched or sealed (i.e. electronic/heat/compressed) at seams created during assembly; and the seal provided by the wrist and ankle cuffs. Additionally, as the barrier provided by PPE increases, the ability of the PPE to allow air exchange (i.e. breathable) with the environmental decreases. This decrease in the breathability of PPE is correlated to a

reduction in the ability of the body to dissipate heat, which can lead to an increase in body temperature, perspiration, and heat stress. This information must be considered during the PPE selection process where the user will be required to weigh the protection needed verses potential comfort concerns.

- 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 HSC:
    - Breathable with minimal moisture resistance PPE – Disposable polypropylene jumpsuits, and gowns, smocks, shoe covers, and hair nets.
        - Generally worn in BSL1 laboratories and ABSL1 animal facilities and is designed to provide a simply 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, BSL3 facilities, and operating rooms.
    - Non-breathable moisture impervious – Tyvek and Tychem jumpsuits, aprons, smocks, sleeve covers, shoe covers, and head covers, as well as, rubber aprons andsmocks.
        - Fluid impervious materials that provides 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 ofPPE:
    - o 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.

- Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin conditions or wounds. Avoid working with BSL2, BSL2+ or other potentially infectious materials if non-intact skin cannot be adequately covered.

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

PPE	BSL1	BSL2	BSL2+
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.	Safety glasses are required. Mask with visors, face shield, and safety goggles may be required based on laboratory operations and the hazards present or utilized	The potential exposure risks to agents with high overall health consequences, requires BSL3 personnel to wear face protection. This could include face shield, PAPR shrouds, or safety goggles with an N-95 respirator.
Respiratory Protection All those who may wear a respirator must be enrolled in the HSC Respiratory Protection Program.		Filtering Face Piece particulate respirators (N-95s) may be required when working with pathogens that are spread by aerosol or droplets (e.g. influenza, Neisseria).	The use of respiratory protective equipment such as a powered air purifying respirator (PAPR) will be required. The use of PAPRs is required for response and cleanup of a BL3 spill.
Other		Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons, and household rubber gloves will be recommended on a case by case basis. Generally, additional protective clothing is required whenever there is a high potential for splashing of potentially infectious material, such as organ harvesting or large spill response and clean up.	

Signage and Labeling

Laboratory Entrance Doors

All laboratory entry doors must have a sign similar to what we have shown in figure 3. . 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.
- Required procedures for entering and exiting the laboratory.
- Agent specific information should be posted in accordance with the institutional policy.

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)

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.

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

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.

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.

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 impacts 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).

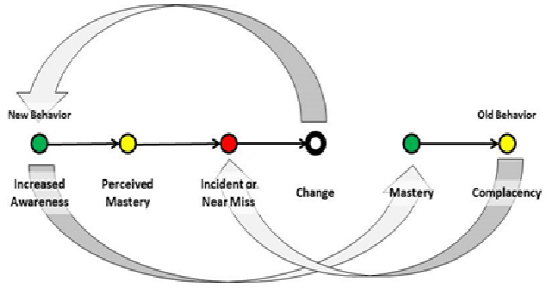


Figure 6. Change and Train – Utilize Lessons Learned

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 his/her 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 ofaccidents.
- 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 work surfaces.

Operational Standards for HSC 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.

Biosafety Level 1 (BSL1) – Figure 7

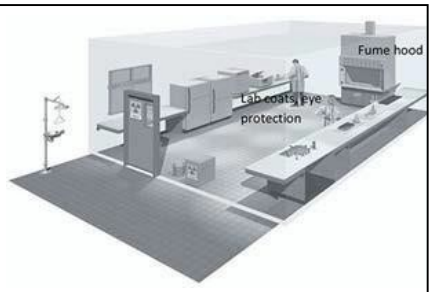


Figure 7. Biosafety Level 1 laboratory.

- Keep laboratory door closed.
- Wear laboratory coats, gloves, and eye protection.
- Do not reuse disposable PPE.
- Use procedures that minimize aerosol formation.
- Do not smoke, eat, drink or store food inlaboratories.
- Do not mouth pipette, use mechanical pipettingdevices.
- Critically evaluate the use and need for hypodermic needles.
- Implement sharps protection procedures.
- Receiving training on and properly use the fume hood 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 aspill.
- Autoclave all solid biological wastes before discard.
- Liquid culture waste and unused/unwanted culture stocks must be treated with bleach to a final concentration of 10% v/v. Allow at least 15 minutes for the bleach to inactivate viable material prior to discharging this material to the sanitarydrain.
- Decontaminate other non-disposable contaminated materials before washing orreuse
- 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).
- 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 thePI.
- Keep animals and plants not used in experiments out of thelaboratory
- Keep areas neat and clean.
- Control insect and rodent infestations.

Biosafety Level 2 (BSL2) – Figure 9

- 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. respirator, Tyvek sleeves, faceshield) 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.

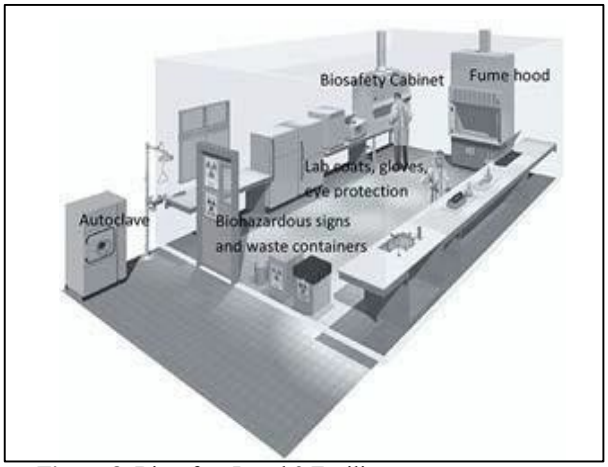


Figure 8. Biosafety Level 2 Facility

- 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-producingequipment/procedures.

Tissue Culture Laboratory – Figure 9

Tissue culture laboratories are commonly found within HSC research buildings and in clinical laboratory facilities. According to institutional standards, tissue culture laboratories are operated as biosafety level 2 facilities.

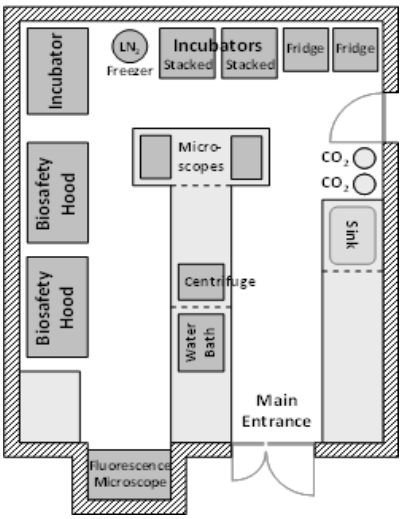


Figure 9. Tissue Culture Laboratory

As the majority of tissue culture and infectious agent work occurs in tissue culture rooms, specialized equipment and procedural controls are necessary to ensure infectious materials are properly contained, handled, and disposed. Additionally, as these facilities are typically outfitted with biosafety cabinets, laboratory procedures must include the proper use of these units.

Contamination is common concern in tissue culture rooms. The implementation of good laboratory practices and sterile techniques are key methods in the prevention of culture and product contamination. Proper use of the biosafety cabinet and strict decontamination procedures are key components of sterile technique. Additional methods include maintaining a clean laboratory coat that is reserved solely for cell culture work.

Biosafety Level 2+ (BSL2+)

BSL2+ 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 BSL2+ work. It is preferred that the BSL2+ 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 BSL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment has been decontaminated, the sign is removed and the laboratory is returned to standard BSL2 or BSL1 use if the work is notroutine.

Biosafety Level 3 – Figure 10

Pathogens requiring BSL3 containment, equipment, and practices are much too complex for a simple description in this manual. The IBC will require detailed SOPs be generated to define the required containment, specialized equipment, unique PPE, and the intricate biosafety practices necessary to work in a BSL3. Working with agents requiring BSL3 containment is currently prohibited at HSC as HSC does not have any BSL3 labs.

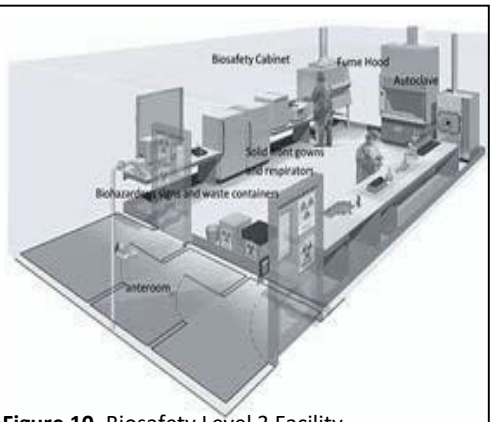


Figure 10. Biosafety Level 3 Facility

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	Open bench top, sink required
2	Associated with human 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 +: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing beforelaundering • Baseline serum	Class I or II BCSs or other physical containment devices used for all manipulations of agents PPE: protective lab clothing, gloves, respiratory protection as needed	BSL2 + : • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	Dangerous or exotic agents which pose high risk of life threatening disease, aerosol transmitted lab infections, or related agents with unknown risk of transmission	BSL3 practices plus: • Clothing change before entering, shower onexit. • All material decontaminated on exit from facility	All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air supplied, positive pressure personnel suit	BSL3 +: • Separate building or isolated zone • Dedicated supply/exhaust, vacuum, and deconsystems • Other requirementsoutlined in BMBL

*Adapted from the CDC Office of Health and Safety

Safety Considerations When Using Engineering Controls and Equipment

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 sealed 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 to be handled within a Biosafety Cabinet. This will include but is not limited to the followingprocesses:

- Inoculating culture media with stocks of infectious agents (e.g. plating, seed cultures, overnight cultures, generating freezer stocks, etc.).
- Prepping culture material for further processing (e.g. 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 moreinformation.
- 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 andblower.
- Disinfect all interior surfaces with 70% ethanol or 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 singleprocedure.
- After procedure, allow cabinet to run 2-3 minutes before removing materials.
- Wipe down all work surfaces with 70% ethanol or suitable disinfectant.
- Turn off fluorescent light and blower if desired.

Many BSCs are equipped with germicidal ultraviolet (UV) lamps. 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. 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 biosafetybarrier.
    - The use of Bunsen burners inside BSCs is high discouraged as this can create hazardous conditions which may cause serious fires (Figure 13). Alternative technologies such as electric incinerators, pilotless burners, or touch-plate microburners is encouraged.

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 adequatelyfilled.
    - 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 expeditework.
    - Prep a BSC biological waste container
        - Example: Place a 24”x12” autoclave bag into a 4liter plasticbeaker.
    - Prep a pipet tray with disinfectant (2-5% dilutedbleach)
- Prepare the work BSC worksurface:
    - Create a 2 x 2-foot work area between the clean (supplies) and dirty areas (waste containers) of the cabinet
    - Place the prepped waste container and pipet tray into theBSC.
- Working in the BSC:
    - Keep materials at least 4 inches inside workarea.
    - Once inside the cabinet, all movements must be slow and methodical.
    - Work should proceed from clean to contaminatedareas.
- 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 (i.e. volume of disinfectant must be appropriate for volume of contaminated liquid waste). 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 tubingbefore

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.

Fume Hoods

A fume hood is a ventilated enclosure designed to contain and exhaust fumes, vapors, mists and particulate matter generated within its interior. The function of a fume hood is dependent on the building exhaust system and HEPA filtration is not standard.

During laboratory surveys, EH&S will evaluate the status of laboratory fume hoods and will use anemometers to verify that the hood is functioning. During this evaluation EH&S surveyors will determine if the being operated in accordance with the Chemical Safety Manual.

The containment provided by a fume hood depends upon the behavior of the operator, the type of hazardous material utilized, and the placement of items on the hoods work surface. Prior to working in a fume hood, users must receive hands on training from their PI or EH&S. The following information is provided to assist the proper use of BSCs.

Important points to remember:

- Like BSCs Fume hoods are not storage areas.
- The sash (Glass Barrier) should be set no higher than 18 inches from the bottom of the hood.
- All fume hoods pull airflow inward directly across the work surface away from the user.
- Fume hoods do not offer product protection as the air moving across the work surface is not filtered and contain ambient air contaminants.
- Functions to protect user only if the linear face velocity (lfv) is maintained at or above 60 linear feet per minute (lfm).
  - All activities should be completed at least 6 inches from the sash.

Centrifuges: Procedures for Centrifugation

All centrifugation 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.
- 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.
- 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.
- 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. If the rotor does not fit in the BSC, use the fume hood.

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.
- 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.
- 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.

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).

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 chemical disinfectant to chemical trap flasks. Allow the aspirated fluids to complete the dilution. (For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard.)
- Vacuum line VacuGuard 0.2µm filter must be used and shall be examined and replaced if clogged or if liquid makes contact with the filter. Used filters shall be discarded in the medical waste stream.

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 theinoculum.
- Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget 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 biohazardbag.
- 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 puncturewounds.
- 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 beige sharps container. DO NOT bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable, use a one handed method. DO NOT discard syringes into a red bucket or biohazardbag.

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.

Important points to remember:

- Pipetting of toxic chemicals should be performed in a chemical fumehood.
- Infectious or toxic materials should never be forcefully expelled from apipette.
- 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 aheight.
- 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, and replaced by a clean pan with fresh disinfectant.

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 prior to sonic cleaning due to possible aerosolgeneration.

Important points to remember:

- 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 anappropriate

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 aerosolcloud.
- Grinding of infected tissues or materials with any open device is best done within a BSC.

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 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.

Microtome/Cryostat

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

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

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 theblade.
- Dislodge stuck blocks using mechanical means such as forceps and/or dissecting probes.
- Wear appropriate PPE such as a lab coat or gown, mask, safety glasses or goggles, surgical grade Kevlar gloves that provide dexterity and cut protection, and examination gloves to protect againstbiohazards.
- 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 appropriatedisinfectant.
- Consider trimmings and sections of tissue as contaminated and discard in the appropriate waste stream.
- Do not move or transport microtome with knife inposition.
- Do not leave knives out of containers when not in use.
- Do not leave motorized microtomes running unattended.

Warm Room

Warm Rooms (37°C) 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 they can become to silent bio-incubators leading to the generation of unknown bio-specimens which can perpetuate unknown contaminates and exposures.

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

- The departments should 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 warmrooms.
  - 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.
- Equipment:
  - All equipment that utilizes liquids should be monitored for leaksdaily.
  - All equipment should be monitored for mold growth asnecessary.
- disciplinary actions from management.
- Proper Storage:
  - No Cardboard should be stored in warmrooms.
  - 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.

- Equipment:
  - All equipment that utilizes liquids should be monitored for leaksdaily.
  - All equipment should be monitored for mold growth as necessary.
- 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 in a manner that allows aeration, if necessary, to prevent leakage of contents.
  - It is recommended that flasks, tubes, or containers be filled to no more than 35% capacity when shaking is required.

Spill Response

Spills of Human Pathogen (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.

- Spill must be immediately reported to the supervisor or Warm Room oversightperson.
- Spilled material must be immediately absorbed with absorbent material
- Liberally coat the absorbent material with a 10% (1-10 concentrated) bleachsolution
- Allow the material to incubate/disinfect for no less than 30 minutes.
- Collect all spill material in an autoclave bag and autoclaveimmediately.
- Reassess area for missed material: under mats, equipment, shakers, plates, flask, etc. and disinfect as described above.
- Discard cleaning material into a biohazard bag andautoclave.
- 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 Human Pathogens (Risk Group 2) 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 Warm Room oversightperson.
- Department must contact EH&S.
- Warm room must be deactivated and cleaned according to EH&S's recommendations.

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. These conditions are typically acerbated by bad habits and habitual misuse of these areas. These activities increase the potential for mold growth which cause eventual experimental contamination, as well as

may serve as a reservoir for pathogenic fungus. These fungi can create environments leading to exposure to active pathogens and/or their bio-products which can serve as allergens.

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 coldrooms
- Media, solid and/or liquid, stored in cold rooms should be dated and removed after 3 months or at listed expiration.
- All equipment that utilizes liquids should be monitored for leaksdaily.
- All equipment should be monitored for mold growth.
- Equipment must be cleaned after each use.
- Spilt material must be absorbed immediately.
- Ice or other baths utilized to store equipment, culture, etc. must be within secondary containment.
- Media must be immediately discarded if contaminated or expired.
- Cold rooms should be deactivated, disinfected, and cleaned on a schedule as determined by use and departmental oversight.

Spills of Human Pathogens (Risk Group 2-4) 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 respiratoryexposure.
- Must be immediately reported to the supervisor or Warm Room oversightperson.
- Spilled material must be immediately absorbed with absorbent material.
- Liberally coat the absorbent material with a 10% (1-10 concentrated) bleachsolution.
- 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 Human Pathogens (Risk Group 2-4) 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 oversightperson.
- Department must contact EH&S.
- Cold room must be deactivated and cleaned according to EH&S's recommendations.

Miscellaneous Equipment (Water baths, Cold Storage, Shakers)

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

Deep freeze, liquid nitrogen, and dry ice chests as well as refrigerators should be checked, cleaned out periodically to remove any broken ampoules, tubes, etc. containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deep freezers should be properly labeled. Security measures should be commensurate with the hazards.

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

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

Safety Considerations During Experimental Procedures

Pathogen Cultivation, Manipulation, and Storage

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.

Culture Plates, Tubes and Bottles

Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a BSC.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is 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. The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wireloop.

Water of syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. If plates are obviously wet, they should be opened in the BSC.

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

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

Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created. Aerosol creation should be prevented or minimized; 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. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a BSC and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted cotton. 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 cotton, 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.

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:

- Use designated and appropriately labeled incubators for infected tissueculture.
- The volume per flask must be determined so that horizontal placement during incubation does not cause media leakage.
- Do not fill cell culture plate wells past 50% capacity.
- 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 TC container.
- Plate lids should be secured with tape on at least two sides of the plate. When using the microscope, plate lids must be sealed with parafilm.
- If a vacuum flask is used for tissue culture spent mediaremoval:
  - Vacuum flask not contained within the BSC must be in secondarycontainment.
  - Vacuum hosing must be monitored for contamination. If contaminated hosing should be autoclaved prior to disposal
  - Flask should contain enough concentration disinfectant (Bleach) to neutralize any agents extracted with the waste media.
  - Flask should be emptied daily and restocked with fresh disinfectant prior to next use.
- It is required that a VacuGuard 0.2µm 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).
- All waste generated during tissue culture activities must be treated as biohazardous and autoclaved prior to disposal.

Microscopy work with infectious agents

Any culture vessel carrying infectious material should be treated with the upmost care and focus to ensure movement of these materials does not lead to an adverse event. 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 themicroscope.

- Demonstrate care in how you set the vessel onto work surfaces including the microscope platform.
- Care must be employed if the lid must be removed for examinations that occur outside of a BSC.
- Immediately return all culture vessels to their origin after the examination is completed.

Safety Precautions During Work with Animals

The use of biological agents/materials, biotoxins, and prions in animals requires additional risk analysis and assessment for which the IBC and IACUC have oversight. Once a viable bacterial agent enters a susceptible host, this host may become a bio-factory for these organisms and thus can 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, blood, tissue excretion, extracted, and/or otherwise obtained from the animal must be considered hazardous
- Contaminated animal dander, bedding, and food dust.
- Contaminated water.

In addition, 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:

- 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 biotoxins in that it is likely not shed post administration or injection, however, these materials do actively replicate and to some degree create tumor masses in the animal model. Although exposure following inoculation is likely handled by our immune system as foreign, 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. In that we try to achieve the best experimental results associated with exposure of animal to various biological material and agents, we in turn increase the risk associated with direct and/or indirect exposure. Specifically, the following are examples of increased risk factors:

- 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.
- 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 (intox unit). 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 must be considered hazardous and handled appropriately. This creates an additional hazard as the bedding must be considered hazardous for the extent of the experiment.
- Topical administration or superficial injection of biological agents possesses a risk to animal-handling and laboratory staff 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 as it has become common practice. The risk involving work with transgenic animals is typically low in comparison to other hazards. However, the environmental impact and the spread of disease is a significant risk and must be considered along with Federal, State, and local regulations. 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.

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.

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.

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.

ABSL3

ABSL3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease. ABSL3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL2. ABSL3 laboratory has special engineering and design features.

ABSL3 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment

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

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 be immediately notified prior to any transport of infected and/or contaminated live animals from the DLAMfacility.

Transgenic Animals
ABSL1 containment and standard DLAM conditions are sufficient for breeding, rearing, and housing transgenic animals. 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.

Human Cells, Risk Group 1 Organisms, and Viral Vectors Administered Through Stereotactic Injection

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.

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 his/her designee and EH&S/DLAM.

A cage change must be completed no sooner than 48hrs 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.

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 caging systems available for animals exposed to human pathogens 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 prestart or pre-continuation meeting between the PI and EH&S/DLAM.

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.

Some Risk Group 2-3 pathogen work might require ABSL3 containment, equipment, and practices as determined by IBC. These procedures are much too complex for a simple description in this manual. The IBC will require detailed SOPs be generated to define the required containment, specialized equipment, unique PPE, and the intricate biosafety practices necessary to work in a ABSL3. If interested in learning more about ABSL3 containment reference the BMBL 5th Edition.

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 who 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 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.

Use of Radioactive Materials

All the research work involving usage of radioactive material in animals MUST be approved by UNTHSC 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.

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.

Hazard Transportation

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. *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 Policy and Procedures.
- 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 with agent name.
  - 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. Styrofoam) so it can be easily disinfected.
  - A biohazard sticker with the name of the agent and the name and phone number of the PI on the outside of thecontainer.
  - Absorbent (paper towels) between the secondary and tertiary containers suitable for the volume transported.
  - Do not use gloves when handling the tertiary container.
- Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.

- 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.
- Cultures removed from BSL2 facilities and/or equipment may require the use of specialized PPE. Refer to your approved hazard registration for and committee approval for information on required procedures for work with registered hazards.

Decontamination, Disinfection, and Sterilization

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.
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 "overkill'' and therefore lacks the margin of safety achieved by sterilization procedures.

Liquid Decontaminants
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. Unfortunately, the more active the decontaminant the more likely it will possess undesirable characteristics such as corrosiveness. 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.

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.

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.

Decontamination Using Vapors and Gases

A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde (FA), 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 resists penetration by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; rooms, buildings and associated air-handling systems.

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). Dry heat at 160°C to 170°C for periods of two to four hours is suitable for destruction of viable agents on an impermeable non- organic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat for decontamination. Incineration serves as an efficient means of disposal for human and animal pathological wastes.

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 (i.e. simple or complex, solid or porous surface, and/or airborne)?
- What is the highest concentration of organisms anticipated to be encountered?
- Can the disinfectant, either as a liquid, vapor, or gas, be expected to contact the organism and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility ofmaterials?

- 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.

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 contacttime.
  - Each disinfectant will be prepared in dedicated containers in accordance with the manufacturer's recommendations, and its working concentration marked on the container.

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.)

In general:

- Solid waste that may have been contaminated (bench paper, towels, gloves, used containers) **must** be disposed of in biological waste and sterilized by autoclaving.
- Solutions containing biological material **must** be treated with 10% bleach for at least 15 minutes and then poured down thedrain.

Containers

Containers must be appropriate for the contents and interchangeable use of containers is not permitted (i.e., broken glass box or medical waste 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- sharpscontainers.
- *Broken glassware*. All glassware must be disposed of in either a broken glass container or sturdy cardboard box. Seal securely and clearly label "BROKENGLASS".
- *Solid biohazardous waste*. Only use Orange or Clear autoclave bags. Place bags in a secondary heavy duty plastic container clearly labelled with a biohazardsticker.
- *Medical waste.* 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 forcontent(s).

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 limitedto:

- 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 waste container.
- Minimizing the amount of bleach that is present in materials to be autoclaved. In most cases bleached material, after at least a 20-30 minute incubation, can be poured down the sanitary drain. Bleach produces corrosive vapors which will damage the stainless steel components of theautoclave.

Training

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

departmental autoclaves. The laboratory PI/supervisor is encouraged to use the Autoclave Use and Verification manual to train his/her employees.

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.

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 loadtreated.
- Ensure that the autoclave parameters are met for each load.
- Perform a monthly biological indicator test to ensure that the autoclave equipment is functioning correctly.
- Follow proper procedures when the equipment fails.

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. Autoclaved waste is sent to a municipal landfill, but bulk human material cannot be passed through this waste stream even if it has been sterilized. EH&S provides Medical Waste boxes for these types of materials. Items that **must** be disposed of in a Medical Waste box include, but are not limitedto:

- Bulk human material.
- 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.
- Cups, paper, plastic, and/or any other material not contaminated with human blood or bodily

fluids.
- Food items.
- Large volumes of liquid (e.g., urine).

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 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 eventually collected by a contracted vendor and incinerated. If any chemicals are found in these boxes, we are 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.

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.

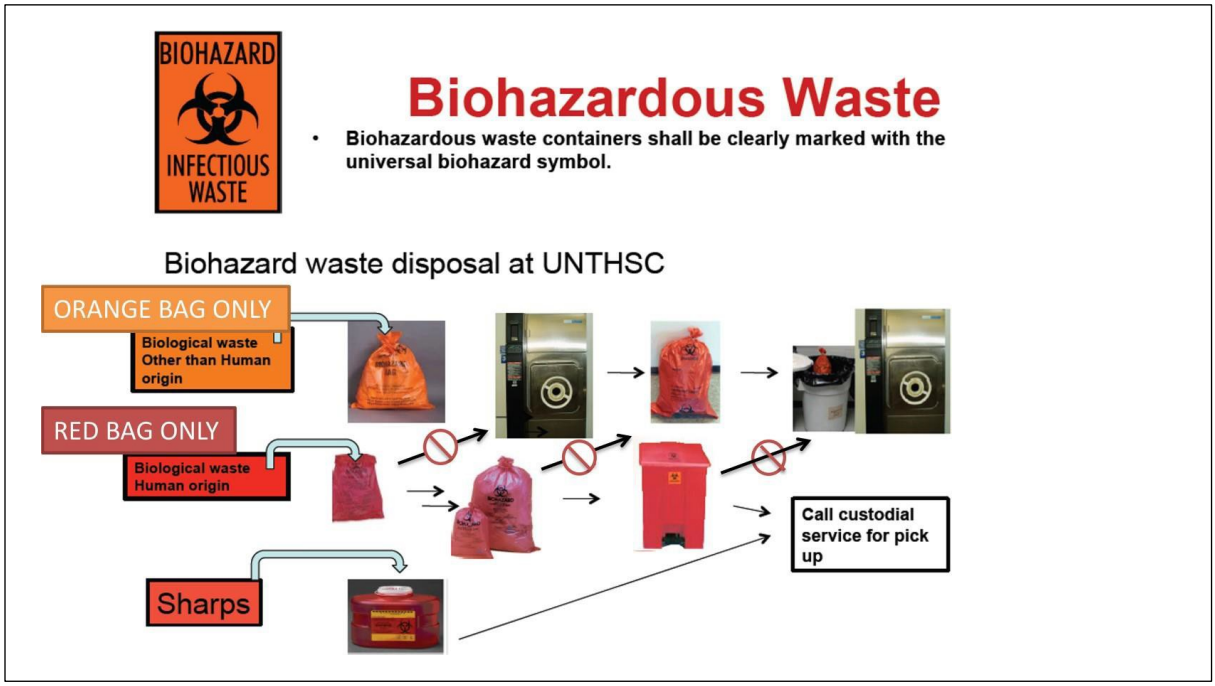


Figure 11- Summary of biowaste management process at UNTHSC

Special Considerations

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 you should consult your PI or supervisor. 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 offer an opportunity to 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 (generally with actual pathogens, not necessarily for only other potentially infectious materials such as blood or body fluids) should be given. Also, Investigators actively working with reproductive hazards explain the risk assessment at time ofhire.

Reproductive Biological Hazards

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

- Cytomegalovirus (CMV)
- Hepatitis B virus (HBV)
- Hepatitis E virus
- Human Immunodeficiency virus (HIV)
- Human parvovirus B19
- Rubella (German Measles)
- Lymphocytic Choriomeningitisvirus
- Toxoplasma gondii(Toxoplasmosis)
- Listeria monocytogenes
- Varicella-zoster virus (chicken pox)

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. Examples of conditions that might warrant special precautions are HIV infection, 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 and/or your PI about the situation.

Immunizations

In certain situations, personnel engaged in particular research activities would be immunized with appropriate vaccines, such as rabies, rubella and measles. Vaccines not commonly available will be obtained, whenever possible, for those engaged in specific research with potential exposure to the agent in question. Please contact your PI for further information on vaccines available.

Emergency Response

HSC requires that significant 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 biohazardous material/agents, recombinant or synthetic DNA, and/or transgenic animals.

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 Occupational Health Department.
- If ocular and/or mucosal exposure is suspected, rinse the area for no less than 15 minutes, and seek an immediate medical consultation from Occupational HealthDepartment.
- 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. EH&S and Occupational Health must be notified within 24 hours of the event.

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 spillremediation.
- 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 otherapplications.

  - Spills greater than 100mls and/or involves the grills or plenum spaces. Contact EH&S to discuss and implement the appropriate decontaminationprocess.

- Spills occurring outside of primary containment equipment (e.g., BSC):
  - *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.

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:

- 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 BSL2+ agents, EH&S must be notified and will assume responsibility, in conjunction with the PI, to clear the laboratory forre-entry.

Spills involving blood products

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

- 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.
- Using a detergent solution, clean the spill site of all visibleblood.
- Spray the spill site with a 10% bleach solution (made fresh) and allow contact for 15 minutes.
- Wipe the area down with disinfectant-soaked paper towels.
- Discard all disposable materials used to decontaminate the spill and any contaminated PPE into a biohazard bag.
- Wash area with soap and water followed by 70% Ethanol.

Spill of a Biohazardous Radioactive Material

A biohazardous spill involving radioactive material requires emergency procedures that are different from the procedures used for either material alone. Use procedures that protect you from the radiochemical while you disinfect the biological material. Before any clean up, consider the type of radioisotope, characteristics of the microorganism, and the volume of the spill. Contact the Radiation Safety Office (817-735-5431) for isotope clean- up procedures.

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post a warning sign.
- Remove contaminated clothing, turning exposed areas inward, 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 disinfectant, following it with a three-minute water rinse.

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 fordisposal.

- Staff **must** thoroughly wash their hands, wrist, and forearms following PPE removal.

Investigation of Laboratory Accidents

EH&S, in cooperation with the PI and his/her 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.

Infectious Material Incidents (Including recombinant DeoxyriboNucleic Acid (rDNA) and Infected Animals)

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 UNTHSC PD or Safety Office, or from non- campus agencies such as the Fort Worth Fire Department, is to be reported immediately to UNTHSC PD (817-735-2600).
Reports should provide the dispatcher with the following information:

- Where and what type 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 he/she is calling and who and where someone will meet response personnel upon their arrival.

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.