UNIVERSITY OF TEXAS ARLINGTON | ENVIRONMENTAL HEALTH & SAFETY

Biosafety Manual

THE U N I V E R S I T Y OF TEXAS AT ARLINGTON

Biosafety Manual

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Culture of Safety

"No regulation or guideline can ensure safe practices. Individual and organizational attitudes regarding safety will influence all aspects of safe practice, including willingness to report concerns, response to incidents, and communication of risk. Each organization should strive to develop a culture of safety that is open and nonpunitive, encourages questions, and is willing to be self-critical. Persons and organizations must be committed to safety, be aware of risks, behave in ways that enhance safety, and be adaptable. Scientists understand that practices should be refined as observations are made, hypotheses tested, findings published, and technical progress achieved. The same holds true for safety in the laboratory, which should evolve as experience is gained and as laboratory activities change. As laboratorians gain more knowledge over time concerning how to recognize and control hazards, the level of risk that is considered acceptable should become smaller, with the goal of moving continuously to eliminate or reduce risk to the lowest reasonably acceptable level."

Citation: Centers for Disease Control and Prevention. Guidelines for Biosafety Laboratory Competency. MMWR 2011; 60(Suppl.):3-4.

Contents

1 FOREWORD

The University of Texas at Arlington (UTA) Biosafety Manual has been prepared by the Environmental Health and Safety Office (EH&S) as a guideline for biological research at UTA. The manual provides a core set of biosafety practices and procedures for the safe handling of known biohazards and potentially infectious materials. Laboratories that work with microorganisms, technologies involving recombinant or synthetic nucleic acid molecules, laboratory animals, toxins of biological origin, venoms, human blood, other potentially infectious materials (OPIM) including human cell lines/unfixed tissue, etc., or bloodborne pathogens are special and often require unique work environments. These laboratories must be managed to reduce the potential for personnel exposure and environmental release. Waste generated from these laboratories that biohazards are worked with must also be handled uniquely.

In general, the handling and manipulation of biological agents and toxins, as well as recombinant or synthetic nucleic acid molecules, requires the use of various precautionary measures depending on the material(s) involved. The UTA Biosafety Manual will provide assistance in the evaluation, containment, and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary. EH&S is available to assist in this endeavor.

The UTA Biosafety Manual focuses on biosafety levels (BSL) 1 and 2 since no work with BSL-3 or -4 agents is conducted at UTA at the moment. There are currently no facilities for this type of research. The manual provides university-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. It also provides information about registration and training along with details on work practices, safety equipment and facility design. It is the responsibility of the Principal Investigator (PI) to ensure that his/her laboratory is in compliance. That responsibility includes identification of the risks or hazards associated with their research or laboratory work and the application of appropriate safety procedures. The UTA Biosafety Manual can be used as a guide to reach compliance within the laboratory. The PIs should consult the sections relevant to their research/laboratory work and apply the appropriate safety procedures. Although the implementation of these procedures is the responsibility of the PI, a successful Biosafety Program depends largely on the combined efforts of all members of the laboratory. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used.

The EH&S Biological Safety Specialist can be reached at 817-272-2185 for consultation, or to address questions or concerns with any aspect of the Biosafety Program.

2 SCOPE

The UTA Biosafety Manual applies to all laboratory personnel, volunteers, or other individuals working at or on the premises of all UTA-operated facilities.

Biohazards include infectious or etiologic (disease causing) agents of humans, animals and plants, toxins of biological origin, human-derived materials, recombinant/synthetic nucleic acid molecules, and any materials potentially containing infectious agents or biohazards. Biohazardous agents may include, but are not limited to: certain bacteria, fungi, viruses, rickettsiae, *Chlamydia* bacteria, parasites, recombinant/synthetic products, allergens, cultured human cells and the potentially infectious agents these cells may contain, viroids, prions and other infectious agents as outlined in laws, regulations, or guidelines.

The purpose of the UTA Biosafety Manual is to give guidance how to:

- protect personnel from exposure to infectious agents or other viable biological materials that may cause harm to laboratory personnel themselves or others after secondary transmission;

- protect all employees and others not employed by UTA who may be on the premises or in proximity of biohazards;
- prevent waste from contaminating the environment;
- provide an environment for high quality research and teaching while maintaining a safe work place; and
- comply with applicable federal, state, and local guidelines and requirements.

3 RULES, REGULATIONS, AND GUIDELINES

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents, and recombinant/synthetic nucleic acid molecules:

Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH): Biosafety in Microbiological and Biomedical Laboratories (BMBL)

In 1984, the CDC/NIH published the first edition of the BMBL. This document describes combinations of standards and special microbiological practices, safety equipment, and facilities that constitute BSL-1-4, which are recommended for working with a variety of infectious agents in different laboratory settings. This document also outlines requirements for animal biosafety levels. The BMBL has been revised several times (last revision was done December 2009) and is commonly seen as the standard for biosafety. EH&S has used the BMBL as the basis for the UTA Biosafety Manual.

Centers for Disease Control and Prevention (CDC) Website

National Institute of Health (NIH): Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

These guidelines address the safe conduct of research that involves construction and handling of recombinant/synthetic nucleic acid molecules and organisms containing them. In 1974, the rDNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. As a result of the committee's activity, the initial version of the NIH Guidelines was published in 1976, and it has been amended and revised many times since then. Included in the NIH Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed research using the NIH Guidelines as a minimum standard. For more information, please refer to the section 5.2.3.3 of this manual: Recombinant/Synthetic Nucleic Acid Molecules Research/Experiments at UTA, the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), and the Regulatory Services website about research involving rDNA molecules.

Occupational Safety and Health Administration (OSHA): Bloodborne Pathogens Standard 29 CFR 1910.1030

In 1991, OSHA promulgated a rule to deal with the occupational health risks caused by exposure to human blood and OPIM. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions. EH&S at UTA has established an Exposure Control Plan to protect employees at UTA from exposure to human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens. For more information, please refer to the UTA Exposure Control Plan for Bloodborne Pathogens.

United States Department of Agriculture (USDA): Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins.

The USDA has established a set of rules that require facilities and institutions to be registered and approved in order to transfer or receive certain biological agents and toxins. The most current list of restricted agents and toxins covered under this rule: Health and Human Services (HHS)/USDA Select Agents and Toxins List.

Texas Commission on Environmental Quality (TCEQ) Regulatory Guidance

TCEQ regulations on medical waste covers rules for handling, treatment, and proper disposal of sharps, samples, and other wastes of concern from health care related facilities and similar settings. Medical waste may be treated on-site, or off-site at an authorized treatment facility, following the requirements of Title 30 Texas Administrative Code (30 TAC), Chapter 330, Section (§) 330.3 and explained further in 25 TAC, Part 1, Chapter 1, Subchapter K, §1.132 . The Medical Waste Regulations in Texas define how producing facilities (generators of medical waste) must handle the waste from the point at which it becomes medical waste, to the point of its ultimate disposal. UTA's compliance with TCEQ Regulatory Guidance is outlined in the Biological (or Special) Waste section 8 of this manual.

Texas Commission on Environmental Quality (TCEQ) Website.

Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens, biological products and genetically modified organisms are addressed in the following rules and guidelines:

United Nations
Recommendations of the Committee of Experts on the Transportation of Dangerous Goods

International Civil Aviation Organization (ICAO)
Technical Instructions for the Safe Transport of Dangerous Goods by Air

International Air Transport Association (IATA)
Dangerous Goods Regulations (DGR)

U.S. Department of Transportation (DOT)
Code of Federal Regulations, Title 49 CFR, Parts 171-180

Department of Health and Human Services 42 CFR Parts 72 and 73 Office of Inspector General 42 CFR Part 1003 Possession, Use, and Transfer of Select Agents and Toxins; Final Rule

Importation permits are required for certain infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine. In addition, the USDA Animal and Plant Health Inspection Service (APHIS), 7 CFR Part 340, requires permits for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms.

4 DEFINITIONS / ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
Aerosols	Colloids of liquid or solid particles suspended in gas.
Aerosol, Transmitted	Particles or droplets of biologically active agents or microorganisms that are transmitted in nature by dissemination into the ambient air with subsequent deposition upon receptive areas of the body exposed to the ambient air (mucous membranes, lungs, eye conjunctiva, cut or abraded skin).
Animal Biosafety Level (ABSL)	Laboratory practices, techniques, safety equipment and laboratory facilities appropriate for the operations performed and the hazards posed by the particular biohazard material when working in animal research facilities. The NIH and the CDC define four levels of animal biosafety in the U.S. Department of Health and Human Services Publication, Biosafety in Microbiological and Biomedical Laboratories, 2009. This publication recommends animal biosafety levels for work with particular microorganisms.
ANSI	American National Standards Institute
APHIS	Animal and Plant Health Inspection Services
Autoclave	Device designed to sterilize equipment, fluids, or solid materials by means of heat, steam, and pressure within a chamber.
Biohazardous Agent	Biologically active particle or particles, usually microorganisms, capable of causing disease, illness or injury in or to humans, animals, plants, or the ecosystem.
Biohazardous Material	Substance that harbors biohazardous agents, including human or animal blood, body fluids, or cells/tissue that may be contaminated with biologically active agents.
Biological Barrier	Impediment (naturally occurring or artificially contrived) to the infection, transmission and/or survival of a biohazardous agent.
Biosafety Cabinet (BSC)	Ventilated cabinet, which serves as a primary containment device for operations involving biohazardous materials. The Class II vertical laminar flow BSC is a ventilated cabinet with an air barrier curtain at its open-front sash. This type of cabinet offers product protection, user protection, and environmental protection when properly operated. Exhaust air from the workspace is filtered with a high efficiency particulate air (HEPA)/ultra-low particulate air (ULPA) filter, prior to discharge. The cabinet provides a sterile working environment by also supplying HEPA/ULPA filtered downward airflow within the workspace. Class II BSCs are further classified as type A1, A2, B1, B2, and C1.
BMBL	Biosafety in Microbiological and Biomedical Laboratories
Biosafety Level (BSL)	Laboratory practices, techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazards posed by the particular biohazard material. The NIH and the CDC define four levels of biosafety in the U.S. Department of Health and Human Services Publication, Biosafety in Microbiological and Biomedical Laboratories, 2009. This publication recommends biosafety levels for work with particular microorganisms.
Blood	Used here to mean human blood, blood components, viable tissues, body fluids, viable cells, and other products made from viable materials that may contain blood.

Bloodborne Pathogens (BBP)	Pathogenic microorganisms that are present in human or non-human primate blood or other potentially infectious materials (OPIM) and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and the human immunodeficiency virus (HIV).
CDC	Centers for Disease Control and Prevention
CEMS	Chemical Environmental Management System
Certification	Procedure by which BSC meets national standards of physical testing which include but is not limited to air balancing, filter integrity, velocity measurements, and electrical grounding.
CFR	Code of Federal Regulations
Containment	Confinement of a biohazardous agent that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include physical and biological barriers and inactivation using physical or chemical means.
Contamination	Presence or reasonably anticipated presence of microorganisms or other potentially infectious materials in or on a surface.
Contaminated Sharps	Contaminated object or device having rigid corners, edges, or protuberances capable of cutting or piercing skin including, but not limited to all of the following: hypodermic needles, blades, pipettes, and broken glass or sharp-edged plastic items contaminated with microorganisms or other potentially infectious materials.
Decontamination	The reduction of microorganisms on a surface or item to an acceptable level by the use of physical or chemical means so that the organisms are no longer capable of transmitting infectious particles and the surface or item is rendered safe for routine handling, use, or disposal.
DGR	Dangerous Goods Regulations
DMSO	Dimethyl sulfoxide
Disinfection	Process by which viable microorganisms are reduced to a level which is unlikely to cause disease in healthy people, plants, or animals.
DNA	Deoxyribonucleic acid
DURC	Dual Use Research of Concern
Engineering Controls	Mechanical equipment, facility characteristics, or other physical controls that isolate or remove the hazards from the workplace.
EH&S	Environmental Health and Safety Office
EPA	Environmental Protection Agency
ERA	Electronic Research Administration
Ethidium Bromide (EtBr)	Ethidium bromide is the most commonly used stain for detecting DNA/RNA in molecular biology laboratories.
Exhaust	Withdrawal of air from a cabinet or room by means of a motor/blower system; that portion of air volume which is designed to be discharged from a cabinet or a room to a separate air system or to the outside of a building.
Exposure Incident	Unanticipated, specific eye, mouth, mucous membrane, non-intact skin, inhalation, or parenteral contact with blood, other potentially infectious materials, or microorganisms during the course of an employee's duties.
FDA	U.S. Food and Drug Administration

FWA	Federal Wide Assurance
GMOs	Genetically modified organisms
The Guide	National Research Council's Guide for the Care and Use of Laboratory Animals
Hand Washing Facility	Facility providing an adequate supply of running potable water, soap, and single use towels or hot air drying machines.
HBV	Hepatitis B virus
High Efficiency Particulate Air (HEPA) Filter	Disposable, extended, pleated, dry filter which has rigid casing enclosing the full depth of the pleat. Minimum particulate removal is 99.97% for particles with a diameter of 0.3 μm.
HHS	US Department of Health and Human Services
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
HVAC	Heating, ventilation, and air-conditioning
IATA	International Air Transport Association
ICAO	International Civil Aviation Organization
Inactivation	Process that destroys the ability of a specific biohazardous agent to self-replicate.
Institutional Animal Care and Use Committee (IACUC)	At the University of Texas at Arlington (UTA), the Provost appoints members of the Committee and the Vice President (VP) for Research has ultimate responsibility for all animal use by UTA. Functionally, the IACUC reports to the VP for Research. The IACUC advises the VP for Research on issues related to animal care and use and makes recommendations for change in the program or facilities.
Institutional Biosafety Committee (IBC)	In accordance with the NIH Guidelines, UTA has established an Institutional Biosafety Committee (IBC) responsible for the review of all research involving recombinant or synthetic nucleic acid molecules. The IBC is a university-wide standing committee advisory to the VP for Research. On behalf of the Institution, and in accordance with the NIH Guidelines, the IBC is responsible for oversight and review of recombinant/synthetic nucleic acid molecules research including independent assessment of: containment levels, laboratory facilities, procedures, practices, health surveillance, and training and expertise of personnel involved in the recombinant/synthetic nucleic acid molecules research.
IMDG Code	The International Maritime Dangerous Goods Code
IRB	Institutional Review Board
IODURC	The United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern
LAI(s)	Laboratory-associated infection(s)
Laminar Airflow	Unidirectional airflow through the work area often referred to as turbulence-free airflow.
MMWR	Morbidity and Mortality Weekly Report
NaOCl	Sodium hypochlorite
NIH	National Institutes of Health
NSF	NSF International

Occupational Exposure	Reasonably anticipated eye, mouth, mucous membrane, non-intact skin, inhalation, or parenteral contact with blood, other potentially infectious materials, or microorganisms that could result from the performance of an employee's duties, if measures are not taken to prevent it.
Office of Biotechnology Activities (OBA)	Office within the National Institutes of Health that is responsible for reviewing experimental activities related to recombinant/synthetic nucleic acid molecules.
OHRP	Office of Human Research Protection
OSHA	Occupational Safety and Health Administration
Other Potentially Infectious Materials (OPIM)	Human or non-human primate body fluids: seminal and vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, other body fluids that are visibly contaminated with blood such as saliva or vomit. The definition includes all body fluids, viable research and clinical samples where it is difficult or impossible to determine if the material is contaminated with blood or blood components. Any unfixed or viable human tissue or organ from a human or non-human primate (living or dead). Cells or tissue culture, organ cultures, and culture medium or other solutions, blood, organs, or other tissues that are not known to not contain human or non-human primate bloodborne pathogens. Any viable (unfixed) material within *in vitro* (tubes or flasks) or *in vivo* (animals) experimental systems that have been deliberately infected with bloodborne pathogens from human or non-human primates (including HIV and HBV).
Parenteral	Descriptive of piercing mucous membranes or the skin barrier through events such as needle sticks, human bites, cuts, and abrasions.
Pathogen	Agent containing sufficient genetic information, which upon expression of such information is capable of producing disease in healthy people, plants or animals.
Personal Protective Equipment (PPE)	Specialized clothing or equipment worn by a laboratory worker for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) are not intended to function as protection against a hazard and are not considered to be personal protective equipment.
pH	A measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity. The pH scale commonly in use ranges from 0 to 14.
Physical Barrier	Equipment, facility, device, or physical condition that is designed to achieve containment or exclusion of biohazards.
Plenum	Enclosure for flowing gases inside a biosafety cabinet in which the static pressure at all points is relatively uniform.
PI	Principal Investigator
psi	Pounds per square inch
Quats	Quaternary Ammonium Compounds
Recombinant DNA (rDNA)	Molecules constructed outside living cells by joining natural or synthetic deoxyribonucleic acid (DNA) segments to DNA molecules that can replicate in a living cell; or molecules that result from the replication of those described in above.

Recombinant DNA Advisory Committee (RAC)	Public advisory committee that advises the Director of the NIH on recombinant or synthetic nucleic acid molecules matters. Renamed later the Novel and Exceptional Technology and Research Advisory Committee (NExTRAC)
Regulated Waste	Liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; pathological and microbiological wastes containing blood or other potentially infectious materials.
Release	Discharge of a biohazardous agent from a containment system; can be incidental or accidental.
Risk Level	Classification scheme established by federal and world governmental bodies to rate the relative risks associated with exposure to specific biological agents and microorganisms.
RNA	Ribonucleic acid
SDS	Safety Data Sheet
SOP(s)	Standard Operating Procedure(s)
Sterilize	Use of a physical or chemical procedure to destroy all microbial life including highly resistant spores.
TAC	Texas Administrative Code
TB	Tuberculosis or TB (short for *tubercle bacillus*) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*. Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have an active *Mycobacterium tuberculosis* infection cough, sneeze, or otherwise transmit their saliva through the air.
TDG	Transport of Dangerous Goods Regulations
TDSHS	Texas Department of State Health Services
ULPA	Ultra Low Particulate Air (filter). An ULPA filter can remove from the air at least 99.999% of dust, pollen, mold, bacteria and any airborne particles with a size of 100 nanometers (0.1 μm) or larger.
Ultraviolet (UV) Light	An invisible band of radiation at the upper end of the visible light spectrum. With wavelengths from 10 to 400 nm, ultraviolet (UV) starts at the end of visible light and ends at the beginning of X-rays. The primary source of ultraviolet light is the sun, and most of the UV that reaches earth is in the lower-frequency, longer-wavelength ultraviolet region.
Universal Precautions	Safe work practice controls for infection control. All human and non-human primate blood, OPIM, and cells/tissue are to be treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.
UTA	The University of Texas at Arlington
UVGI	Ultraviolet germicidal irradiation (UVGI) is a disinfection method that uses UV light at sufficiently short wavelength to kill microorganisms.
VBM	Valuable biological materials
VP for Research	Vice President for Research
WHO	World Health Organization

Work Practice Controls	Procedures and practices that reduce the likelihood of exposure by altering the manner in which a task is performed (e.g., prohibiting recapping of needles by a two-handed method).
Zoonotic	Ability of a natural pathogen of an animal species or a genetically engineered natural pathogen of that species to migrate outside of its species of origin and move across a human somatic cell membrane.
Zoonotic Disease	Disease that naturally infects animals that is pathogenic for humans.

5 GENERAL PRINCIPLES OF BIOSAFETY

5.1 INTRODUCTION

Since its publication in 1984, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) has become the cornerstone of biosafety practice in the United States. The principles of biosafety introduced in the first edition of BMBL and carried through in the fifth edition (2007; revised 2009) address the safe handling and containment of infectious microorganisms and hazardous biological materials. These principles are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections (LAIs).

Work with infectious agents has expanded and PIs are compelled to evaluate and ensure the effectiveness of their biosafety programs, the proficiency of their laboratory workers, as well as the capability of equipment, facilities, and management practices to provide containment and security of microbiological agents. Similarly, individual workers/students who handle pathogenic microorganisms must understand the containment conditions under which infectious agents can be safely manipulated and secured. The use of vaccines may provide an increased level of personal protection. Application of all this knowledge and the use of appropriate techniques and equipment will enable the microbiological and biomedical community to prevent personal, laboratory, and environmental exposure to potentially infectious agents or other biohazards.

The handling and manipulation of infectious biological agents (bacterial/fungal/parasitic/rickettsial/viral agents, arboviruses and zoonotic viruses, prions, biological toxins, recombinant/synthetic nucleic acid molecules, and other viable materials) requires the use of precautionary measures. This manual provides assistance in the evaluation, containment, and control of biohazards associated with safety planning and concerns related to the safe use and handling of biohazardous agents. Everyone working with these materials at or on the premises of UTA facilities should become familiar with this manual and is encouraged to seek additional advice or training when necessary.

5.2 BIOLOGICAL RISK ASSESSMENT

Biological risk assessment that can very well be called the backbone of biosafety is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection. Biological risk assessment is an important responsibility of PIs of microbiological

and biomedical laboratories. The IBC, the Institutional Animal Care and Use Committee (IACUC), biological safety professionals, and laboratory animal veterinarians share this responsibility.

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

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

While there are many tools available to assist in the assessment of risk for a given procedure or experiment, the most important component is professional judgment. Risk assessments should be performed by the individual(s) most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available. The PI is responsible for ensuring that adequate risk assessments are performed, and for working closely with the IBC, the IACUC, and EH&S personnel to ensure that appropriate equipment and facilities are available to support the work under consideration. Risk assessment should be reviewed routinely and revised when necessary, taking into consideration the acquisition of new data and other relevant new information from the scientific literature.

5.2.1 RISK GROUPS

One of the most helpful tools available for performing a microbiological risk assessment is the listing of risk groups for microbiological agents. The World Health Organization (WHO) and the NIH have classified human etiological agents into four risk groups (RG) based on hazards to both the individual and the community. The higher the risk group level, the more requirements there are for containment devices and containment practices (containment barriers) when handling the material in question. The four risk groups correlate to but are not equivalent to biosafety levels. Determining the risk group of a biological agent can be part of the biological risk assessment and helps in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BSL-2. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions.

RG classification takes into account characteristics of the microorganism and its potential to do harm to health care workers, the public health of the nation, the environment, the national economy, or the agriculture products of the country. In some cases where the RG level has not been established, individual researchers and peer reviewers are required to establish risk groups associated with the biological materials that are handled at UTA. The following are the criteria for classifying agents into four risk groups:

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

The risk group level should be assessed first without consideration of containment barriers. Once the risk group level is agreed upon, natural and contrived biological barriers should be considered and physical containment barriers should be devised to ensure a reasonable degree of protection appropriate to the risk group level.

There are, however, other factors that need to be considered in addition to RG for a particular agent when conducting a biological risk assessment. These include:

- Agent's biological and physical nature
- Capability to infect and cause disease in a susceptible human or animal host (pathogenicity of the agent)
- Infective dose
- Virulence as measured by the severity of disease
- Agent's endemic nature
- Origin of the agent (non-indigenous agents are of special concern because of their potential to introduce risk of transmission or spread of human and animal infectious diseases from foreign countries into the United States)
- Natural route of infection
- Probable other routes of transmission resulting from laboratory manipulations (laboratory activity planned: sonication, aerosolization, centrifugation, etc.)
- Routes of infection resulting from laboratory manipulations (parenteral, airborne, ingestion)
- Concentration of the agent and volume of concentrated material to be manipulated
- Stability in the environment
- Availability of preventive measures and effective treatments for the disease
- Information available from animal studies and reports of LAIs or clinical reports
- Potential outcome of exposure
- Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment

The agent summary statements contained in BMBL identify the primary agent and procedure hazards for specific pathogens and recommended precautions for their control.

On the basis of the information ascertained during the risk assessment, a biosafety level can be assigned to the planned work, appropriate personal protective equipment (PPE) selected, and standard operating procedures (SOPs) developed to ensure the safest possible work conditions.

5.2.2 AGENTS FOR WHICH THERE IS LIMITED INFORMATION

The risk assessment procedure described above works well when there is adequate information available. However, there are situations when the information is insufficient to perform an appropriate risk assessment. In these cases, it is prudent to take a cautious approach to agent manipulation:

- Standard precautions should always be followed, and barrier protections applied (gloves, gowns, eye protection)
- Basic containment – BSL-2 practices and procedures should be the minimum requirement for handling the agent
- Transport of agent(s) needs to follow national and/or international rules and regulations

5.2.3 RISK ASSESSMENT AND RECOMBINANT/SYNTHETIC NUCLEIC ACID MOLECULE TECHNOLOGY

Recombinant/synthetic nucleic acid molecule technology or genetic engineering involves combining genetic material from different sources thereby creating genetically modified organisms (GMOs) that may have never existed in nature before. When conducting a risk assessment of GMOs, consideration of the same factors used in risk assessment of the wild-type organism should be done. However, it is important to address the possibility that the genetic modification could alter (i.e., increase or decrease) the pathogenicity of the agent or affect its susceptibility to antibiotics or other treatments. Sometimes, important information may not be available for newly engineered organisms and the risk assessment may be difficult or incomplete. In these cases, due diligence should be practiced and the biosafety level assignment should be made conservatively. Once more information is available, another risk assessment should be completed.

Research experience has demonstrated that genetic engineering may be conducted in a safe manner when an appropriate risk assessment has been performed and adequate safety measures are used. Whether the recombinant/synthetic nucleic acid molecule technology is used to clone DNA/RNA segments in bacterial hosts or to create GMOs such as transgenic and "knock-out" animals and transgenic plants, the following should be taken into consideration in a biological risk assessment:

- The pathogenic properties and any potential hazards associated with such organisms may be novel and not well-characterized
- The properties of the donor organism
- The nature of the DNA/RNA sequences that will be transferred
- The properties of the recipient organism
- The properties of the environment

These factors should help determine the BSL that is required for the safe handling of the resulting GMO, and identify the biological and physical containment systems that should be used.

5.2.3.1 TRANSGENIC AND "KNOCK-OUT" ANIMALS

Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes ("knock-out" animals) do not generally present particular biological hazards.

5.2.3.2 TRANSGENIC PLANTS

A risk assessment should determine the appropriate BSL for the production of transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects.

5.2.3.3 RECOMBINANT/ SYNTHETIC NUCLEIC ACID MOLECULE RESEARCH/EXPERIMENTS AT UTA

In the past several years, recombinant/synthetic nucleic acid molecules have become widely used in many fields of research.

In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:

(i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;

(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
(iii) molecules that result from the replication of those described in (i) or (ii) above.

Synthetic nucleic acid molecule segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural nucleic acid molecule counterpart. Genomic DNA/RNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant nucleic acid molecule.

The NIH has established regulations on the use and containment of recombinant/synthetic nucleic acid molecules in the laboratory. Regulations require persons conducting such research to file a registration form with the IBC which must approve the protocols related to recombinant/synthetic nucleic acid molecules.

UTA complies with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, effective April 2019. In addition, UTA follows the guidance found in BMBL, CDC/NIH, Fifth Edition, revised December 2009. All projects involving recombinant/synthetic nucleic acid molecules, regardless of funding, must be reviewed by the UTA IBC prior to initiation. The PI must submit a Protocol Application for Research Involving Recombinant or Synthetic Nucleic Acid Molecules to the IBC. This can be found on the Regulatory Services website.

Responsibilities of the PI **prior** to initiating recombinant/synthetic nucleic acid molecules research are:

- Make available to all laboratory staff the protocols that describe the potential hazards and the precautions to be taken
- Instruct and train laboratory staff in the practices and techniques required to ensure safety and procedures for dealing with accidents
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested

Responsibilities of the PI **during the conduct** of recombinant/synthetic nucleic acid molecules research are:

- Supervise the safety performance of the laboratory personnel to ensure that the required safety practices and techniques are employed
- Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to appropriate authorities
- Correct work errors and conditions that may result in the release of recombinant/synthetic nucleic acid molecules
- Ensure the integrity of the physical containment that is achieved through the use of laboratory practices, containment equipment (e.g., biosafety cabinets), and special laboratory design. Emphasis needs to be placed on **primary means** of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a **secondary means** of protection against the accidental release of organisms outside the laboratory or to the environment.

The PI is responsible for full compliance with the NIH Guidelines in the conduct of recombinant/synthetic nucleic acid molecules research. Please refer to the most recent edition of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules for more information.

5.2.4 HAZARDS OF TISSUE CULTURE / CELL LINES

Human cells and tissues have the potential to harbor latent infectious agents and personnel who handle these materials are at risk for possible exposure. Research laboratories where work involves handling human tissue/cells shall have card-reader(s) to limit access to only those who have completed the appropriate requirements. For additional information and requirements for working with human cell cultures, please refer to UTA Exposure Control Plan for Bloodborne Pathogens Manual.

5.2.5 BIOSAFETY CONSIDERATIONS FOR BIOLOGICAL EXPRESSION SYSTEMS

Biological expression systems that consist of vectors and host cells can many times be used safely in experiments at BSL-1, provided the inserted foreign DNA/RNA expression products do not require higher biosafety levels. Higher biosafety levels may, however, be required when:

- The expression of DNA/RNA sequences derived from pathogenic organisms may increase the virulence of the GMO
- Inserted DNA/RNA sequences are not well characterized
- Gene products have potential pharmacological activity
- Gene products code for toxins

5.2.6 VIRAL VECTORS FOR GENE TRANSFER

Viral vectors that are used for the transfer of genes to other cells lack certain virus replication genes and are propagated in cell lines that complement the defect. Stocks of such vectors may be contaminated with replication-competent viruses, generated by rare spontaneous recombination events in the propagating cell lines, or may derive from insufficient purification. These vectors should thus be handled at the same BSL as the parent virus from which they are derived.

Common viral vector systems include retroviruses, lentiviruses, adenoviruses, poxviruses, herpesviruses, alphaviruses, and baculoviruses. The following viruses are examples of risk group 1 agents: Retroviruses, baculoviruses. Adenoviruses, poxviruses, herpesviruses are examples of risk group 2 agents.

5.2.7 RISK ASSESSMENT FOR AGRICULTURAL RESEARCH

Risk assessment for agriculture (plant and animal) infectious disease research has different criteria than that for human and/or zoonotic infectious disease research. Risk management strategies for work with agricultural pathogens must focus on biocontainment and environmental protection in addition to laboratory personnel protection. For most agriculture pathogens, the U.S. CDC/NIH publication BMBL can be used to establish standards for an appropriate level of biocontainment.

5.2.7.1 CONTAINMENT FOR PLANTS AND PLANT PESTS

Plants and plant pests rarely infect or infest healthy humans and therefore pose little direct risk to lab personnel. Some, however, can pose a significant threat to agricultural production, forests or natural ecosystems. As a result it is important that personnel working with plants and plant pests or facilities housing these organisms take steps to prevent the accidental escape of potentially damaging plants or plant pests into the environment. The containment requirements for a particular plant or plant pest are often project-specific and are determined after assessing the risk factors associated with the biology of the plant or plant pest and the impact an escape might have.

5.2.8 GUIDELINES REGARDING SELECT AGENTS AND TOXINS

The purpose of the CDC's Select Agents regulation (42CFR72) is to provide means of accountability for the use of select agents/biological agents that are considered by the federal government as potential bio-terrorist threats. The HHS and USDA published final rules for the possession, use, and transfer of select agents and toxins in the Federal Register on March 18, 2005. All provisions of these final rules supersede those contained in the interim final rules and became effective on April 18, 2005.

Select agents and toxins have been determined by the HHS and USDA to have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products. The Federal Select Agent Program is jointly comprised of the CDC/Division of Select Agents and Toxins and the Animal and Plant Health Inspection Service (APHIS)/Agriculture Select Agent Services. The Federal Select Agent Program regulates the possession, use, and transfer of 67 specific biological agents and toxins (Select Agents and Toxins List).

5.2.8.1 REGISTRATION OF SELECT AGENT AND TOXIN POSSESSION

All individuals who possess select agents/toxins must register with the CDC and/or APHIS. The registration process is rigorous and includes many provisions such as:

- Description of research space including heating, ventilation, and air-conditioning (HVAC) details, safety equipment and security features
- Research summary outlining use of agent/toxin
- Agent-specific safety and biocontainment procedures
- Safety and technical training of lab personnel
- Security and emergency response plans
- Security risk assessment, including U.S. Attorney General background check of personnel with access to agent

A facility inspection may or may not be required prior to registration, depending on documentation supplied by the applicant. Once the registration document is prepared and submitted to the appropriate federal authorities, the turnaround time for approval is expected to be at least two months. If CDC approves the registration, a unique registration number will be issued. Those facilities not pre-inspected will be inspected following registration. All registered facilities will be inspected subsequently on a periodic basis. For new registrations, the agent/toxin cannot be transferred to research facilities until approval is granted by the CDC and/or APHIS.

Currently, there are no research projects on the UTA campus utilizing select agents or toxins. Use of these materials will require involvement and approval by UTA Office of Research Administration. If one is considering using any of the agents or toxins as described by the CDC and/or USDA, please notify the Office of Research Administration.

5.2.8.2 CONSIDERATIONS FOR UTA DEPARTMENTS

It is critical for departments to identify any potential for use or possession of select agents/toxins by research personnel in order to protect both the University and the researcher from unknowingly violating a regulatory requirement that bears both civil and criminal penalties. The following actions can be taken to prevent this from happening:

- Screen all research materials received in order to assure that no items on the Select Agents and Toxins List have inadvertently been sent to campus. This is especially true for items received from foreign countries because the select agent regulations apply to the United States. International colleagues may not be aware of these restrictions.
- Query all visiting research personnel, or newly recruited faculty before they come to campus to assure that they are not planning to bring any materials that are restricted under the select agents/toxins regulations. Again, international colleagues may not be aware of these restrictions.
- If any researcher plans to pursue grant money for research involving select agents/toxins, in order to plan for this potential work, research personnel need to be aware of the scope of regulatory requirements and limitations associated with this type of work.

5.2.8.3 GUIDELINES FOR HANDLING EXEMPT STRAINS OF SELECT AGENTS / THE USE OF EXEMPT LEVELS OF SELECT AGENT TOXINS

The HHS and the USDA regulations for the possession, use and transfer of select agents and toxins have also established a procedure by which an attenuated strain of a select agent that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the regulations when used for specific purposes. It should be noted, however, that if an excluded attenuated strain is manipulated in such a way that virulence is restored or enhanced, or if factors associated with virulence are reintroduced, it will then be subject to the regulations.

Several toxins that appear on the Select Agents and Toxins List may be used in reduced quantities without completing the rigorous registration. Following is a current list of the Select Toxins and the permissible toxin amounts that are allowed to be possessed by each PI in order to remain exempt from federal registration.

Permissible Toxin Amounts per PI for exemption	**Amount**
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

5.2.8.3.1 SECURITY REQUIREMENTS FOR HANDLING EXEMPT STRAINS OF SELECT AGENTS

Specific security measures are not only appropriate, but prudent practice for handling exempt strains of select agents. An accurate and up-to-date inventory must be maintained and the following information included in the inventory:

- Date of use
- Name of person using the materials

- Beginning amount of material
- Amount of material used for procedure
- End amount of material
- Procedure the material was used for

All exempt strains of select agents (stock solutions, working solutions, etc.) must be stored in a lockable storage unit. Storage units that house exempt strains of select agents must be kept locked when not actively being used, and only those people approved by the PI may have access to the strains. Any inconsistencies in the inventory must be investigated.

5.2.8.4 DUAL USE RESEARCH OF CONCERN

Dual Use Research of Concern (DURC) is a subset of dual use research defined as life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security.

The United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (IODURC) articulates the practices and procedures required to ensure that dual use research of concern is identified at the institutional level and risk mitigation measures are implemented as necessary.

It is the responsibility of the PI to identify his or her research involving one or more of the agents or toxins listed in IODURC Section 6.2.1 and notify EH&S of that research to be reviewed for its DURC potential.

5.3 HIGH RISK INDIVIDUALS / PRENATAL CONSIDERATIONS

The PI must determine special hazards and exceptions and he/she is primarily responsible for establishing the safety of personnel under his/her supervision. Persons who are immunocompromised or otherwise particularly susceptible to infection need to be identified so that additional precautions for microbiological safety can be taken when necessary. It may be inadvisable for a person in an immunocompromised condition to work with microorganisms. This includes individuals under systemic corticosteroid therapy, chemotherapy for malignancies, radiation therapy, and those who have certain diseases (e.g., lymphomas, leukemia, and Acquired Immunodeficiency Syndrome, AIDS) which induce severe impairment of immune competence. One should seek medical advice regarding possible work restrictions. Persons who have a medical condition that they feel might be compromised by exposure to reagents or cultures in the laboratory are encouraged to discuss the matter with their PI.

Additionally, certain microbes such as *Toxoplasma gondii*, rubella virus, cytomegalovirus, and vesicular stomatitis virus pose a hazard to pregnant women who should carefully evaluate the risk of working with or near these agents. Toxoplasmosis is a disease acquired from cats that if acquired by a pregnant woman during pregnancy can cause birth defects and other disorders in a fetus. Pregnant women are also known to be at high risk of infection by *Listeria monocytogenes*. Therefore, for her own safety, any female student or staff member who is or thinks that she may be pregnant should discuss the matter with the PI prior to commencing work with *L. monocytogenes.*

Female personnel who are pregnant or become pregnant while involved in the animal care and use program may need to take certain precautions during the time of pregnancy due to the risks associated with animals, biohazardous materials, radiation, or chemical agents. One is required to obtain a release from their personal care physician, to be provided to the Occupational Health Nurse (please, see: Medical Health Questionnaire).

5.4 REGISTRATION OF MATERIALS POTENTIALLY INFECTIOUS FOR HUMANS

Biological safety or biohazard control is management of biological hazards through proper application of engineered containment and administrative controls. Biological safety at UTA is a team effort involving the PI, research and teaching lab personnel, IBC, IACUC, and EH&S.

The UTA EH&S maintains a registry of all laboratories and personnel working with biohazardous agents such as microbial pathogens, toxins, and/or human blood, body fluids, and cells/tissues. UTA complies with the CDC recommendations in BMBL, 5th Edition, revised 2009.

The PI is responsible for completing the appropriate parts of the Human Pathogen Registration (HPR) and forwarding it to EH&S. After receiving the registration, EH&S conducts a laboratory inspection using Biosafety Level 2 (BSL-2) Commissioning Checklist to ensure compliance with local, state, and federal regulations and to improve safety. The PI is also responsible for notifying EH&S when the project has terminated or when other significant changes occur using the Human Pathogen Registration Update (HPRU). All three forms mentioned in this paragraph can be found on the EH&S - Biological Safety website under "Forms".

All projects involving recombinant/synthetic nucleic acid molecules must be reviewed by the UTA IBC prior to initiation. UTA complies with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

5.5 BIOSAFETY LEVELS

Governmental bodies, the CDC and the NIH, have developed standard procedures providing protection against biological hazards. These physical containment barriers for handling biohazards are called biosafety levels (BSLs). BMBL provides specific descriptions of combinations of microbiological practices, laboratory facilities, and safety equipment and recommends their use in four biosafety levels of operation with infectious agents. The BSLs described in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules are based on and consistent with the biosafety levels presented here.

A BSL is based on the potential hazard of the agent and the functions of the laboratory. BSL-1 is for work with agents that pose the least hazard and BSL-4 is for work with agents that pose the greatest hazard. The table below summarizes the recommended BSLs:

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	No primary barriers required Personal protective equipment (PPE): Laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required

2	Agents associated with human disease, routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practices plus: • Limited access (card-reader(s) when work involves handling human blood, OPIM, or tissue/cells) • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = biosafety cabinets (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 and eye protection, as needed	BSL-1 plus: • Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practices plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering	Primary barriers = BSCs or other physical containment devices used for all open manipulations of agents PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory • Entry through airlock or anteroom • Hand washing sink near laboratory exit
4	Dangerous/exotic agents which pose high individual risk	BSL-3 practices plus:	Primary barriers = All procedures conducted in Class	BSL-3 plus:

	of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level Related agents with unknown risk of transmission	• Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	• Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamina-tion systems

A good starting point for selecting a physical containment level is to equate the risk group level of the specific wild-type microorganism to the corresponding biosafety level (i.e., RG-2 = BSL-2). Depending upon the specific microorganism, its contrived genetic alterations, the types of manipulations intended, and the proximity of the research/work to sensitive areas, the BSL may be increased or lowered. For example, the handling of blood and body fluids in laboratories requires Universal Precautions for Bloodborne Pathogens. Universal Precautions equates to BSL-2 practices.

At UTA, BSL-1 is the minimum facility containment level and BSL-1 practices (standard microbiological practices) are the minimum acceptable work practices for all laboratories, rooms, or other areas where containers of viable biological materials are opened.

UTA does not have BSL-3 or BSL-4 containment facilities, and therefore will not allow the intentional handling of microorganisms requiring this level of containment. **Because UTA only has BSL-1 and BSL-2 containment facilities, only these levels are addressed in this manual.** All work with infectious agents at the University should follow the CDC/NIH guidelines. If you are uncertain under which BSL your work should be performed, please contact EH&S at 817-272-2185 for assistance. It should be emphasized that no system of risk assessment or containment barriers can guarantee there will be no risk.

The BSLs existing at UTA are described in the following sections.

5.5.1 BIOSAFETY LEVEL 1 (BSL-1)

BSL-1 is appropriate for undergraduate and secondary educational training and teaching laboratories, and for research laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adults, and which are of minimal potential hazard to laboratory personnel and the environment. Exempt organisms under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules fall in this category. Many agents not ordinarily associated with disease

processes in humans are opportunistic pathogens and may cause infections in the young, aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

BSL-1 represents a basic level of containment that relies on standard microbiological practices. The laboratory is not necessarily separated from the general traffic patterns in the building, and special containment equipment or facility design is not required nor generally used. Work is mostly conducted on open bench tops using standard microbiological practices. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a PI with general training in microbiology or a related science.

5.5.1.1 STANDARD MICROBIOLOGICAL PRACTICES (BSL-1)

- The laboratory supervisor must enforce the institutional policies that control access to the laboratory. Access to the laboratory is limited or restricted when experiments or work with cultures and specimens are in progress.

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

- Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Persons who wear contact lenses in laboratories should also wear safety glasses with side shields, goggles or a face shield. Food/drinks are stored outside the work area in cabinets or refrigerators designated (and labeled) for this purpose only.

- Mouth pipetting is prohibited; mechanical pipetting devices are used.

- All procedures are performed carefully to minimize the creation of splashes and/or aerosols.

- Work surfaces are decontaminated at least once a day and after any spill of viable material.

- Special care needs to be practiced when using "sharps", in other words, syringes, needles, Pasteur pipettes, capillary tubes, scalpels, and other sharp instruments or when handling broken glassware to reduce risk of sharps injuries.

- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container. The container needs to be closed during the transport from the laboratory. Materials to be decontaminated offsite from the laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility. If you need assistance in acquiring the appropriate container, or have questions concerning disposal, contact EH&S, 817-272-2185.

- An effective integrated pest management program is required.

- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

5.5.1.2 SPECIAL PRACTICES (BLS-1)

None required

5.5.1.3 SAFETY EQUIPMENT (PRIMARY BARRIERS) (BSL-1)

- Special containment devices or equipment such as a BSC is generally not required for manipulations of agents assigned to BSL-1.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Gloves must be worn to protect hands from exposure to hazardous materials. This is especially important if the skin on the hands is broken or if a rash exists. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available.
- Protective eyewear should always be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Contact lenses should not be worn in laboratories.

5.5.1.4 LABORATORY FACILITIES (SECONDARY BARRIERS) (BSL-1)

- Laboratory door(s) is/are used for access control.
- Laboratories must have a sink for hand washing.
- The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Bench tops are impervious to water and resistant to moderate heat, acids, alkalis, organic solvents, and other chemicals.
- Laboratory windows that open to the exterior should be fitted with insect-proof screens.

5.5.1.5 EXAMPLES OF BSL-1 AGENTS

Bacterial Agents

- *Bacillus subtilis*
- *Bacillus thuringiensis*
- Nonpathogenic *Escherichia coli, e.g.,* strain K12
- *Lactobacillus acidophilus*
- *Micrococcus luteus*
- *Pseudomonas fluorescence*
- *Serratia marcescens*

Fungal Agents

- *Aspergillus niger*
- *Saccharomyces cerevisiae*

Viruses

- Infectious canine hepatitis virus

5.5.2 BIOSAFETY LEVEL 2 (BSL-2)

BSL-2 practices, equipment, and facility design apply to research and teaching laboratories when moderate-risk agents are handled. It should be remembered that the agents not listed in RG-2 are not automatically classified in RG-1. A risk assessment must be conducted based on the known and potential properties of the agents.

BSL-2 builds upon BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and environment. It differs from BSL-1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures
- Access to the laboratory is restricted when work is being conducted (research laboratories where work involves handling human blood, OPIM, tissue/cells shall have card-reader(s) to limit access to only those who have completed the appropriate requirements)
- Extreme precautions are taken with contaminated sharp items
- All procedures in which infectious aerosols or splashes may be created are conducted in BSC or other physical containment equipment

5.5.2.1 STANDARD MICROBIOLOGICAL PRACTICES (BSL-2)

Same as BSL-1.

5.5.2.2 SPECIAL PRACTICES (BSL-2)

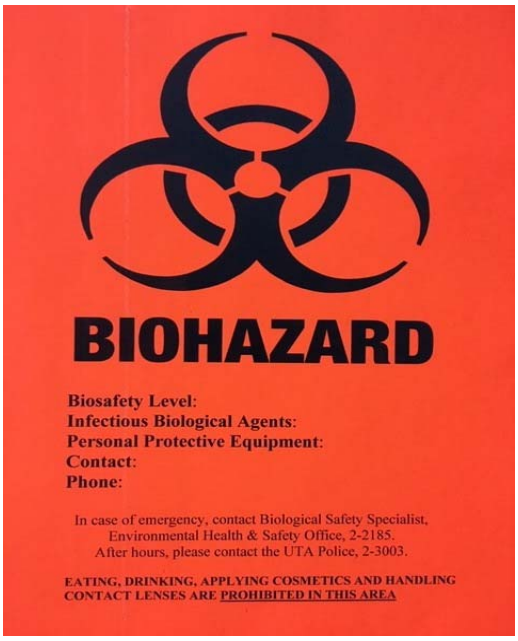
- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

- Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory (for example, hepatitis B vaccine).

- Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

- When the infectious agent(s) in use in the laboratory require special provisions for entry, the universal biohazard sign must be posted on the entrance door(s) to the laboratory. The appropriate information to be posted on the entrance door includes:

- Biosafety level
- List of infectious agent(s)
- Required PPE that must be worn in the laboratory
- Name(s) and telephone number(s) of the PI(s)
- Contact information after work hours



- Site-specific biosafety procedures are prepared and adopted in addition to this UTA EH&S Biosafety Manual. The site-specific procedures should advise personnel about special hazards and practices/procedures that are required to be followed when working in a particular laboratory.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.
- High degree of precaution should always be taken with any contaminated sharp item, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
- Needles and syringes or other sharp instruments should be used only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.

- Only needle-locking syringes or disposable syringe-needle units should be used (in other words, needle is integral to the syringe) for the injection or aspiration of infectious materials.
    - Do not bend, shear, break, recap or remove needles from disposable syringes, or otherwise manipulate them by hand before disposal.
    - Place used disposable needles and syringes carefully in puncture-resistant containers used for sharps disposal. These sharps containers are supplied and removed by EH&S for disposal.

    - Place non-disposable sharps in a hard-walled container for transport to a decontamination area, in other words, autoclave room.
    - Do not handle broken glassware directly. Use a brush and dustpan, tongs, or forceps.
    - Plastic ware should be substituted for glassware whenever possible.
- Potentially infectious materials (cultures, tissues, specimens of body fluids) must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within the facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination with an appropriate disinfectant. Equipment must be decontaminated before repair, maintenance, or removal form the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately reported to the PI/laboratory supervisor and EH&S, 817-272-2185, and evaluated. Medical evaluation, surveillance, and treatment should be provided and appropriate written records maintained.
- All procedures involving the manipulation of infectious materials that may generate aerosols or splashes should be conducted within a BSC or other physical containment equipment.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.

5.5.2.3 SAFETY EQUIPMENT (PRIMARY BARRIERS) (BSL-2)

- Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices are used whenever:
    - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening

containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryos.

- High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a BSC.

- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC or other containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse.

- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or deposited for laundering. It is recommended that personnel do not take laboratory clothing home. If it needs to be laundered, clothing must first be autoclaved.

- Gloves must be worn to protect hands from exposure to hazardous materials.
  - Glove selection should be based on an appropriate risk assessment.
  - Alternatives to latex gloves should be available.
  - Wearing two pairs of gloves may be appropriate. If a spill or splatter occurs, hands will be protected after the contaminated gloves are removed.
  - Gloves need to be changed when contaminated, their integrity has been compromised, or when otherwise necessary.
  - Gloves should be removed and hands washed when work with hazardous materials has been completed and before leaving the laboratory.
  - Disposable gloves should never be washed or reused. They should be disposed with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
  - Disposable gloves should not be worn outside the laboratory, or UTA glove procedures should be followed.

5.5.2.4 LABORATORY FACILITIES (SECONDARY BARRIERS) (BSL-2)

- Laboratory doors must be lockable. Research laboratories where work involves handling human blood, OPIM, or tissue/cells shall have card-reader(s) to limit access to only those who have completed the appropriate requirements.

- Laboratories must have a sink for hand washing.

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

- Laboratory furniture must be capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily cleaned and decontaminated.

- Bench tops are impervious to water and resistant to moderate heat, acids, alkalis, organic solvents, and other chemicals.

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

- BSCs should be installed in such a manner that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

- Vacuum lines should be protected with liquid disinfectant traps.

- High Efficiency Particulate Air (HEPA)/Ultra Low Particulate Air (ULPA) filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) or a direct (hard) connection.

- An eyewash station is available.

- A method for decontamination of infectious or regulated laboratory wastes is available (for example, autoclave, chemical disinfection, incinerator, or other approved decontamination method).

- There are no specific ventilation requirements. However, when planning new facilities, mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory should be considered.

5.5.2.5 EXAMPLES OF BSL-2 AGENTS

Bacterial Agents

- *Bordetella* spp., including *B. pertussis*
- *Campylobacter jejuni* subsp. *jejuni*
- *Clostridium tetani*
- *Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- *Klebsiella* - all species except *K. oxytoca* (RG-1)
- *Listeria monocytogenes*

- *Mycobacterium (*except those listed as RG-3) including *M. avium* complex, *M. asiaticum, M. bovis* Bacille Calmette-Guérin vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
- *Salmonella* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
- *Shigella* spp., including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
- *Vibrio cholera*
- *Yersinia enterocolitica*

Fungal Agents

- *Blastomyces dermatitidis*
- *Cladosporium bantianum, C. (Xylohypha) trichoides*
- *Penicillium marneffei*
- *Sporothrix schenckii*
- *Trichophyton*

Parasitic Agents

- *Cryptosporidium* including *C. parvum*
- *Giardia* including *G. lamblia*
- *Microsporidium*
- *Toxoplasma* including *T. gondii*
- *Trichinella spiralis*

Viruses

- Adenoviruses, human - all types
- Hepatitis A, B, C, D, and E viruses
- Herpesviruses - except *Herpesvirus simiae* (Monkey B virus) (RG-4)
- Papovaviruses - all human papilloma viruses
- Paramyxoviruses - Newcastle disease virus, measles virus, mumps virus
- Parvoviruses - human parvovirus (B19)
- Picornaviruses - coxsackie viruses types A and B, polioviruses - all types, wild and attenuated
- Smallpox, and whitepox
- Reoviruses - all types including coltivirus, human rotavirus, and orbivirus (Colorado tick fever virus)
- Rhabdoviruses - rabies virus - all strains

5.6 BLOODBORNE PATHOGENS AND UNIVERSAL PRECAUTIONS

In December 1991, OSHA promulgated the final rule for occupational exposure to bloodborne pathogens (BBP). The rule, commonly referred to as the Bloodborne Pathogens Standard, became effective March 6, 1992. The objective is to provide guidelines to eliminate or minimize employee exposure to human BBP. Although UTA is not covered by OSHA's Bloodborne Pathogen standard, the University is covered by the Health and Safety Code, which requires the Texas Department of State Health Services (TDSHS) to establish an exposure control plan designed to minimize exposure of governmental entity employees to BBP.

A human BBP is a pathogenic microorganism present in human blood that can cause disease in humans. Employees face a significant health risk from occupational exposure to blood and OPIM considering that these materials may contain BBP, including HBV that causes hepatitis B, a serious liver disease, and HIV, which causes AIDS.

The standard includes the CDC guidelines referred to as Universal Precautions. The concept behind Universal Precautions is to treat all human blood and certain human body fluids as if known to be infected with HIV, HBV, and other BBP.

In an effort to eliminate or minimize exposure to BBP by employees who may be reasonably anticipated to be exposed to blood or OPIM during the performance of their duties, the standard requires employers to institute:

- A program of engineering and work practice controls
- Personal protective clothing and equipment
- Informational training
- Hepatitis B vaccination
- Post exposure evaluation and follow-up
- Sign and label programs

Tissue cultures may also unknowingly contain human pathogens, and this is why all human (and non-human primate) specimens and cell cultures must be handled using Universal Precautions. Contact EH&S at 817-272-2185 for information regarding Bloodborne Pathogens for Laboratory Research Personnel Training if, during the course of your work, you have a potential for coming into contact with human blood or OPIM.

Universal Precautions are summarized below and should be practiced whenever encountering human blood.

UNIVERSAL PRECAUTIONS / BBP PRACTICES
Bloodborne Pathogen Training needs to be taken annually. Taking the course online will automatically document completion of the training.
Identification of those that are at risk is important. If job duties have changed, and you now know that you are at risk, please notify EH&S of the change.
An Exposure Control Plan for Bloodborne Pathogens can be found on EH&S website. Review of this manual and completion of the online Bloodborne Pathogens for Laboratory Research Personnel Training (https://uta-ehs.org/) meets the Universal Precautions/BBP requirements for research areas.
Hepatitis B vaccination and prophylaxis for needle sticks or other exposure incidents involving BBP should be made available.
Extreme sharps precautions, protective clothing and glove use, splash protection, and disinfection: refer to the Special Practices (BLS-2) (section 5.5.2.2) and Safety Equipment (Primary Barriers) (BSL-2) (section 5.5.2.3) for BSL-2 laboratories in this manual.

5.6.1 UTA – EXPOSURE CONTROL PLAN FOR BLOODBORNE PATHOGENS

EH&S provides support in the effort to adequately protect UTA personnel from occupational exposure to blood and OPIM to achieve regulatory compliance. EH&S has developed Exposure Control Plan for Bloodborne Pathogens in accordance with Texas Department of State Health Services Exposure Control Plan, Health and Safety Code 81.304 to be analogous with the OSHA Bloodborne Pathogens Standard, 29 CFR 1910.1030.

A bloodborne pathogen exposure determination concerning UTA BSL-2 research laboratories is made without regard to the use of PPE. Persons whose expected job functions / activities in research work include possible exposure to human blood / OPIM (including tissue and cells) are considered to be exposed even if they wear PPE.

Principal investigators / supervisors are responsible to enforce compliance with the Exposure Control Plan for Bloodborne Pathogens for BSL-2 research laboratories handling human blood / OPIM for all applicable persons:

- Engineering controls and equipment shall be utilized to eliminate or minimize exposure to persons (sharps containers, specimen containers, containers for reusable sharps, biosafety cabinets, sharps with engineered sharps injury protection, hand washing facilities). Where potential for exposure still exists after implementation of these controls, PPE shall also be utilized.

- Work area controls and procedures shall be utilized to eliminate or minimize exposure to persons (work area restrictions for research facilities, contaminated equipment, housekeeping, regulated waste, laundry procedures). Prerequisite for access to laboratory area that is a BSL-2 entity where human blood / OPIM are stored / worked with: Hepatitis B Vaccination / Hepatitis B vaccine Waiver / Exemption (CO-LS-F12).

5.6.1.1 HEPATITIS B VACCINATION PROGRAM

IT IS HIGHLY RECOMMENDED THAT ALL PERSONS WITH POTENTIAL EXPOSURE TO BLOODBORNE PATHOGENS THROUGH HANDLING HUMAN BLOOD / OPIM RECEIVE THE HEPATITIS B VACCINATION. EMPLOYEES SHALL BE OFFERED THE HEPATITIS B VACCINATION FREE OF CHARGE. EMPLOYEES MAY REFUSE THE HEPATITIS B VACCINE, BUT THEY NEED TO BE PROPERLY INFORMED OF ITS BENEFITS THROUGH APPROPRIATE TRAINING. IF THE EMPLOYEE REFUSES THE VACCINE, HE/SHE MUST SIGN A DECLINATION FORM (HEPATITIS B VACCINATION / HEPATITIS B VACCINE WAIVER / EXEMPTION) (INDEX CO-LS-F12).5.7 LABORATORY ANIMALS

The use of animals in research and teaching is subject to state and federal laws and guidelines. UTA has adopted the National Research Council's Guide for the Care and Use of Laboratory Animals (the Guide) as a primary reference on animal care and use. The goal of the Guide is to promote humane care of animals used in research, teaching, and testing. UTA's animal care and use program is managed in accordance with this Guide and in compliance with applicable federal, state, and local laws and regulations, such as the Animal Welfare Act and Animal Welfare Regulations and Public Health Service Policy on Humane Care and Use of Laboratory Animals.

The IACUC at UTA is a review committee that is charged with reviewing research protocols for the humane use of experimental animals. The IACUC must approve the work before experimental work with animals can be started. When the animal research will involve biohazards (potentially infectious or pathogenic materials), the investigator must register using the Human Pathogen Registration (HPR) (CO-LS-F7) prior to initiation of work. Thus, work with experimental animals is strictly regulated. Only those authorized can enter animal facilities or work with experimental animals in the facility. Access to the animal facility is controlled by an ID badge key card entry. Before key card is activated, personnel working with live, vertebrate animals must get trained.

In accordance with animal welfare regulations, the IACUC is responsible for ensuring that PIs and other personnel are appropriately qualified and experienced for conducting procedures on living animals, including the proper and humane care and use of laboratory animals. All individuals conducting research with animals must complete the online training modules for basic care, ethical background, and occupational health and safety.

Additional hands-on training is required to ensure that personnel have the appropriate level of experience. To accomplish this, the IACUC has initiated a 3-Tier Hands-On Training Program.

- Tier 1: Basic Hands-On Training and Orientation – All researchers working with live animals will first complete the Basic Training, provided by the Animal Care Facility (ACF) Manager (acf@uta.edu). Basic Hands-On Training and Orientation will cover topics such as emergency procedures, PPE, security, hazards, basic animal handling, and will include a tour of the ACF. Basic Hands-On Training and Orientation is required before obtaining card access to the ACF and working with animals.
- Tier 2: Protocol-Specific Techniques - During review of the protocol, the IACUC will identify specific procedures that will require additional personnel training. Some examples of protocol-specific techniques are injections, administering euthanasia, restraint, transportation of animals, etc. These special protocol-specific techniques will be added to the personnel's requirement list for Basic Training and will be provided by the ACF Manager.
- Tier 3: Survival Surgery Training - The need for this training will be identified during protocol review by the IACUC. The training will be scheduled and provided by the ACF Manager, and must be completed prior to performing any survival surgery procedures.

The additional requirements for training under Tier 2 and Tier 3 are at the discretion of the IACUC, and may not be required if the PI adequately demonstrates or describes proficiency with the specific techniques within the protocol application.

For questions or concerns, please call 817-272-3723 or email at regulatoryservices@uta.edu.

Another required training is Hazard Communication and Waste Management – Academic Training (a state requirement that needs to be completed by all UTA employees and students who use, handle, or transfer hazardous chemicals at their workplace). Depending on the research project, other UTA training(s) such as Biosafety Level 2 (BSL-2) Training, Vaccinia Virus Training, and Bloodborne Pathogens for Laboratory Research Personnel Training might be needed. The link to the EH&S Training Management site: https://uta-ehs.org.

PIs are responsible for the humane treatment of animals under their supervision, and for adherence to applicable university, state, and federal regulations. Appropriate PPE (gloves, masks, laboratory coats) must be worn whenever entering an area where experimental animals are housed. Guidelines are available for safely working with laboratory animals and can be obtained by referring to the Guide or contacting the ACF Manager at 817-272-5236.

5.8 OCCUPATIONAL HEALTH AND SAFETY IN THE ANIMAL CARE AND USE PROGRAM

Due to occupational job duties in the ACF, one may be at risk of exposure to potentially infectious materials and/or blood or blood products that may put one at risk for acquiring diseases. Certain accommodations may be required for personal safety, including immunizations, use of respirator, special protective equipment or clothing, etc. Some animals can carry pathogens that can be transmitted to humans through contact with their body fluids, similar to human bloodborne pathogens. This contact can occur through biting, spitting, or contamination of broken skin or mucus membranes with bodily secretions from the animal. The potential hazards such as animal bites, chemical cleaning agents, allergens, and zoonoses that are inherent in or intrinsic to animal use should be identified and evaluated. UTA research groups conducting animal research take part in the Occupational Health and Safety Program which ensures that the risks associated with the experimental use of animals are reduced to acceptable levels. Individuals conducting research with animals at UTA must be listed in the authorized personnel section of an animal research protocol, or be added to a previously approved protocol. The listed individuals must complete the

occupational health requirements. These involve obtaining tetanus immunization, completing the Medical Health Questionnaire, and submitting the information to the Occupational Health Nurse. Details on these requirements can be found in Authorization to Work with Animals. The Occupational Health Nurse will review the information and working conditions and conduct a risk assessment. The person may be contacted for additional questions or follow-up. Depending on this assessment, one may be required to perform additional training, obtain additional immunizations, or be instructed to follow certain procedures or utilize specific PPE. These instructions will be communicated to the person in question directly by the Occupational Health Nurse.

For questions pertaining to occupational health and safety (medical evaluation, health information, immunization requirements, health and safety protective measures), please contact the Occupational Health Nurse at 214-505-7660 .

5.9 ANIMAL BIOSAFETY LEVELS

Four biosafety levels ranging from animal biosafety level one (ABSL-1) through ABSL-4 are described for animal experimentation with biological agents in BMBL. The table below summarizes these recommended ABSLs.

ABSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each animal species. PPE: laboratory coats and gloves; eye, face protection, as needed	Standard ACF: • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available
2	Agents associated with human disease Hazard = percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual • Decontamination of all infectious wastes and animal cages prior to washing	ABSL-1 equipment plus primary barriers: • Containment equipment appropriate for animal species • PPE: laboratory coats, gloves, face, eye and respiratory protection, as needed	ABSL-1 plus: • Autoclave available • Mechanical cage washer recommended • Negative airflow into animal and procedure rooms recommended

3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	ABSL-2 practice plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding is removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I, II or III BSCs available for manipulative procedures that may create infectious aerosols • PPE: appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations and windows • Autoclave available in facility • Entry through ante-room or airlock • Negative airflow into animal and procedure rooms • Hand washing sink near exit of animal or procedure room
4	Dangerous /exotic agents which pose high risk aerosol transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments Agents with a close or identical antigenic relationship to an agent	ABSL-3 practices plus: • Entrance through change room where personal clothing is removed and laboratory clothing is put on • Shower on exiting • All wastes are decontaminated before removal from the facility	ABSL-3 equipment plus: Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure suit) used for all procedures and activities	ABSL-3 facility plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamin-ation systems

	requiring BSL-4 until data are available to redesignate the level Related agents with unknown risk of transmission			

Both facility requirements and practices are stricter in animal experimentation (*in vivo*) work than in laboratory work because of several factors. First, animals are more likely to bite and scratch, so the probability of parenteral exposure is greater than in laboratory experiments (*in vitro*). Second, animals can carry endogenous pathogens or catch them from their human caretakers and transmit them to others (zoonosis). Third, animals are not as hygienic as humans: their feces, saliva, urine, and other body fluids can coat their skin or fur. Fourth, animals living in confined quarters (cages), are more likely to be stressed, and therefore are more likely to be susceptible to disease. Finally, the particulate nature of animal bedding and the cage changing / cleaning operations add to the load of particles in the ambient air in animal colonies. The more microorganisms floating in the air, the greater the chance animal researchers will inhale them. This is especially important when the organism of study naturally causes disease by eye conjunctiva contact, nasal mucus membrane contact, or inhalation.

As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable. However, it should be remembered that the animal room can present some unique problems. In the microbiological laboratory, hazardous conditions are caused by personnel or by the equipment being used. In the animal room, the activities of the animals themselves can present new hazards. As stated earlier, animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic disease.

Ideally, facilities for laboratory animals used in studies of infectious or noninfectious disease should be physically separate from other activities such as animal production and quarantine. Traffic flow that will minimize the risk of cross contamination should be considered in the plans. A "clean/dirty hall" layout may be useful to minimize this risk.

UTA has only ABSL-1 and ABSL-2 facilities and thus only ABSL-1 and ABSL-2 containment level experiments can presently be performed at UTA. ABSL-1 is the minimum facility containment level and BSL-1 practices (standard microbiological practices) are the minimum acceptable work practices for UTA vivaria. The recommendations detailed below describe two combinations of practices, safety equipment, and facilities for experiments with animals infected with agents that cause, or may cause, human infection. These two combinations, designated ABSL-1 and ABSL-2, provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The two ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to BSL-1 and BSL-2, respectively. PIs inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this type of special work.

5.9.1. ANIMAL BIOSAFETY LEVEL 1 (ABSL-1)

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

5.9.1.1 STANDARD PRACTICES (ABSL-1)

- The ACF Manager establishes policies, procedures, and protocols according to institutional polices and makes sure that plans are in place for emergencies. Prior to beginning a study involving animals, protocols must be reviewed and approved by the IACUC and if applicable, the IBC. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
- A safety manual specific to the ACF is prepared or adopted and must be available/accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.
- The ACF Manager must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
- An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Personnel using respirators must be enrolled in the Respiratory Protection Program.
- Only those persons required for program or support purposes are authorized to enter the ACF. Facility personnel, service workers, and visitors are all advised of the potential hazards and are instructed on the appropriate safeguards. Access to the animal rooms is limited.
- PPE is available and used according to the established policies; see 5.9.1.3 Safety Equipment (Primary Barriers) (ABSL-1).
- Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consuption are not permitted in animal or procedure rooms. Food/drinks must be stored outside of the ACF in cabinets or refrigerators designed and used for this purpose.
- All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Please, refer to 5.11.4.3.5 Sharps for information.
- Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
- Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
- All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

- Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
- An effective integrated pest management program is required.

5.9.1.2. SPECIAL PRACTICES (ABSL-1)

None required.

5.9.1.3 SAFETY EQUIPMENT (PRIMARY BARRIERS) (ABSL-1)

- A risk assessment should determine the appropriate type of PPE to be utilized. Special containment devices or equipment may not be required as determined by appropriate risk assessment.
- Laboratory coats, gowns, or uniforms are recommended to be worn in the ACF to prevent contamination of personal clothing. Protective outer clothing should not be worn outside areas where infectious materials and/or animals are housed or manipulated. Laboratory coats, gowns, and uniforms are not worn outside the ACF.
- Protective eyewear / face protection is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations of airborne particulates.
- Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
- Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

5.9.1.4 FACILITIES (SECONDARY BARRIERS) (ABSL-1)

- The ACF is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the ACF is restricted.
- The ACF is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water-resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. It is recommended that penetrations in floors, walls, and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
- Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
- Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris.
- External windows are not recommended; if present, windows must be resistant to breakage. Where possible, windows should be sealed. If the ACF has windows that open, they are fitted with insect screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

- If floor drains are provided, the traps are always filled with water and/or an appropriate disinfectant to prevent the migration of vermin and gases.
- Ventilation should be provided in accordance with the Guide. No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow.
- The ACF must have a sink for hand washing.
- Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F (82.2°C). If manual cage washing is utilized, it must be ensured that appropriate disinfectants are selected.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- Emergency eyewash and shower are readily available.

5.9.2 ANIMAL BIOSAFETY LEVEL 2 (ABSL-2)

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

5.9.2.1 STANDARD PRACTICES (ABSL-2)

- Aside from the standard policies, procedures, and protocols for emergency situations established by the ACF Manager, appropriate special policies and procedures should be developed as needed and approved by the IACUC and if applicable, by the IBC. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
- A safety manual specific to the ACF is prepared or adopted and must be available/accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.
- The ACF Manager must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
- An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Personnel using respirators must be enrolled in the Respiratory Protection Program.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign identifies the animal biosafety level, the infectious agent(s) in use, PPE requirements, lists the name and telephone number of the responsible person(s), and indicates any special requirements (e.g., the need for immunizations and respirators) for entering the animal room.
- Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the ACF and the areas where infectious materials and/or animals are housed or manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

- PPE is available and used according to the established policies (see 5.9.2.3 Safety Equipment (Primary Barriers) (ABSL-2).
- Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal or procedure rooms. Food/drinks must be stored outside of the ACF in cabinets or refrigerators designed and used for this purpose.
- All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Please, refer to 5.11.4.3.5 Sharps for information.
- Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent and especially after overt spills, splashes, or other overt contamination.
- Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
- All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
- Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
- An effective integrated pest management program is required.

5.9.2.2 SPECIAL PRACTICES (ABSL-2)

- Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a base line serum sample should be stored**.** In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the ACF unless special procedures can eliminate the extra risk.
- Procedures involving a high potential for generating aerosols should be conducted within a BSC or other physical containment device. When a procedure cannot be performed within a BSC, a combination of PPE and other containment devices must be used.
- Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.
- All materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated (including animal tissues, carcasses, and bedding) must be placed in durable, leak proof container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

- Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Incidents that may result in exposure to infectious materials must immediately be reported to the ACF Manager. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

5.9.2.3 SAFETY EQUIPMENT (PRIMARY BARRIERS) (ABSL-2)

- Properly maintained BSCs, PPE and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. A risk assessment should determine the appropriate type of PPE to be utilized.
- When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages with filter tops for rodents.
- Laboratory coats, gowns, or uniforms are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting.
- Eye and face protection (goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or other containment device. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
- Respiratory protection is worn based upon risk assessment.
- Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
    - Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary
    - Gloves must not be worn outside the animal rooms
    - Gloves and other PPE should be removed in a manner that prevents transfer of infectious materials
    - Disposable gloves are not washed or reused. Used gloves needs to be disposed with other contaminated waste
- Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

5.9.2.4 FACILITIES (SECONDARY BARRIERS) (ABSL-2)

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

- The ACF is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water-resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. It is recommended that penetrations in floors, walls, and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris.
- Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
- External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the ACF has windows that open, they are fitted with insect screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.
- If floor drains are present, they must be maintained and filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
- Ventilation should be provided in accordance with the Guide. The direction of airflow in the animal facility is inward; animal rooms maintain negative pressure compared to adjoining hallways. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from "clean" areas and toward "contaminated" areas.
- Cages should be autoclaved or otherwise decontaminated prior to washing. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F (82.2°C). The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
- An autoclave should be present in the ACF to facilitate decontamination of infectious materials and waste.
- A handwashing sink is available in the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA (ULPA) filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance. All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
- If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.

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

5.10 USE OF HUMAN SUBJECTS IN RESEARCH

UTA has established an Institutional Review Board (IRB) to review human subject research. The IRB reviews, approves, and monitors research that is conducted or supported by the UTA faculty, students or staff in order to determine that the rights and welfare of the human subjects are adequately protected. The IRB is guided by the ethical principles described in the 'Belmont Report' and by the regulations of the U.S. Department of Health and Human Services (HHS) found at Title 45 CFR, Part 46. UTA maintains an approved Federalwide Assurance (FWA) for the Protection of Human Subjects of Compliance with HHS and the Office for Human Research Protections (OHRP).

It is the responsibility of the PI to assure that all research involving human subjects is reviewed and approved by the IRB prior to initiation. All personnel with a reasonable anticipated risk of exposure to BBP through the contact with human blood or OPIM must complete Bloodborne Pathogens for Laboratory Research Personnel Training (https://uta-ehs.org/) and renew the training annually.

5.11 CONTAINMENT BARRIERS

There are three types of containment barriers: natural biological barriers, contrived biological containment barriers, and physical containment barriers. All three types should be considered when planning biological experiments or when developing procedures involving handling of viable or biologically active materials.

5.11.1 NATURAL BIOLOGICAL BARRIERS

Understanding the natural mode of transmission of microorganisms and the natural immunity and health status of the laboratory personnel are important considerations when assessing the protective impact of natural biological barriers. Application of this knowledge to the design of laboratories and practices/procedures enhances the containment of biohazards in the work environment.

5.11.1.1 ROUTES OF TRANSMISSION

An infection occurs when microorganisms enter the human body in sufficient numbers by a particular route of transmission. Not all infections cause harm. Some infections may be beneficial, such as infections with live vaccine strains designed to bestow immunity.

When a particular infection is known to be harmful or when all the effects of an infection are unknown, it is necessary to block the natural route of transmission of that microorganism. All work with viable biological agents should be designed to reduce known and predicted routes of transmission to unintended recipients to the greatest extent possible.

The following are common routes of transmission for laboratory-acquired infections. Risk assessment can reveal laboratory procedure hazards. Most microorganisms are transmitted by more than one route. Knowing the natural routes of transmission of a microorganism is a first step in determining the types and extent of contrived biological and physical containment barriers needed for its safe handling.

5.11.1.1.1 DIRECT CONTACT WITH ABRADED/CUT SKIN OR MUCOSAL MEMBRANE (INCLUDES THE EYES, NOSE, AND MOUTH) EXPOSURE

Spilled material can come into direct contact on the skin, as can droplets produced by pipetting, removal of screw caps, and vortex mixing of unsealed tubes.

The control of a contact exposure is accomplished through wearing of appropriate PPE such as a face shield, gloves, safety glasses/goggles, a mask, and laboratory coat. Other ways to control contact exposure include using absorbent paper on the workbench, performing all procedures carefully, and frequently wiping work surfaces with an appropriate disinfectant.

It is also important to keep all non-essential items away from the area where work is being performed to protect personal items from contamination. Handle and store all contaminated wastes properly to prevent contact exposure of lab personnel as well as housekeeping staff and waste handlers.

> **Example:** Adenoviruses are naturally transmitted by contact with the eye conjunctiva, in addition to other modes. Goggles, glasses or other eye protection should be worn to minimize accidental hand to eye contact or splashes to the eye.

5.11.1.1.2 INOCULATION

Parenteral inoculation in a laboratory usually occurs with a needle and syringe (accidental inoculation) or with other contaminated sharp or by bites and scratches from infected animals, and arthropod vectors.

Extreme caution needs to be exercised whenever using a needle. Needle use should be restricted: whenever an alternative to a needle is possible, it should be used. Inoculation can also occur through other sharps such as Pasteur pipettes and razor blades.

The control of an inoculation hazard is accomplished by the safe use, handling, and storage of needles and other sharps. Please, refer to the section 5.11.4.3.5 “Sharps” for more information.

> **Example:** HIV and other retroviruses are transmitted from blood to blood. Retrovirus experiments should include sharps precautions to minimize the possibility of accidental inoculation.

5.11.1.1.3 INGESTION

Ingestion may occur either directly or indirectly. Exposure may occur from mouth pipetting or splashing from a container into the mouth or by contaminating the hands and then touching the mouth or items, such as a coffee cup, food, or lip balm, which then touches the mouth.

The control of an ingestion exposure is accomplished through the use of mechanical pipetting devices whenever pipetting and by practicing good personal hygiene, such as washing hands frequently throughout the day and not eating or drinking in the laboratory. Food items/drinks cannot be stored in refrigerators that contain hazardous materials or in the laboratory where work with infectious agents is being performed.

> **Example:** *Escherichia coli* is naturally transmitted by ingestion. All work with *E. coli* should include procedures that minimize the risk of ingestion, such as frequent hand washing and glove use, no eating or drinking in the laboratory, and keeping hands away from face and mouth.

5.11.1.1.4 INHALATION

It is generally known that aerosols are the primary means by which infectious diseases are spread and contracted. An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. An aerosol can be either a liquid or a dry particle. An aerosol with a diameter of five microns or less can easily be inhaled and carried to the alveoli of the lungs. These aerosols can remain airborne for a long period and can spread wide distances, especially after entering the building's ventilation system. Procedures and equipment that create aerosols also create larger droplets (particles with a diameter larger than five microns) that rapidly settle out of the air. These droplets can settle on surfaces and therefore contaminate the skin, gloved hands, work spaces and mucous membranes.

Aerosols, or respirable sized particles, are extremely hazardous because they are generated in many commonly performed laboratory procedures and are usually undetected. The creation of infectious aerosols places the person carrying out the procedure and others in the laboratory at risk. Any procedure that breaks the surface tension of a liquid will produce aerosols. Pipetting, heating inoculating loops, using a blender, centrifuging with a non-self-contained centrifuge, using sonicators and vortex mixers, and changing animal bedding all produce aerosols.

> **Example:** *Mycobacterium* species are transmitted by very small particles expelled from infectious patient's mouth and inhaled by unprotected bystanders. Respiratory protection is recommended when working with this agent. When *Mycobacterium*-infected rodents are handled, care should be taken to guard against inhalation of feces-contaminated bedding aerosols.

The control of an inhalation exposure is accomplished by a combination of using the appropriate safety equipment such as BSCs and by performing procedures carefully to minimize the creation of aerosols. Refer to the section 5.11.4.3 "Laboratory Safety Equipment / Other Laboratory Equipment" for additional information.

5.11.2 NATURAL OR ACQUIRED IMMUNITY AND HEALTH STATUS

Once transmitted, the individual's natural or acquired immunity may diminish the harmful effects of a microorganism to the recipient. The recipient, if healthy and immune competent will muster self-defenses to kill or eliminate the microorganism from their body.

> **Example:** Most working adults have developed natural immunity to common childhood diseases, such as common cold viruses. This natural immunity may protect them from disease if they are exposed to these viruses in the work environment. Young children may not yet have developed natural immunity. Care should be taken to block the transmission of these viruses to employees' young children at home by removing their work environment outerwear (laboratory coats or gowns) before leaving work each day.

> **Example:** Personnel who handle human tissue/cells, blood, and body fluids in the work environment are at greater risk of transmission of HBV. Receiving hepatitis B vaccine will give workers an acquired immune response to hepatitis B and probably prevent them from developing the disease.

5.11.3 CONTRIVED BIOLOGICAL CONTAINMENT BARRIERS

Contrived biological barriers are created when infectious materials are altered in the laboratory to render them less likely to cause disease or other biological effects in unintended recipient.

Today it is possible to intentionally rearrange, add to, or eliminate some of the genes responsible for the harmful effects of microorganisms thus making them less harmful to humans. The genetically altered microorganisms are called vectors, vaccines, or gene therapy agents because they are used as carriers for human genes that are packaged inside the microbial cells. These vectors can still infect human cells, but once inside the cells they are predicted to

be less harmful than the wild-type agents. It is still important to contain these vectors by blocking their transmission from laboratories because of their potential long-term effects if released into the environment and community-at-large.

5.11.4 PHYSICAL CONTAINMENT BARRIERS

Physical containment barriers represent administrative, engineering, and PPE controls designed to control biological materials.

The objective of physical containment is to confine biohazards thus reducing the potential harmful exposure of the laboratory worker, persons outside of the laboratory, institutional visitors, the community-at-large, and the environment. Physical barriers are established in addition to the identified natural and contrived barriers. They function as a fail-safe if one or more of the biological barriers fail.

There are two functional types of physical containment:

Primary Containment: Barriers erected for the protection of personnel within the immediate laboratory. This type generally includes PPE and safety equipment.

Secondary Containment: Barriers erected for the protection of the environment external to the immediate handling area. This type generally includes facility features.

In general (but not always), primary containment barriers are the minimum requirement when the only natural mode of transmission of a microorganism or other biohazardous material is via direct contact, ingestion, or parenteral inoculation. Secondary containment barriers are always required, in addition to primary containment barriers, when an agent that is naturally transmitted by inhalation or droplet dispersal is handled, or when splashes or spills of contact-transmitted agents are possible during handling.

5.11.4.1 PERSONAL PROTECTIVE EQUIPMENT (PPE)

PPE is defined as specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (for example, uniforms, pants, shirts, or blouses) are not intended to function as protection against a hazard and are not considered PPE. The type of PPE required in microbiological/biochemical laboratories will depend upon the assigned BSL for that laboratory (see “Biosafety Levels” section 5.5).

Appropriate PPE is used to prohibit blood or OPIM to pass through to or reach the laboratory worker’s work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time that the protective equipment will be used.

All PPE shall be removed prior to leaving the laboratory.

5.11.4.1.1 LABORATORY CLOTHING

This category includes laboratory coats, gowns, aprons, and other protective body clothing. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated.

A laboratory coat is recommended for all work at BSL-1 and it or other suitable protective clothing is required when handling potentially infectious materials at BSL-2. Additional criteria for selecting clothing are: comfort, closure types and location, antistatic properties, flammability, and durability. Protective clothing must be removed and left in the

laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or autoclaved before it is taken home for laundering.

5.11.4.1.2 CLEANING, LAUNDERING, AND/OR DISPOSAL OF PERSONAL PROTECTIVE EQUIPMENT

If personal protective clothing becomes contaminated (garment is penetrated e.g., by blood or OPIM) it should be removed immediately or as soon as feasible. Contaminated laundry should be handled as little as possible with a minimum of agitation and be bagged (in red bags) or containerized without sorting or rinsing in the location of use. If contaminated laundry is sent to a facility that does not utilize Universal Precautions in the handling of all laundry, the department must ensure that the red bags are labeled with the universal biohazard symbol and the word "biohazard".

Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through or of leakage from the bag or container, the laundry should be placed and transported in bags or containers that prevent soak-through and/or leakage of fluids to the exterior.

5.11.4.1.3 GLOVES

Gloves must be worn when working with biohazards, toxic substances, or any other hazardous agents. Gloves must be selected based on the hazards involved and the activity to be conducted.

Disposable gloves are by far some of the most widely used safety products. There are many things to consider when choosing gloves, such as quality, amount of protection needed, and any allergies one may have to certain materials. The following is a brief breakdown of three types of disposable gloves:

- Nitrile – Disposable nitrile gloves are made of synthetic latex and are extremely resistant to punctures and tears. Nitrile protects against a broad range of chemicals. Another reason why nitrile gloves are a popular option in hand protection is that nitrile does not contain any natural rubber latex, so it can be used by workers with latex allergies. Nitrile gloves have replaced latex gloves in many laboratories, and nitrile long sleeve (11 ½ - 12 inch) gloves are an excellent choice since they are able to cover the user's wrist areas properly.

- Latex – Latex offers the best fit of the three glove materials presented here, which is why it is most often used by surgeons and other medical professionals. Latex provides superior dexterity and barrier protection, making it the most trusted glove material among many. However, the protein in latex gloves can cause an allergic reaction and irritation in many people. Nitrile is the best alternative if you do have a latex allergy.

- Vinyl – Vinyl is the least expensive of the three disposable glove materials presented here. It is an economical choice for use in activities that do not require the highest degree of tactile precision, such as food service applications and jobs where product protection is necessary. Vinyl does not offer as much dexterity as nitrile or latex, and tends to tear more easily and should be used only for low-risk applications.

    - It should be kept in mind that isopropanol has been shown to permeate through latex and vinyl gloves in less than 10 minutes. These types of gloves were also permeated by ethanol, but with a much lower rate. Glutaraldedyde based disinfectants were not shown to be able to permeate latex or vinyl.

Disposable, single-use gloves should be replaced as soon as possible after they have become contaminated, when their integrity has been compromised (they are torn or punctured) or when their ability to function as a barrier is compromised. Hands should be properly washed with soap and warm water after removing gloves. Disposable gloves are meant to be used only once - they must not be washed or reused. Latex gloves used in a wet procedure should be replaced after one hour of use.

Gloves should be removed and hands washed when work with potentially infectious materials is complete or when leaving the laboratory (How to safely remove disposable gloves). If you are transporting potentially infectious materials (i.e., cultures, waste, etc.) to another part of the building, use the one glove rule: use one gloved hand for handling the materials and use the other ungloved hand for touching common surfaces such as door knobs and elevator buttons. It is advisable to use carts, bottle carriers, or secondary containment trays for transportation of hazardous materials (Glove Procedures – UTA).

Temperature resistant gloves must be worn when handling hot materials or dry ice.

Utility gloves may be decontaminated for reuse if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, exhibit other signs of deterioration, or when their ability to function as a barrier is compromised.

5.11.4.1.4 MASKS, EYE PROTECTION, AND FACE SHIELDS

Masks in combination with eye protection devices, such as goggles or safety glasses with solid side shields, or chin-length face shields, have to be worn whenever splashes, spray, spatter, or droplets of blood, OPIM, or other biohazardous materials may be generated and eye, nose, or mouth contamination can be reasonably anticipated.

Whenever possible, laboratory operations should be performed in containment devices such as a BSC or fume hood, or behind a bench-top shield in order to minimize the potential for skin or mucous membrane contact with a hazardous splash. If procedures do not permit containment of the hazard with a containment device, then appropriate PPE must be worn as outlined:

- Splash goggles are the only form of eye protection approved for splash hazards. If a biological splash hazard exists (or chemical splash including bleach), splash goggles must be worn.

- Full face protection (i.e., face shield) must be used for procedures that have anticipated splashes or sprays of infectious (or other hazardous) materials to the face, or if there is a high potential for aerosol generation.

5.11.4.1.5 RESPIRATORS

For certain protocols/projects, additional PPE such as respiratory protection may be required. Respirators should be worn in rooms containing animals that have been infected with pathogens and housed in open cages. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection need to contact EH&S for assistance in selecting proper equipment, medical evaluation, initial training, and fit-testing (Respiratory Protection Program).

5.11.4.2 HOUSEKEEPING

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas that are free of significant sources of contamination. Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. To facilitate decontamination, the laboratory should be kept neat and free

of clutter - surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers, exits, and fire extinguishers must not be blocked. The storage of combustibles, e.g., cardboard boxes and paper needs to be kept to a minimum. Combustibles must not be stored within 24”of the ceiling in non-sprinkled buildings or within 18” of the sprinkled head in a sprinkled building.

5.11.4.3 LABORATORY SAFETY EQUIPMENT / OTHER LABORATORY EQUIPMENT

5.11.4.3.1 BIOSAFETY CABINETS (BSCS)

BSCs that have high efficiency particulate air (HEPA)/ultra-low particulate air (ULPA) filters are used as primary barriers against exposure to infectious biological agents. The airflow in a BSC is laminar, in other words the air moves with uniform velocity in one direction along parallel flow lines. A BSC must be used in conjunction with safe laboratory techniques, because potentially dangerous aerosols can still escape.

Depending on the design, a BSC may be vented to the outside or the air may be exhausted into the room. BSCs are not chemical fume hoods. A percentage of the air is recirculated in most types of BSCs. Therefore, the levels of explosive, flammable, or toxic materials will be concentrated within the cabinet. HEPA/ULPA filters only trap particulates, allowing any contaminant in non-particulate form to pass through the filter.

5.11.4.3.1.1 CLASSIFICATION OF BSCS

BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed. Three types of BSCs, designated as Class I, II, and III, have been developed to meet varying research and clinical needs (see Table 1 below).

5.11.4.3.1.1.1 CLASS I

Class I BSC is a ventilated cabinet for personnel protection with an unrecirculated inward airflow away from the operator. Class I BSC is similar in terms of air movement to a chemical fume hood, but has a filter in the exhaust system to protect the environment. Since the exhaust air is filtered, the user and the environment are protected, but the product inside the cabinet is not because unsterilized room air is drawn over the work surface through the front opening. The air from the cabinet is exhausted through a filter (a) into the laboratory and then to the outside of the building exhaust, (b) to the outside through the building exhaust, or (c) directly outside.

With a Class I BSC, the user's hands and arms while inside the cabinet are exposed to the infectious materials. The Class I BSC is designed for general microbiological research with low to moderate risk agents, and is useful for containment of mixers, blenders, and other equipment. The Class I BSC can also be used for work with radionuclides and volatile toxic chemicals when exhausted outdoors.

5.11.4.3.1.1.2 CLASS II

Class II BSC is a ventilated cabinet for personnel, product and environmental protection which provides inward airflow and filtered supply and exhaust air. The Class II BSC has five designs depending on how much air is recirculated and/or exhausted and if the BSC is hard-ducted to the ventilation system or not. Class II cabinets may be of use with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radionuclides; however, care must be exercised in selecting the correct Class II cabinet design for these purposes. Class II cabinets are the type most commonly used.

5.11.4.3.1.1.3 CLASS III

The Class III BSCs are often referred to as glove boxes since access to the work surface is by means of heavy duty rubber gloves, which are attached to ports in the cabinet. These BSCs are gas-tight and under negative pressure providing the highest level of personnel protection and are most suitable for work with agents that require BSL-3 or BSL-4 containment. The supply air of Class III BSC is filtered and exhaust air passes through two filters. The Class III BSC should have an attached pass-through box that can be sterilized and is equipped with a filtered exhaust. The Class III BSC may be connected to a double-door autoclave used to decontaminate all materials entering or exiting the cabinet. Several glove boxes can be joined together to extend the work surface.

Table 1: Characteristics of BSC classes/types

BSC Class, Type	Face Velocity (fpm)	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front; exhausted through HEPA to the outside or into the room	Yes	When exhausted outdoors[1,2]
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit	Yes (minute amounts)	No
II, A2	100	Similar to II, A1, but has 100 linear fpm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to outside through a canopy unit	Yes	When exhausted outdoors (formerly "B3") (minute amounts)[1,2]
II, B1	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	Yes	Yes (minute amounts)[1,2]
II, B2	100	No recirculation; total exhaust to the outside through a HEPA filter	Yes	Yes (small amounts)[1,2]
II, C1	100	BSC that can be used in a recirculating Type A-mode for standard microbiological work, or can be connected to an exhaust system to function in Type-B mode for handling hazardous chemical vapors or radionuclides		
III	N/A	Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection	Yes	Yes (small amounts)[1,2]

[1] Installation requires a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

[2] In no instance should the chemical concentration approach the lower explosion limits of the compounds.

Source: adapted from BMBL, fifth edition, Appendix A, Table 2.

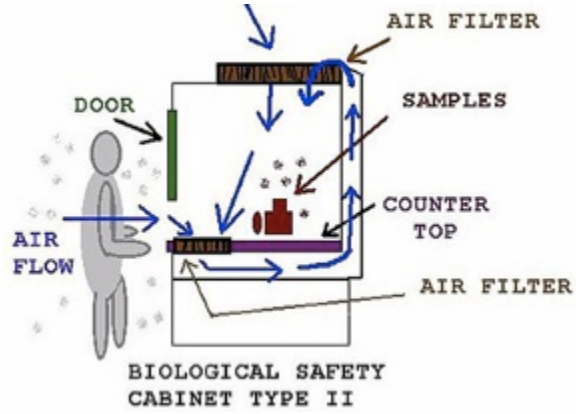


Figure 1: Class II BSCs provide an effective partial barrier system for the safe manipulation of low- and moderate-risk microorganisms. Class II cabinets are the most frequently used in research and clinical laboratories.

See also: Class II Biosafety Cabinets: Part 1 - Introduction & Type A2.

5.11.4.3.1.2 SELECTION OF A BSC

A BSC should be selected primarily in accordance with the type of protection needed: Product protection, personnel protection against different risk group microorganisms, personnel protection against exposure to radionuclides and volatile toxic chemicals, or a combination of these (see Table 2 below).

Volatile or toxic chemicals should not be used in BSCs that recirculate exhaust air to the room, i.e. Class I BSCs that are not ducted to building exhaust systems, Class II A1 BSCs, or not ducted Class II A2 BSCs. Class II A2 (ducted) and Class II B1 BSCs are acceptable for work with minute amounts of volatile chemicals and radionuclides. A Class II B2 BSC, also called a total exhaust cabinet, is necessary when significant amounts of radionuclides and volatile chemicals are expected to be used.

Table 2: Selection of a BSC through risk assessment

	Protection Provided			
Biological Risk Assessed	Personnel	Product	Environmental	BSC Class

BSL-1 - 3	Yes	No	Yes	I
BSL-1 - 3	Yes	Yes	Yes	II (A1, A2, B1, B2)
BSL-4	Yes	Yes	Yes	III; II—When used in suit room with suit

Source: adapted from BMBL, fifth edition, Appendix A, Table 1.

5.11.4.3.1.3 LOCATION OF A BSC

The velocity of air flowing through the front opening into a Class II BSC should be at minimum 100 ft/min (0.51 m/s. At this velocity the integrity of the directional air inflow can still easily be disrupted by air currents generated by people walking close to the BSC, open windows, air supply registers, and opening and shutting doors. Ideally, BSCs should be situated in a location away from traffic and potentially disturbing air currents.

BSCs not connected to an exhaust system should have at least 12 inches (30 cm) clearance from the filter face and any overhead obstructions when the cabinet is in its final operating position, to allow for testing of the exhaust HEPA/ULPA filter. Whenever possible a 6 inches (15 cm) clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance.

5.11.4.3.1.4 CERTIFICATION OF BSCS

NSF International Standard/American National Standard for Biosafety Cabinetry - NSF/ANSI 49-2018 "Biosafety Cabinetry: Design, Construction, Performance, and Field Certification" applies to Class II (laminar flow) biosafety cabinetry designed to minimize hazards inherent in work with agents assigned to BSLs 1, 2, 3, or 4. It also defines the tests that shall be passed by such cabinetry to meet this standard. This standard includes basic requirements for the design, construction, and performance of BSCs that are intended to provide personnel, product, and environmental protection; reliable operation; durability and structural stability; cleanability; limitations on noise level; illumination; vibration; and motor / blower performance. Cabinets that meet the standard and are certified by NSF bear an NSF mark.

The operational integrity of a BSC must be validated before it is placed into service, after it has been repaired or relocated, and annually thereafter by qualified technicians. BMBL strongly recommends that accredited field certifiers be used to test and certify BSCs. Certification of a BSC includes tests for cabinet integrity, HEPA/ULPA filter leaks, down flow velocity profile, face velocity, negative pressure/ventilation rate, air-flow smoke pattern, and alarms and interlocks. The certification sticker is placed on the front panel of the BSC.

EH&S employs an outside contractor to perform all BSC and clean air bench certifications. All BSCs shall be certified annually or whenever a significant change has been made to the operational characteristics of the system or as per request or newly installed units or units being moved. EH&S keeps an inventory list of BSCs on the UTA campus and monitors due dates, sends reminders to the PIs for the annual certifications, and maintains records of these. The UTA departments/PIs are responsible for the certification/decontamination/repair/replacement part(s) costs. PIs/research personnel should notify EH&S at 817-272-2185 when any new BSCs have been purchased.

5.11.4.3.1.5 DECONTAMINATION OF BSCS

BSCs must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination is performed by a qualified professional.

5.11.4.3.1.6 WORKING IN A CLASS II BSC

As with work on open bench tops, work performed within a BSC must be performed carefully and safely. To avoid contamination and the risk of personnel exposure, best practices should be followed to reduce and control splatter and aerosol generation and arranging the workflow "from clean to contaminated". In particular, open flames cause disruption of the airflow inside, and they are not necessary within the clean environment of a Class II BSC. Once work inside a BSC has been completed, it is necessary to decontaminate the surfaces of the BSC as also other laboratory equipment and materials. Suggested work practices and procedures for minimizing risks when working in Class II BSCs are detailed in Class II Biosafety Cabinets: Proper Use.

5.11.4.3.1.7 OPERATION AND MAINTENANCE OF BSCS

Most BSCs are designed to permit operation 24 hours a day and continuous operation can help to control the levels of dust and particulate materials in the laboratory. Class II A1 and II A2 BSCs exhausting to the room or connected by thimble connections to dedicated exhaust ducts can be turned off when not in use. Other types such as Class II B1 and II B2 BSCs, which have hard-duct installations, must have airflow through them at all times to help maintain room air balance. Cabinets should be turned on for several minutes before beginning work and after completion of work to allow the cabinet to “purge” (i.e. to allow time for contaminated air to be removed from the cabinet environment).

All repairs of BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported to the PI and the BSC repaired before it is used again.

5.11.4.3.1.8 ULTRAVIOLET LIGHTS

UV lights are not required in BSCs even though many BSCs are equipped with them. If they are used, they must be cleaned weekly with an alcohol and water mixture to remove any dust/dirt/film that may inhibit the germicidal effectiveness of the UV light. UV bulb should not be cleaned when it is on or hot; UV bulb should be turned off and allowed to come to room temperature. UV bulb should not be touched with ungloved hands as oils from hands can dirty the bulb.

The intensity of the UV light diminishes over time. The lamps should be checked periodically with a UV meter to ensure the appropriate UV intensity. UV light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate. UV lights must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure. The BSC sash must be closed when the UV lamp is on. Some units are interlocked so that the sash must be closed before the UV light will go on. When lights are left on for extended periods of time (e.g.

overnight may be much longer than necessary), this will decrease the effective life span of the lamp. UV light may damage materials stored within the BSC, including plastics and rubber materials, e.g. aspirator tubing. It is recommended that materials are **not** stored in the BSC when not in use.

UV germicidal light limitations also include:

- **Penetration:** UV light does not penetrate soil or dust. Microorganisms beneath any dirt or dust particles are not affected by the UV radiation. Areas hidden or shadowed from the light are not disinfected.

- **Relative humidity:** Humidity decreases the effectiveness of UV light. Antimicrobial effects of UV light drops off precipitously above 70% relative humidity.

- **Temperature and air movement:** Optimum temperature for UV light output is 77 to 80°F (25 to 27°C). Temperatures below this optimum temperature result in reduced output of the antimicrobial wavelength. Moving air tends to cool the lamp below its optimum operating temperature and result in reduced output, therefore the BSC blower should be off when the UV light is on.

5.11.4.3.1.9 OPEN FLAMES / STERILE, DISPOSABLE INOCULATING LOOPS, NEEDLES, AND CELL SPREADERS

As mentioned earlier, open flames disrupt the airflow patterns of a BSC. Additionally, the heat produced by the continuous flame may damage the HEPA/ULPA filter. Sterile, disposable inoculating loops, needles and cell spreaders are available as an alternative to using open flames in the BSC for sterilizing equipment. Sterilization of inoculating loops or needles in an open flame also generates small particle aerosols which may contain viable microorganisms. The disposable transfer loops, needles, and cell spreaders should be placed in disinfectant after use and discarded as described in the Biological (or Special waste) Section 8.

5.11.4.3.1.10 SPILLS

When a spill of biohazardous material occurs within a BSC, cleanup should begin immediately, while the cabinet continues to operate. See the “Biohazard Spills inside a Biological Safety Cabinet” section 6.2 for information on spill cleanup procedures.

5.11.4.3.1.11 ALARMS

BSCs can be equipped with one of two kinds of alarms. Sash alarms are found only on cabinets with sliding sash. The alarm signifies that the sash has been moved to an improper position. Airflow alarms indicate a disruption in the cabinet’s normal airflow pattern. This represents an immediate danger to the operator or product. When an airflow alarm sounds, work should cease immediately and the PI should be notified. Manufacturer’s instruction manuals provide further details.

5.11.4.3.2 CLEAN BENCHES

Clean benches are not considered laboratory safety equipment. However, they deserve to be mentioned because they may be confused with BSCs. Clean benches direct filtered air over the work area to protect biological specimens from particulate contamination by bathing the work area with filtered air that is free of particulate contamination.

Because they do not provide protection to the user, they should not be used in conjunction with biohazardous material, toxins, or radionuclides.

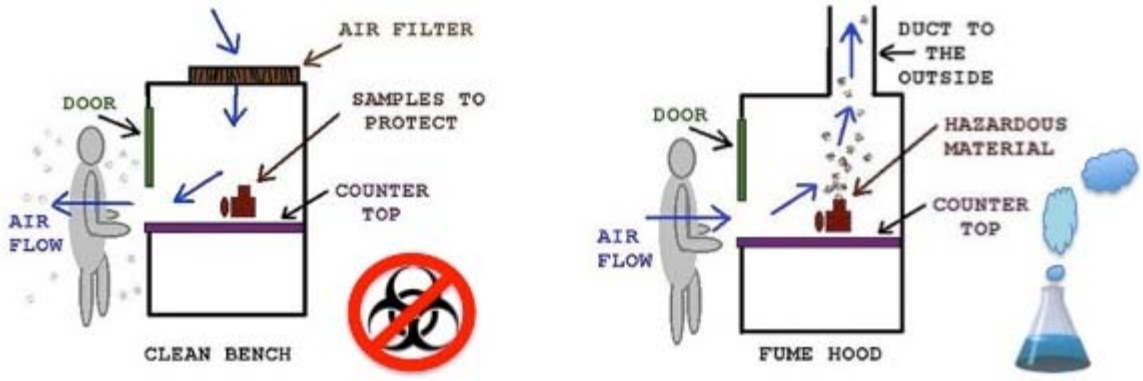


Figure 2: Clean benches protect research products from contamination but do not afford protection to the user. Thus, clean benches should not be used when working with potentially infectious materials, chemical hazards or radioactivity. Use fume hoods when working with hazardous chemicals.

5.11.4.3.3 PIPETTES AND PIPETTING DEVICES

Pipettes are among the most commonly-used pieces of equipment in the biological laboratory, and their misuse has been related to a significant number of LAIs. Regrettably, many laboratory workers were taught to pipette by mouth, even after the associated hazards were recognized. With the availability of mechanical pipetting devices, **mouth pipetting is now strictly prohibited**. Even harmless materials should never be mouth-pipetted since one may, at some time, mistakenly mouth-pipet something that is hazardous.

Aerosols can be generated when a liquid is dropped from a pipette onto a work surface, when cultures are mixed by alternate sucking and blowing, and when the last drop is blown out of a pipette. Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette. To minimize aerosol production, drain a pipet with the tip against the inner wall of the receiving vessel. Never forcibly expel any biohazardous material from a pipette. The inhalation of aerosols unavoidably generated during pipetting operations can be prevented by working in a BSC.

Pipetting devices should be selected with care. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean. Plugged (aerosol resistant) pipette tips should be used when manipulating microorganisms and cell cultures.

5.11.4.3.4 CENTRIFUGES, SONICATORS, HOMOGENIZERS, SHAKERS, AND BLENDERS

All of these instruments can create aerosols, and this must be considered with each use. If hazardous materials such as carcinogens, highly toxic, or infectious agents are going to be placed in any of these instruments, then precautions must be taken to prevent an exposure of laboratory personnel to aerosols or liquids.

5.11.4.3.4.1 CENTRIFUGES

Centrifugation is an operation that involves a lot of energy and finely-tuned mechanical instruments. There are many documented cases of occupational laboratory exposures due to centrifugation accidents, which nearly always result from aerosol exposure to the room occupants. To minimize the risk of mechanical failure, centrifuges should be on a strict maintenance schedule, used according to the manufacturer's instructions, and be routinely inspected to ensure leakage is not occurring. Users should be properly trained in centrifuge operation, and safety precautions should be prominently posted on each unit.

Aerosols are also created by activities such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatants, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube with infectious materials breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the procedures in Table 4 should be followed:

Table 4: Working safely with centrifuges

Use sealed tubes placed inside of capped safety buckets (safety cups) that seal with O-rings. Before use, inspect tubes, O-rings, cups, and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil or loose caps to close centrifuge tubes.
Fill, open, and seal centrifuge tubes, rotors, and accessories inside a BSC. Avoid overfilling of centrifuge tubes so closures will not become wet while spinning in a horizontal or angled position.
After tubes are filled and sealed, wipe them down with disinfectant before placing them in the rotor. Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
Always balance buckets, tubes, and rotors properly before centrifugation.
Select the type of centrifuge tube (glass type, or plastic-polymer type) that is best suited to the chemicals you will be using and the speed at which you are spinning to avoid melted or shattered tubes.
Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosols to settle before opening the tube.
Small low-speed centrifuges may be placed in a BSC during use to reduce exposure to escaped aerosols.

5.11.4.3.4.2 SONICATORS, HOMOGENIZERS, SHAKERS, AND BLENDERS

Operation of sonicators, homogenizers, shakers, and blenders or similar instruments may create hazardous aerosols and lead to exposure of personnel unless extreme caution is exercised. Depending on the nature of the material being used in these instruments, it may be necessary for them to be used or opened only in a BSC.

Sonicators should be operated in BSCs or covered with shields during use. The shields and outsides of sonicator should be decontaminated after use. In addition, hearing protection may be required while using a sonicator.

Kitchen homogenizers are not sealed and they release aerosols. Only equipment designed for laboratory use should be used. Their construction minimizes or prevents release of aerosols.

Biological shakers are instruments used to agitate a collection of biological samples simultaneously. Shakers consist of a motor attached to a flat surface with fasteners for securing laboratory ware whose contents require mixing. All

points on the surface move in the same fashion within the x-y plane, either back and forth (reciprocal shakers) or in a circular motion (orbital shakers).The principal application of shakers is for growing yeast, bacteria, or mammalian cells in specialized containers known as shaker bottles. Shaking promotes the growth of cells and microorganisms by improving aeration and oxygen transfer. Shakers that start or stop abruptly will cause fluid to splash up, creating opportunities for contamination and loss of material.

Household blenders do not prevent the spread of aerosols, and thus only safety blenders should be used. Safety blenders have been designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. Blenders should be loaded, operated and unloaded in a BSC when used in conjunction with potentially infectious materials. The use of glass blender jars is not recommended because of the breakage potential. A towel moistened with disinfectant should be placed over the top of the blender during use. Blender jars should be allowed to rest for at least one minute to allow the aerosols to settle before opening them. The device should be decontaminated promptly after use. The lid and gasket should be inspected routinely to ensure that they are in good condition and that the lid fits tightly.

5.11.4.3.5 SHARPS

Generally, the use of sharps should be restricted to procedures for which there is no alternative. Situations where the use of sharps may be appropriate include parenteral injection, phlebotomy, and aspiration of fluids. Plastic alternatives should be substituted for glassware whenever possible to prevent the unnecessary potential for sharps related exposure incidents.

The following practices should be adhered to if it has been determined that the use of sharps is unavoidable:

- All personnel should be trained in safe sharps handling procedures.
- Use disposable sharps devices (i.e., scalpels, needles) if possible.
- Procedures should be organized in a manner that limits personnel exposure to the sharp device. For example:
    - Do not expose/unsheathe sharp devices until the procedure actually requires the use of these items.
    - Do not leave exposed sharp items unattended.
    - If feasible, place a sharps container within arm's reach of the point of use to allow for immediate disposal.
    - For reusable sharps, use a hard-walled container that encloses the sharp end of the device for storage until processing for reuse.
- To avoid accidental sticks, hypodermic needles must be placed directly into the containers and not recapped, bent, broken, clipped, or removed from disposable syringes.
- Do not handle sharps with two hands.
- Dispose of waste sharps in a properly labeled sharps container. See the “Biological (or Special waste)” Section 8: 8.4 “Sharps”.

- Permanently close and make request for disposal of sharps containers when they are ¾ full through CEMS by following the instructions in the Standard Operating Procedure – Request for Biological Waste Removal. Do not overfill or shake containers because these actions can result in accidental sharps exposure.

- Broken glassware should be handled with a mechanical device, such as tongs, forceps, or a broom and dustpan, never directly by hand.

5.11.4.3.6 RECOMMENDED WORK PRACTICES: LYOPHILIZERS AND AMPOULES

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 BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized, and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

6 EMERGENCY PROCEDURES FOR BIOHAZARDOUS SPILLS

If there is an emergency or if anyone is in danger, immediately call the UTA Police Dispatch, 817-272-3003, for assistance. Explain the nature and the extent of the emergency being as specific and detailed as possible. Emergency personnel will be dispatched to help you. A list of emergency numbers should be posted in the laboratory.

If, however, there is no immediate threat to health, you should use your best judgment to decide whether to call for help or to address the matter yourself. The guidelines below are intended to help you make that determination.

Prioritize your actions to deal with the spill:

- Call UTA Police Dispatch at 817- 272-3003 for help if there is an emergency.

- Notify EH&S, 817-272-2185.

- Notify your PI.

- Determine exactly what has been spilled and proceed as stated below.

Biohazardous materials must first enter the body through a specific exposure route. Once in the body the host's immune response determines whether infection will occur. Knowing the identity of the infectious agent and the route of exposure is essential in being able to clean up spilled biohazardous materials safely.

However, if the spilled material contains a mixture of chemical, biological, and radioactive materials, consider the threats separately and address them in the following order:

- Chemical hazards. Many chemicals can cause immediate injury and you should address these first.
- Biological hazards. Address these second.
- Radioactive hazards. Radioactive materials can spread very easily, and can be difficult to clean up. Time of exposure and distance from the material are key factors in determining a dose; the shorter the exposure time and the further away from it you are, the smaller the dose. Address radioactive hazards as soon as possible. Refer to the UTA Radiation Safety Manual.

Advanced preparation by the PI to deal with a biohazardous spill is essential. Spill cleanup procedures that are appropriate for the materials used in the laboratory need to be developed. Having a carefully planned biological hazards spill kit stored in a suitable place and updated regularly will make cleanup of accidently spilled materials easier and also give a peace of mind in the laboratory. Anyone working with biohazardous materials must receive training in spill cleanup appropriate for materials routinely used. Later in this chapter is a list of recommended items for a biological hazards spill kit (section 6.6).

The procedures below should be followed to address a spill of biohazardous materials, either outside or inside a BSC, outside the laboratory during transport, or when the spill happens in a centrifuge.

6.1 BIOHAZARD SPILLS OUTSIDE A BIOSAFETY CABINET

The following guidance is general in nature and the exact clean-up and decontamination procedures will depend on the biological organisms of concern, the size of the spill, the concentration of the organisms, and the spill location, etc. Decontamination is defined as: reduction of microorganisms on a surface or item to an acceptable level by the use of physical or chemical means so that the organisms are no longer capable of transmitting infectious particles and the surface or item is rendered safe for routine handling, use, or disposal. If you need assistance and are not in an emergency situation, consult with the Biological Safety Specialist 817-272-2185.

- Energetic spills (e.g. dropping a culture flask with 100 ml of liquid that breaks, or dropping liquid alone) will create airborne droplets of culture. Avoid breathing any airborne materials (aerosols). Holding your breath, leave the room immediately and close the door(s).
- Warn others not to enter the contaminated area and request assistance.
- If clothing is known or suspected to be contaminated, remove garments with care (gently folding the contaminated area inward), and store them in an autoclavable biohazard bag or properly-labeled closable container before autoclaving according to standard directions. If you suspect that your shoes have been contaminated, remove them and place in a separate biohazard bag for decontamination.
- Thoroughly wash your hands, face, and any other exposed areas of the body. If the spilled material soaked through laboratory clothing, take a complete body shower. Use safety showers and eyewashes as appropriate. If the spill involves potential exposure to BBPs, follow UTA's bloodborne pathogen policy (Exposure Control Plan for Bloodborne Pathogens) for managing and reporting these exposures.

- Wait at least 30 minutes to allow aerosols to settle before entering the contaminated area.

- Put on appropriate PPE and equipment (lab coat/gown, gloves, mask/eye protection, face shield, and respiratory protection if necessary) after assembling the needed cleanup materials and before re-entering the room. If your gloves are not puncture-resistant, be especially careful if the spill involves broken glass or other sharps. Remove any sharp, contaminated objects from the spill area using mechanical means (like tongs or forceps), **never with hands**. Discard contaminated sharps in a sturdy, leakproof, with a biohazard sign labeled sharps container.

- Apply appropriate disinfectant (follow the labeled directions for mixing) for the agent involved in the spill with a gentle flooding action to avoid secondary aerosols.

- Cover excess liquids with absorbent material, such as paper towels soaked with the disinfectant.

- Allow an adequate contact time for disinfectant to work (follow the labeled directions for contact time). Then wipe up the spill working toward the center of the spill.

- Use disinfectant solution to wipe over surrounding areas that are likely to have been contaminated with aerosols and splashes.

- Decontaminate boots, or if using shoe covers discard them. Discard the gloves, mask, and either discard or decontaminate eye protection, face shield, and clothing used during the cleanup.

- Place all contaminated spill cleanup materials (paper towels, gloves, etc.) into an autoclavable bag/container and autoclave it according to standard directions, or send a request for waste disposal through the Chemical Environmental Management System (CEMS).

- Wash hands thoroughly.

6.2 BIOHAZARD SPILLS INSIDE A BIOSAFETY CABINET

- Spill cleanup procedures should be initiated at once while the BSC continues to operate to prevent escape of contaminants from the cabinet. Notify the PI before starting the spill cleanup.

- If the spill in the BSC is quite substantial (spill flows past the work surface through the front or rear grilles), it may be necessary to decontaminate the cabinet's fans, filters, and airflow plenums. An outside company must do this. Call EH&S at 817-272-2185 for assistance.

- Put on appropriate PPE and equipment (lab coat/gown, gloves, mask/eye protection, face shield, and respiratory protection if necessary).

- Cover small spill with paper towels or other absorbent material. Slowly pour disinfectant solution which is appropriate for the agent involved around the spill and allow to flow into the spill. Paper towels soaked with the disinfectant may also be used to cover the area. Flood the top work surface tray and, if it is a Class II BSC, the drain pans and catch basins below the work surface with the decontaminant. At least thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. The directions of the disinfectant manufacturer should be followed.

- Iodophores are recommended for research laboratories at a 0.5% concentration. Iodophores are active against Gram-negative and -positive bacteria, viruses, fungi, yeast, *M. tuberculosis*, and many bacterial and fungal spores. Iodophores are also non-staining, nontoxic, and active in hard water. Household bleach in 1:10 dilution (0.5% sodium hypochlorite) is not recommended for BSC decontamination since bleach causes corrosion on stainless steel. It is also good to remember that alcohols are not sporicidal and they evaporate quickly.

- Wipe up spill, work surfaces, walls, and any equipment in the cabinet with paper towels dampened with a solution of an appropriate disinfectant for the agent involved. Do not place your head in the cabinet to clean the spill—keep your face behind the sash.

- If your gloves are not puncture-resistant, be especially careful if the spill involves broken glass or other sharps. Remove any sharp, contaminated objects from the spill area using mechanical means (like tongs or forceps), **never with hands**. Discard contaminated sharps in a sturdy, leakproof, with a biohazard sign labeled sharps container.

- Remove excess disinfectant from the tray by wiping with a sponge or cloth soaked in disinfectant. For Class II BSC, drain the tray into the catch basin below the work surface. Use an appropriate container to drain disinfectant from the BSC base and autoclave according to standard procedures.

- Lift out the tray and removable front intake grille and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a decontaminant.

- Replace the tray and front intake grille.

- Remove any contaminated PPE in a manner to avoid cross-contamination and dispose of per standard laboratory practices. Be sure to place reusable items (cloths, cleanup materials) into an autoclavable container to be autoclaved. Place contaminated disposable gloves, paper towels and other spill cleanup materials in autoclavable biohazard bags or autoclavable pans with lids for autoclaving, or send a request for waste disposal through the Chemical Environmental Management System (CEMS).

- Wash hands thoroughly.

6.3 BIOHAZARD SPILLS OUTSIDE THE LABORATORY (DURING TRANSPORT)

If a biohazardous agent spills during transport outside the laboratory, the main difference from the "Biohazard Spills outside a BSC" procedure is to initiate the cleanup **immediately**. The procedures stated earlier for **"**Biohazard Spills outside a BSC" should be followed to clean up the spill. Notify EH&S, 817-272-2185, who can assist you and ensure that the cleanup is done correctly, especially if the spill could affect others in the facility.

6.4 BIOHAZARD SPILLS IN A CENTRIFUGE

When a spill or leak has occurred within a centrifuge, the procedure for cleanup depends upon the risk group of the sample involved as well as the construction of the equipment.

Centrifuges with sealed rotors or buckets that are able to be autoclaved should be sterilized by steam according to the autoclave procedure.

For centrifuges with non-sealed rotors and centrifuges not able to be autoclaved, allow 30 minutes for aerosols to settle first. Place the rotor or bucket in an appropriate non-corrosive disinfectant solution. Keep in mind that bleach will corrode stainless steel if left in contact with it for 30 minutes or more. After disinfection, remove larger pieces of broken glass/sharps using forceps and place in a biohazards sharps container. Carefully wipe the internal surfaces of the centrifuge bowl with disinfectant.

6.5 BLOOD SPILLS

For 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 container for biohazardous sharps.
- Using a detergent solution, clean the spill site of all visible blood.
- Spray the spill site with 10% household bleach and allow to air-dry for 15 minutes.

6.6 BIOLOGICAL HAZARDS SPILL KIT

A well-designed biological hazards spill kit is highly recommended. The following items would be excellent choices for a kit:

- "DO NOT ENTER" sign to be posted on the laboratory door.
- An appropriate chemical disinfectant. In most cases a 10% household bleach solution is a good choice, but keep in mind that bleach will corrode stainless steel if left in contact with it for 30 minutes or more. Whenever you use bleach to clean up spills of an infectious agent, always prepare a fresh solution. Overtime, a bleach and water solution will lose its effectiveness for decontamination. For human blood and body fluids, iodophors or 70% alcohol is appropriate.
- Materials to absorb liquids after decontamination. This could include paper towels, absorbent lab pads, or special materials designed to absorb large volumes of liquid. Keep in mind the volumes of liquid typically used in the laboratory area when selecting an absorbent.
- Appropriate PPE to wear during cleanup. Disposable gloves and a long-sleeved laboratory coat or gown are always necessary. Eye protection is needed. Facial protection should also be considered for large spills.
- A mechanical means for handling broken glass. Broken glass represents a high cutting danger. Do not touch it directly, especially if it is contaminated with a biohazardous agent. Mechanical means could include tongs, forceps, small disposable scoops and sponges, autoclavable dustpans, or any other method that prevents direct contact with the broken glass.

- Biohazard bags, container for biohazardous sharps, and/or other containers to place the material in for further treatment and disposal.

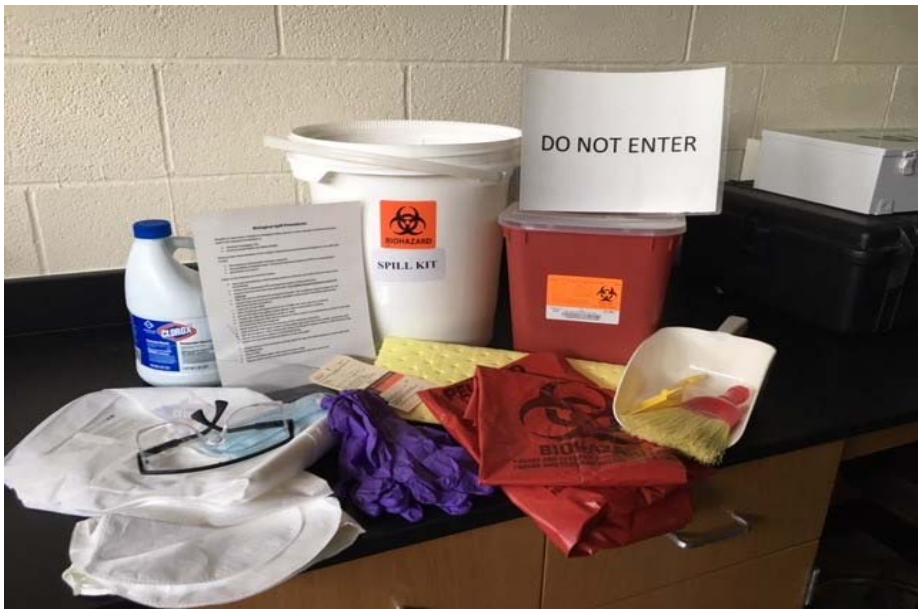


Figure 3: A well-designed biological hazards spill kit is highly recommended.

6.7 AEROSOLIZATION

Aerosolization can happen in the laboratory e.g., when:

- Centrifuges are opened after a bottle/rotor leakage.
- Shaking incubators are opened after a container has leaked or broken.
- Experiments involve working with aerosolization chambers.

The following actions are recommended in the case of aerosolization to address contamination with biohazardous materials:

- Hold your breath and immediately leave the room.
- Remove PPE carefully making sure to turn the exposed areas inward.
- Wash hands well with soap and water.
- Post “DO NOT ENTER” sign on laboratory entry; laboratory should stay evacuated for at least 30 minutes.
- PI must clear laboratory for re-entry. For extensive BSL-2 material contamination (i.e., centrifuge incident), EH&S must be notified and will assume responsibility, in conjunction with the PI, to clear the laboratory for re-entry.

6.8 RADIOACTIVE BIOHAZARD SPILLS

EH&S has established a Radiation Safety Program. Radiation safety is the responsibility of all faculty, staff, and students who are involved in the use of radioactive materials, radiation-producing machines, or laser devices. Please, see Radiation Safety Manual for more information.

A biohazardous spill involving radioactive material requires emergency procedures that are different from the procedures used for either material alone. Anyone working with both radioactive and biohazardous materials should develop a spill cleanup plan appropriate for all materials used. Use procedures that protect you from the radiochemical while you disinfect the biological material. Before any cleanup, consider the type of radionuclide, characteristics of the microorganism, and the volume of the spill. Decontamination procedures involving the use of bleach may be incompatible with some radioactive materials, especially those containing radioiodine. Contact the Radiation Safety Officer at 817-272-2185 for additional information concerning isotope cleanup 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 with water rinse.

6.9 HYGIENE PRACTICES FOLLOWING CLEANUP OF BIOHAZARD SPILLS

- Laboratory personnel must be cognizant of their PPE following the cleanup of biohazard spills to ensure no contaminated material and/or fluid is present that could be aerosolized.
- All disposable PPE should be placed in biohazard bags for disposal.
- Laboratory personnel must completely decontaminate their reusable PPE prior to removal for washing.
- Most importantly, laboratory personnel must thoroughly wash their hands, wrists, and forearms following PPE removal.

7 DECONTAMINATION

Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., laboratory bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

Many different terms are used for disinfection and sterilization. The following are among the most common in biosafety:

- *Antimicrobial* - An agent that kills microorganisms or suppresses their growth and multiplication.
- *Antiseptic* - A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.
- *Biocide* - A general term for any agent that kills organisms.
- *Chemical germicide* - A chemical or a mixture of chemicals used to kill microorganisms.
- *Disinfectant* - A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- *Microbicide* - A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of "biocide", "chemical germicide" or "antimicrobial."
- *Sporocide* - A chemical or mixture of chemicals used to kill microorganisms and spores.

To be effective, sterilization requires time, contact, temperature, and, with steam sterilization, high pressure. The effectiveness of any method of sterilization is also dependent upon four other factors:

- The type of microorganism present: some microorganisms are very difficult to kill while others die easily.
- The number of microorganisms present: it is much easier to kill one organism than many.
- The amount and type of organic material that protects the microorganisms: dirt, blood, tissue remaining, and other organic matter on items to be sterilized act as a shield to microorganisms during the sterilization process.
- The number of cracks and crevices on items to be sterilized might harbor microorganisms: microorganisms collected in scratches, cracks and crevices are protected.

When choosing a method of decontamination, it is important to consider the following aspects:

- Type of biohazardous agents, concentration and potential for exposure.
- Physical and chemical hazards to products, materials, environment and personnel.

7.1 CLEANING LABORATORY MATERIALS BEFORE DECONTAMINATION

Dirt, soil, and organic matter shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides, and disinfectants). Pre-cleaning is essential to achieve proper disinfection or sterilization since many germicidal products claim activity only on pre-cleaned items. Pre-cleaning is used to remove dirt, organic matter, and stains and can be accomplished by brushing, vacuuming, dry dusting, washing or damp mopping with water containing soap or detergent. Pre-cleaning must be carried out with care to avoid exposure to infections agents. Materials used for cleaning must be chemically compatible with the germicides that are applied later. It is quite common to use the same chemical germicide for pre-cleaning and disinfection.

7.2 DIFFERENT WAYS TO DECONTAMINATE

Physical and chemical means of decontamination fall into four main categories:

- Heat
- Liquid chemicals
- Vapors and gases
- Radiation

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide, formaldehyde), radiation, and wet heat (steam sterilization in an autoclave) are used.

7.2.1 HEAT

In order to kill microorganisms, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the microbial cells resulting in shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 250-270°F (121-132°C). This type of heat kills all microbial cells including spores, which are normally heat resistant.

Standard Conditions for Steam Sterilization
Steam sterilization (Gravity): Temperature should be 250°F (121°C), pressure 103 kilopascal (kPa) (15 pounds-force/inch^2 (lbf/in^2 or psi), 20 minutes for unwrapped items, 30 minutes for wrapped items.
Steam sterilization (Gravity): At a higher temperature of 270°F (132°C), pressure should be 207 kPa (30 lbf/in^2 or psi), 15 minutes for wrapped items.
Note: Pressure settings (kPa or lbf/in^2) may vary slightly depending on the sterilizer used. When possible, follow manufacturers' recommendations.

In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 320-338°F (160-170°C) for periods of 2 to 4 hours.

7.2.1.1 DECONTAMINATION OF BIOHAZARDOUS WASTE BY AUTOCLAVING

Autoclaving is accepted as a safe and effective procedure for sterilization. There are several autoclaves on the UTA campus that are used for decontamination of biohazardous waste. To ensure that any biohazardous waste created by the UTA community is properly decontaminated by autoclaving, EH&S tests the performance of these autoclaves on a regular basis. Biological test ampoules with heat resistant spores (*Geobacillus stearothermophilus* spores) are used by EH&S to monitor the autoclave cycle inside the chamber. Chemical indicators (steam chemical integrator strips) are used to indicate that adequate sterilization conditions are reached every time biohazardous waste is decontaminated by autoclaving. Please refer to section 8.3.1 "Steam Sterilization" and section 8.3.1.1 "Autoclaves Used for Biological Waste Treatments".

Persons using autoclaving for decontamination of biohazardous waste are encouraged to take online training: On-Site Biohazardous Waste Management: Autoclaving. The training can be accessed at https://uta-ehs.org/.

7.2.2 CHEMICALS USED AS DISINFECTANTS

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

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

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

The relative resistance to the action of chemical decontaminants may be altered substantially by such factors such as:

- Concentration of active ingredient
- Duration of contact
- PH
- Temperature
- Humidity
- Presence of extrinsic organic matter

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

The appropriate disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when using chemical disinfectants are:

- Nature of surface being disinfected: the more porous and rough the surface, the longer contact time will be needed for a disinfectant to be effective.
- Number of microorganisms present: higher microbial concentrations require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms: microbial agents can be classified according to increasing resistance to disinfectants and heat (see Table 5 below).
- Presence of organic material: the proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature: increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity of disinfectants and longer exposure time might be needed.

Table 5: Increasing Resistance of Microbes to Chemical Disinfectants

LEAST RESISTANT		**Examples**
	LIPID OR MEDIUM-SIZE VIRUSES	HSV Cytomegalovirus HBV HIV
	VEGETATIVE BACTERIA	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella choleraesuis*
	FUNGI	*Trichophuton* sp. *Cryptococcus* sp. *Candida* sp.
	NONLIPID OR SMALL VIRUSES	Poliovirus Coxsackievirus Rhinovirus
\/	**MYCOBACTERIA**	*Mycobacterium tuberculosis* *Mycobacterium bovis*
	BACTERIAL SPORES	*Bacillus subtilis* spores *Clostridium sporogenes* spores
MOST RESISTANT		

7.2.2.1 EPA REGULATIONS OF DISINFECTANTS

The U.S. Environmental Protection Agency (EPA) regulates pesticides, including chemical disinfectants which are required to be registered with the EPA. When using disinfectants, it is important to follow the directions on the manufacturer's label, including those for concentration and contact time, to ensure compliance with the EPA requirements.

7.2.2.2 PROPERTIES OF SOME COMMON DECONTAMINANTS

There are many different disinfectants available under a variety of trade names. In general, these can be categorized as acids, alcohols, aldehydes, alkalines, amines, halogens, heavy metal salts, ketones, and quaternary ammonium compounds. Unfortunately, the most effective disinfectants are often corrosive and toxic. Some of the common decontaminants are presented below.

7.2.2.2.1 ALCOHOLS

Ethyl or isopropyl alcohols in concentration of 70% by weight are good general-use disinfectants. Both lose effectiveness at concentrations below 50% and above 90%. Alcohols denature proteins and are somewhat slow in germicidal action. However, alcohols are effective decontaminants against lipid-containing viruses. A contact time of ten minutes is generally employed in efficacy tests with disinfectants. Due to the high evaporation rate of alcohols, repeated applications may be required to achieve the required ten minute contact time for decontamination. Isopropyl alcohol is generally more effective against vegetative bacteria and ethyl alcohol is a more virucidal agent.

7.2.2.2.2 FORMALDEHYDE

Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration referred to as formalin, or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures, and the pungent, irritating odor makes formaldehyde solutions difficult to use in the laboratory.

Formaldehyde vapor generated from solution is an effective space decontaminant for buildings or rooms, but in the vapor state in the presence of water it tends to polymerize on surfaces to form persistent paraformaldehyde. Heating paraformaldehyde to depolymerize it can liberate formaldehyde gas. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas than is the case in using formaldehyde vapor. Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like BSCs. Applied in closed systems under controlled conditions (e.g., humidity) formaldehyde gas can be used to achieve sterility (formaldehyde vapor and gas possess germicidal properties). Formaldehyde is a toxic substance and a suspected human carcinogen. Formaldehyde creates respiratory problems at low levels of concentration and thus considerable caution must be exercised in handling, storing, and using formaldehyde.

7.2.2.2.3 GLUTARALDEHYDE

This compound, although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehyde are irritating to the eyes, nasal passages and upper respiratory tract. Glutaraldehyde decontaminants should always be used in accordance with the instructions on the label and the appropriate PPE.

7.2.2.2.4 PHENOL AND PHENOL DERIVATIVES

Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Phenol is also toxic and appropriate PPE is necessary during application. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants and they come in various concentrations ranging primarily from 5% to 10%. These derivatives also have an odor which can be somewhat unpleasant. The phenolic disinfectants are used frequently for disinfection of contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including *Mycobacterium tuberculosis*, fungi, and lipid-containing viruses. They are not active against spores or nonlipid viruses.

7.2.2.2.5 QUATERNARY AMMONIUM COMPOUNDS (QUATS)

Quaternary Ammonium Compounds (Quats) are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. They are less active against Gram-negative bacteria and are not active against nonlipid viruses. Quats are easily inactivated by organic materials, anionic detergents (such as soap), or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic, odorless, stable, non-staining, non-corrosive to metals, and inexpensive. Quats can be used for decontamination as well as for general cleaning.

7.2.2.2.6 HALOGENS (CHLORINE AND IODINE)

Chlorine is a universal decontaminant and active against many microorganisms, including bacterial spores. Sodium hypochlorite is the most common base for chlorine disinfectants, and it is found in common household bleach (app. 5% or 50,000 ppm available sodium hypochlorite). A solution of household bleach prepared daily is an inexpensive and effective germicide. Concentrations ranging from approximately 500 ppm (1:100 dilution of household bleach or 1% household bleach solution) sodium hypochlorite to 5,000 ppm (1:10 dilution of household bleach or 10% household bleach solution) sodium hypochlorite are effective depending on the amount of organic material (e.g., blood) present on the surface to be cleaned and disinfected. Diluted solutions, once exposed to light or air, rapidly degrade. Chlorine is a strong oxidizing agent and corrosive to metals. Appropriate PPE must always be used when using chlorine for decontamination. At high concentrations and extended contact time, sodium hypochlorite solutions are considered chemical sterilants since they will inactivate bacterial spores.

The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants for laboratory use is the iodophors (organically bound iodine). Iodophors will kill vegetative bacteria but not spores, bacteria causing tuberculosis, or nonlipid viruses. Clean surfaces or clear water can be effectively treated with 75 ppm available iodine, but difficulties may be experienced if any noticeable amount of protein is present. For iodophors it is critical that the written instructions are followed. Higher concentrations of iodophores are actually less effective, as the iodine is bound to itself or the carrier molecule. For washing hands or for use as a sporicide, iodophors can be diluted 1:10 in 50% ethyl alcohol. This mixture will offer relatively rapid inactivation of many occurring microorganisms. Iodophores are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

7.2.2.3 SELECTING CHEMICAL DISINFECTANTS

No single chemical disinfectant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical disinfectants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to

be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

- What is the target organism(s)?
- What disinfectants, in what form, are known to, or can be expected to, inactivate the target organism(s)?
- What degree of inactivation is required?
- In what menstruum is the organism suspended (i.e. simple or complex, on solid or porous surface, or in air)?
- What is the highest concentration of organisms anticipated to be encountered?
- Can the disinfectant, either as a liquid, vapor, or gas, be expected to contact the organism and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility of materials?
- What is the stability of the disinfectant in used 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?

The primary target of decontamination in the laboratory is the organism(s) under investigation. Laboratory preparations or cultures usually have titers in excess of those normally observed in nature. Inactivation of these materials presents other problems since agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active moieties of chemical disinfectants. Such interference with the desired action of disinfectants may require higher concentrations and longer contact times than those shown to be effective in the test tube.

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 nonlipid viruses and bacteria with a waxy coating, such as *M. tuberculosis*, occupy a midrange of resistance. Spore forms and unconventional (slow) viruses, now known as prions, 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.

Individual investigators should conclusively determine the efficacy of any of the disinfectants. It is readily evident that each of the disinfectants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora.

7.2.3 IONIZING RADIATION STERILIZATION

Gamma and X-rays are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices. UV radiation is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. UV radiation is successfully used in the destruction of airborne microorganisms. The sterilizing capabilities of UV light, such as that found in BSCs, are limited on surfaces because of UV light's lack of penetrating power.

8 BIOLOGICAL (OR SPECIAL) WASTE

8.1 DEFINITION OF BIOLOGICAL (OR SPECIAL) WASTE

The Texas Department of State Health Services (TDSHS) has identified biological or special waste as requiring special handling to protect human health or the environment. The items selected for regulation were deemed to have the highest potential to transmit infectious disease(s) if improperly treated or handled.

The term "biological (or special) waste" refers to regulated waste that includes the following categories:

- microbiological waste
- sharps
- human blood, blood products, and other potentially infectious materials
- pathological waste
- animal waste and bedding of animals intentionally exposed to pathogens

In Texas, disposal of regulated waste is controlled by the Texas Department of State Health Services (TDSHS) and the Texas Commission on Environmental Quality (TCEQ).

8.2 BIOLOGICAL WASTE MANAGEMENT AND DISPOSAL INSTRUCTIONS

Treatment of all laboratory biological waste prior to disposal is good laboratory practice, but biohazardous waste must be treated prior to disposal. Biohazardous waste that is mixed with hazardous chemical waste, radioactive waste, or both must be treated to eliminate the biohazard prior to disposal. After treatment, the waste must be managed as hazardous chemical waste or as radioactive waste through EH&S.

All generators of biohazardous waste and sharps must strictly adhere to the following UTA waste disposal guidelines. Biohazardous waste is defined as all biologically contaminated waste that could potentially cause harm to humans, domestic/wild animals or plants. Examples include human blood, certain body fluids, and cells/tissues, recombinant/synthetic nucleic acid molecules, and human, animal or plant pathogens. Common decontamination methods for biohazardous waste include heat sterilization (e.g., autoclaving), chemical disinfection and incineration.

8.2.1 LIQUID BIOHAZARDOUS MATERIALS (SUCH AS BACTERIAL CULTURES IN LIQUID MEDIA, HUMAN BLOOD, BODY FLUIDS OF ANIMALS EXPERIMENTALLY INFECTED WITH PATHOGENS, ETC.)

Decontaminate by autoclaving (follow Standard Operating Procedure: Steam Autoclaves) or treat with an appropriate chemical disinfectant for the sufficient contact time. After decontamination, liquids may be disposed of by pouring them down the drain to the sanitary sewer if they do not contain hazardous chemicals or radioactive materials. No liquids should be put in regular trash or dumpsters.

8.2.2 DISPOSABLE SOLID ITEMS (NON-SHARPS, AND NOT ANIMAL CARCASSES, TISSUES OR BEDDING)

Collect all non-sharp disposable items (such as gloves, plastic ware, Kim wipes, etc.) contaminated with biohazardous materials in leak-proof autoclavable biohazard bags with universal biohazard symbol. This bag needs to be stored in a container, preferably with a lid, to keep the contents securely inside. Transport biohazardous waste from the laboratory to an autoclave area in a closed, leak proof bag or container. Contain bags in a leak proof tray. Do not leave non-inactivated waste unattended. When getting ready to autoclave, place items to be treated (autoclavable bags with waste or glassware with liquid biowaste) in heat-resistant plastic or metal container. Never place items in direct contact with the bottom of the autoclave. Place the container containing the items to be sterilized on the shelf or rack of the autoclave. Proceed as stated in the Standard Operating Procedure: Steam Autoclaves. Alternatively, collect all non-sharp disposable solid items contaminated with biohazardous materials in biowaste boxes lined with red biohazard bag (Stericycle boxes and liners, available through EH&S). These sturdy, pre-printed cardboard biowaste boxes displaying the biohazard sign are used as the terminal receptacle. Do not overfill the boxes: boxes need to be closable and weigh no more than 43 lbs. While wearing PPE (gloves, safety glasses, and lab coat) gather the four edges of the red bag from the sides of the container. Twist the top of the bag to seal its contents. Secure the seal with a strong, hand-tied single or gooseneck knot to prevent any leakage if inverted. You can also use a zip tie or tape to secure the knot. Ensure that the bag is completely closed. To close the auto-locking Stericycle box, engage the top flaps and secure both sides to produce handles for carrying. The red bag should not be visible after the container has been closed. If red bag is showing, this is considered an improperly closed package and is an U.S. Department of Transportation (DOT) violation. See SOP: How to Properly Package Biohazard (Stericycle) Boxes. In order for the biowaste boxes to be picked up, please send a request for disposal through CEMS by following the instructions in the Standard Operating Procedure – Request for Biological Waste Removal. Stericycle boxes do not need inventory tags as hazardous chemical waste containers do.

8.2.3 RECOMMENDED WORK PRACTICES WITH AUTOCLAVES

The following PPE should be worn when operating an autoclave:

- Heat resistant autoclave gloves for loading and unloading the autoclave.
- Rubber apron in addition to rubber sleeve protectors when removing items from the autoclave.
- Splash goggles/face shield to protect against a splash.

The following procedure is recommended for the decontamination of biohazardous waste that has been gathered in approved autoclavable bags or containers:

- Items in approved autoclavable bags or containers need to be in rigid, autoclavable secondary containers.
- Add one cup of water to each bag of solid waste
- Attach a Steam Chemical Integrator inside each autoclave bag using autoclave tape and close bags only loosely. Steam cannot penetrate closed bags.
- Follow the guidelines set by the posted autoclave parameter signs when setting the cycle time.

- To prevent spills and accidents, be sure that the exhaust setting is appropriate for the type of material you are autoclaving. Fast exhaust should be used for solid items/solid waste and slow exhaust for liquids/liquid waste.

- After the cycle is complete, let the load cool before removing container with treated biowaste from the autoclave.

- Securely close the autoclaved bag.

- Attach: **"Treated in accordance with the provisions of 25 TAC § 1.136(a)"** sticker to the bag that has been autoclaved.

- Place treated autoclave bags into opaque bags and close them securely before disposing with normal trash.

8.2.4 NON-DISPOSABLE OR REUSABLE ITEMS

Decontaminate non-disposable or reusable items (such as equipment, glassware, bench tops, etc.) contaminated with biohazardous materials by autoclaving or by using a chemical disinfectant (such as 10% bleach, a quaternary ammonium compound, an iodophore, etc.). Choose a chemical disinfectant appropriate for the specific biohazardous material being used and allow for sufficient contact time.

8.2.5 ANIMAL CARCASSES, BODY PARTS, TISSUES, BLOOD, SERUM, PLASMA/OR OTHER BLOOD COMPONENTS AND BEDDING INFECTED WITH HUMAN PATHOGENS

Collect solid items in leak-proof autoclavable biohazard bags for incineration or liquid waste in containers for autoclaving. Bags needs to be stored in a container, preferably with a lid, to keep the contents securely inside. If animal carcasses/body parts/tissues are stored frozen before incinerated, they need to be double bagged.

8.3 TREATMENT METHODS

Biohazardous waste can either be treated on-site by laboratory personnel or can be given to EH&S for disposal. Acceptable methods of treatment and disposal of biowaste at UTA include steam sterilization, chemical disinfection, and incineration.

8.3.1 STEAM STERILIZATION

To allow sufficient steam access/penetration to the waste, the waste shall be packaged and loaded into the autoclave chamber according to the instructions given by EH&S in the Standard Operating Procedure: Steam Autoclaves, "Autoclaving Biohazardous Waste Materials Inside Heat resistant Autoclavable bags" section, and autoclave operated according to the recommendations provided by the manufacturer. Strong oxidizing materials (chemicals) must not be autoclaved with organic material: Oxidizer + Organic Material + Heat = Possible Explosion.

When subjecting waste to steam under pressure:

- The temperature in the autoclave chamber must reach at least 250°F (121°C).

- The gauge pressure must be at least 15 lbf/in^2 or psi, 20 minutes for unwrapped items, 30 minutes for wrapped items.

- The treatment time must be at least 50 minutes.

Do not pour melted agarose down the drain. Allow it to cool and solidify, then dispose of as solid waste in waste bags.

8.3.1.1 AUTOCLAVES USED FOR BIOLOGICAL WASTE TREATMENTS

Autoclaves used for biohazardous waste treatments are tested periodically by EH&S for their effectiveness through the use of biological indicators (*Geobacillus stearothermophilus* spores) (Standard Operating Procedure: Steam Chemical Integrators – Sterilization Assurance – Steam Autoclave Kill Cycle).

8.3.2 CHEMICAL DISINFECTION

Use a chemical agent that is registered with the EPA in accordance with the manufacturer's instructions.

8.3.2.1 LIQUID BIOLOGICAL WASTE

One option for disinfecting BSL-1 and BSL-2 liquid waste for drain disposal is to use bleach. Bleach, a sodium hypochlorite solution (NaOCl), is a broad-spectrum disinfectant that is an effective disinfectant for enveloped viruses (e.g. HIV, HBV, herpes simplex virus, HSV), vegetative bacteria (e.g. *Pseudomonas, Staphylococcus, and Salmonella*), fungi (e.g. *Candida*), mycobacterium (e.g. *Mycobacterium tuberculosis* and *M. bovis*), and non-enveloped viruses (e.g. adenovirus and parvovirus).

Liquid biological waste, including blood, blood products, cultures and stocks of etiological agents and viruses, cell culture material, and products of recombinant/synthetic nucleic acid molecules technology, may be disinfected by adding household bleach to the liquid to be decontaminated until a 10% concentration of household bleach is achieved (or approximately 5,000 ppm NaOCl). This mixture is made by adding 1 part household bleach into 9 parts liquid biological waste (e.g., 100 ml of household bleach into 900 ml of biological waste in a 1 L container). Let bleach-waste mixture stand for at least 30 minutes. This time might need to be longer depending on the extent of the organic matter that is present. After an appropriate contact time, the mixture can be poured into a sink drain connected to the campus sewage system. Be sure to follow the treated material with a copious amount of water. Do not pour bleach-waste mixture into a storm drain.

Recommended PPE when handling bleach includes laboratory coat or plastic apron, latex or nitrile gloves, and safety glasses or chemical safety goggles (recommended). 10% bleach solution is caustic and thus direct contact with skin and eyes must be avoided. The bleach solution should be prepared in a well-ventilated area.

Please, see Standard Operating Procedure: Proper Use of Bleach (Sodium Hypochlorite) as a Chemical Disinfectant in Biolaboratories for more information.

8.3.2.2 SOLID BIOLOGICAL WASTE

Decontaminate solid biohazardous materials by immersing the waste in a liquid chemical disinfectant appropriate for the specific biohazard and allow for sufficient contact time.

Waste that has been immersed in a liquid disinfectant (e.g., a freshly prepared solution of household bleach diluted 1:10 with water or a solution of 70% by volume isopropyl alcohol) must be thoroughly drained before disposal.

8.3.3 DISPOSAL OF STEAM STERILIZED OR CHEMICALLY DISINFECTED BIOLOGICAL WASTE

Biological waste that has been treated in accordance with the methods described above can be disposed of through the regular trash as long as the following procedures are followed:

- Place a label on the original bag or container stating:

  "Treated in accordance with the provisions of 25 TAC § 1.136(a)"

  relating to approved methods of treatment and disposition.

- Place the bag or other container into another bag or container that is a different color and opaque, e.g., a black or green trash bag.

Treated biological waste in a liquid form can be disposed of through the sanitary sewer with a copious amount of water.

8.3.4 INCINERATION

The incineration (combustion) of carbon-based materials in an oxygen-rich environment typically at temperatures higher than 850°C, produces a waste gas composed primarily of carbon dioxide (CO2) and water (H2O). Other air emissions are nitrogen oxides, sulphur dioxide, etc. The inorganic content of the waste is converted to ash. This is the most common and well-proven thermal process using a wide variety of fuels.

Incineration is used for disposing of animal carcasses at UTA. The incinerator is located on the roof of the Life Science Building. Log books for the incinerator are located in the Life Science Building, Biology Department Office and ACF and also with EH&S. Please, refer to section 8.8 "Record Keeping" to learn what type of information about the incineration process must be entered into the log books.

8.3.4.1 OPERATIONAL PROCEDURES FOR THE USE OF THE INCINERATOR

- The incinerator is designed to destroy up to 100 pounds of biological waste per hour. The incinerator should not be overloaded.
- The incinerator is operated only during daylight hours.
- Aerosol cans, closed containers, or flammable liquids must not be burned in the incinerator**.**
- The incinerator must not be used to burn paper such as office records, computer paper, or telephone books.
- Before incinerating, waste must be weighed (scale is situated in the Life Science Building 6th floor room 606).
- When loading waste into the incinerator, make sure that all waste is on the burning chamber hearth (not on the door block) and is not obstructing air passages.
- Close the door, set the timer for a minimum of two hours, and let it burn down.

- Do not open the door or add more trash until the load has burned. This could cause two problems:
    - overloading of the incinerator
    - the possibility of explosion which could injure the operator
- If the main charging door is opened while the incinerator is operating, the primary burner is shut off by the door safety switch.
- Always open and close charging door slowly to avoid flame and smoke puffs.
- Do not use water to cool hot refractory or brick.
- Description of incinerated waste needs to be logged in log book (see section 8.8 “Record Keeping”).
- EH&S arranges the cleaning of the incinerator on regular basis (approximately two times a year). If ashes build up above the bottom of the burner port or obstruct air passages, please contact EH&S to arrange the cleaning of the incinerator.

8.4 SHARPS

A sharp is an item that could cause cuts or punctures. Sharps include:

- Hypodermic needles
- Hypodermic syringes with attached needles (syringe body is not considered to be a sharp). If a syringe body is contaminated with medicine it is classified as pharmaceutical waste.
- Scalpels and blades
- Razor blades
- Disposable razors
- Pasteur pipettes
- Broken glassware

Infected sharps are classified as hazardous/special waste. This waste must be stored, transported and disposed of as hazardous/special waste to make sure it does not cause a risk to human health or the environment. Hazardous/special waste must not be mixed with other waste or with other types of hazardous/special waste. Waste must be segregated so that different wastes types do not get contaminated.

Sharps containers need to be kept in each work area where sharps are used. Collect all sharps contaminated with biohazardous materials in rigid, leak proof, puncture resistant red containers which have been labeled with the universal biohazard symbol. These sharps containers are available through EH&S. Follow the instructions given in Tips and Information for Laboratory Personnel to prevent needle sticks. After using a needle, do not re-cap, bend, break, remove it from the syringe, or manipulate it in any way. Many people have been accidentally stuck with a

needle during the process of re-capping it. Simply place the needle and other sharps into a sharps container to prevent any injuries. Please, see: Needle Safety Poster.

Collect sharps that have never been contaminated with biohazardous materials (e.g., used only with chemicals) in containers labeled as "Non-Biological Sharps". Sharps contaminated with biohazardous materials shall be disposed of as infectious waste. EH&S will pick up and dispose of sharps containers at no charge. To have the sharps containers picked up, please send a request for disposal through CEMS by following the instructions in the Standard Operating Procedure – Request for Biological Waste Removal.

When sharps waste is generated it is important that:

- The containers are not overfilled (sharps containers should not be more than ¾ full when picked up)
- Sharps containers are not disposed of with the regular trash
- Sharps containers are not incinerated

8.5 BROKEN GLASS THAT HAS NEVER BEEN CONTAMINATED WITH BIOHAZARDOUS MATERIALS

Place non-contaminated broken glassware into broken glass containers that are provided by Custodial Services, the chemical stockroom (Chemistry Department), or EH&S. Custodial Services will dispose of the full broken glass containers. Please, close and secure the broken glass container lid with tape, mark the container as trash, and deposit outside the laboratory for pickup.

8.6 HUMAN BLOOD, BLOOD PRODUCTS, AND OTHER POTENTIALLY INFECTIOUS MATERIALS

Human blood and blood products mean:

- Discarded human blood waste
- Serum
- Plasma
- Other blood components, materials containing free-flowing blood and blood products

The following human body fluids are referred to as other potentially infectious materials (OPIM) considering that these materials may also contain BBP, including HBV and HIV:

- Semen
- Vaginal secretions
- Cerebrospinal fluid
- Synovial fluid

- Pleural fluid
- Pericardial fluid
- Peritoneal fluid
- Amniotic fluid
- Saliva (in dental procedures)
- Any body fluid that is visibly contaminated with blood
- All body fluids in situations where it is difficult or impossible to differentiate between body fluids

All liquid human blood, blood products, and OPIM need to be gathered in waste containers marked with the biohazard sign and stored in secondary containment. Contents need to be listed on the attached tag.

Items contaminated with human blood, blood products, or OPIM can be incinerated. Materials need to be transported in leakproof containers to the incinerator located on the roof of the Life Science Building. If you need special assistance regarding incineration, contact EH&S at 817-272-2185. Alternatively, collect items contaminated with human blood, blood products, or OPIM in biowaste boxes lined with red biohazard bag (Stericycle boxes, available through EH&S). These biowaste boxes are used as the terminal receptacle.

In order for the liquid blood, blood products, OPIM waste, or biowaste boxes to be picked up, please send a request for disposal through CEMS by following the instructions in the Standard Operating Procedure – Request for Biological Waste Removal. Stericycle boxes do not need waste tags as hazardous chemical waste containers do.

8.7 BEDDING OF ANIMALS INTENTIONALLY EXPOSED TO PATHOGENS AND ANIMAL WASTE

Dispose of all bedding of animals intentionally exposed to pathogens, animal carcasses, body parts, and items contaminated with blood/blood products in the incinerator located on the roof of the Life Science Building. Double-bag all animal waste to prevent leakage when transporting it to the incinerator. If you need special assistance regarding incineration contact EH&S at 817-272-2185.

8.8 RECORD KEEPING

Personnel who treat and dispose special wastes onsite in accordance with the guidelines described in this section must keep the following records:

- Date of treatment (also time for incineration)
- Amount of waste treated
- Method/conditions of treatment
- Name (printed) and initials of person(s) performing treatment
- For generators of more than 50 pounds per month, a written procedure for the operation and testing of any equipment used and a written procedure for the preparation of any chemicals used in treatment

Personnel must maintain records for three years and must have them available for review on request.

Biological Waste Management and Disposal Instructions apply only to biohazardous/biological waste streams. Radioactive waste and EPA regulated chemical waste should be handled as specified in the UTA Radiation Safety Manual and the UTA Laboratory Safety Manual (Chemical Hygiene Plan), respectively.

Contact EH&S at 817-272-2185 if you are unable to treat and dispose of biological waste yourself, and with any questions or concerns regarding waste disposal.

9 SAFETY TRAINING

9.1 LABORATORY SAFETY TRAINING REQUIREMENTS

Laboratory safety training requirements depend on the nature of work being done. Employees, students, and PIs should select training courses based on the types of hazards that may encounter. Everyone must be properly trained before beginning their work, given new assignments, or when new hazards are introduced to guarantee a safe, accident-free, and healthy work environment in the laboratories. Each person is also responsible for knowing the safety requirements and standards for their area or work and to abide by them. PIs must instill a positive attitude and awareness of the Culture of Safety in their laboratory workers through training and adding discussions concerning laboratory safety in their regularly scheduled laboratory meetings. Safety and health is every bit as important as productivity and quality. If a job cannot be done safely, it should not be done.

For the laboratory safety program to be effective, everyone in the laboratory must cooperate and do their part. It is important that communication is kept open at all times. Workers who notice hazards or other safety problems, or feel that they need additional training should notify their PI. PIs and management shall address these concerns and take corrective action when warranted.

Meeting safe work practices and training requirements at UTA is a cooperative effort. Excellent safety and health conditions do not occur by chance. They are the result of diligent work and careful attention to safe work practices by everyone:

- **All laboratory personnel** who work with or around hazardous chemicals are required to take "General Laboratory Safety Trainings" (see below).
- **PIs** are responsible for providing and documenting the initial and continuing safety training necessary to allow employees/students to perform their work appropriately (see "Site Specific Safety Training" below).
- **PIs** should frequently observe employees/students work habits and promptly correct unsafe habits.
- **Employees/students** are responsible for performing their work in a safe and responsible manner. Knowledge of appropriate work practices and health and safety rules is essential.
- **All laboratory personnel** need to stay current up to date with safety and training information by visiting the EH&S website and/or contacting EH&S with questions regarding safety training requirements. EH&S "Maverick Safety Matters" Newsletters are published two times per year and they are an excellent source of safety tips for all UTA employees and students.

9.2 GENERAL LABORATORY SAFETY TRAININGS: THE HAZARD COMMUNICATION AND WASTE MANAGEMENT TRAINING AND BLOODBORNE PATHOGEN TRAINING

The Hazard Communication and Waste Management Training must be completed by all UTA employees and students who use, handle, or transfer hazardous chemicals at their workplace--Hazard Communication training is a state requirement. This requirement also applies to visiting scientist and part-time workers. The training can be accessed at https://uta-ehs.org/.

All personnel/students at UTA with occupational exposure to blood or OPIM need to complete **the Bloodborne Pathogens for Laboratory Research Personnel Training** annually, also located at https://uta-ehs.org.

An UTA email address is required to access both of the above mentioned trainings.

9.3 SITE SPECIFIC SAFETY TRAINING

In addition to general laboratory safety trainings, **Site Specific Safety Training** needs to be conducted and documented on the Site Specific Training Record. It is the responsibility of the PI or his/her representative to conduct Site Specific Safety Training with all laboratory personnel. Site Specific Safety Training must include (but is not limited to) the following topics: Safe handling of any chemical(s) a person might use, that personnel know where the safety data sheets (SDS) info is and how to find and read the info easily, how to handle/label chemicals if taken out of the original containers, where the safety showers/eye washing stations are located, proper use of PPE (laboratory coats, gloves, eye protection, etc.), where the first aid kit/fire extinguisher are located, etc.

Laboratory personnel, including students, working with RG-1/RG-2 agents or in BSL-1/BSL-2 laboratory areas must also be trained and be proficient in BSL-1/BSL-2 practices. A copy of the CDC/NIH publication BMBL must be available/easily accessible to all laboratory personnel in BSL-2 laboratories. All persons working in the laboratory areas must be advised of the hazards and nature of the research being conducted. Standard microbiological practices (see section 10.1 “Standard Microbiological Practices”) are essential to create a safe work environment in BSL-1 and BSL-2 laboratories. It is ideal that training and education on these practices and procedures start at the undergraduate level.

After the Site Specific Safety Training, PI or his/her representative needs to ask the trainee to sign the Site Specific Training Record (Site Specific Training Record), to document the training. A copy needs to be kept for the trainer’s records and the completed original form sent to EH&S, Box 19257 / ehsafety@uta.edu.

9.4 OTHER TRAININGS

9.4.1 BIOSAFETY LEVEL 2 (BSL-2) TRAINING

Biosafety Level 2 (BSL-2) Training must be completed by all UTA employees and students who are getting ready to work in a BSL-2 laboratory. The topics covered are:

- Biosafety containment levels
- Laboratory facilities (secondary barriers)
- Safety equipment (PPE and primary barriers)
- General laboratory procedures in BSL-2 laboratories
- Aerosol-producing procedures
- Biohazard spill cleaning procedures

- Disinfection in biolaboratories
- General cleaning
- Autoclaving
- BSL-2 waste handling and disposal procedures at UTA
- Health hazards – biological exposures
- Shipment on/off campus

The training can be accessed at https://uta-ehs.org/. Use your UTA Net I.D. and password to log in to the training.

9.4.2 WORKING WITH RECOMBINANT/SYNTHETIC NUCLEIC ACID MOLECULES

In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:

(i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;
(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
(iii) molecules that result from the replication of those described in (i) or (ii) above.

Research Administration online training is required for research personnel working with recombinant or synthetic nucleic acid molecules.

9.4.3 VACCINIA TRAINING

Prior to being given access to a laboratory that harbors vaccinia virus and/or other Orthopoxviruses, information needs to be given to employees or research personnel of infectious vaccinia virus. Please, read "Guidelines for Working with Vaccinia Virus and Other Orthopoxviruses" and take the Vaccinia Virus Training that can be accessed at https://uta-ehs.org/.

Using the "Verification of Training on Vaccinia Virus, Recombinant Vaccinia Viruses, or Other Orthopoxviruses that Can Infect Humans" (CO-LS-F9), you must verify that you have been adequately informed, are aware of the potential for exposure, and understand the risks and symptoms associated with the vaccinia virus. Please, submit this completed verification form to EH&S, Box 19257 / ehsafety@uta.edu attention Biological Safety Specialist.

10 SAFETY PRACTICES IN BIOLOGICAL LABORATORIES

This chapter is meant to be a quick reference aid for employees, students, supervisors and PIs. "Safety Practices in Biological Laboratories" have been outlined with safety in mind to help laboratory personnel to find the safest way to perform their work tasks and in the same time comply with safety standards. Modifications or adjustments may be necessary to meet specific laboratory or area objectives. EH&S encourages the use of best available technology and hopes that laboratory personnel will first read these suggestions, and then, if needed, research other ways or other equipment to safely reach their goals.

If you find a better way to safely perform a task, please notify EH&S. If it is applicable to other departments, your safe method may be included in this section of the manual in the future. Alternatively, if you are asked to perform a work task that you feel has potential for biohazard exposure and the topic is not covered herein, please call EH&S at 817-272-2185 for assistance.

The "Safety Practices in Biological Laboratories" section is dynamic and will change often. Please check back for new safety techniques and modifications to existing techniques. Please remember that **a safe work place is the first step in producing quality experimental results.**

10.1 STANDARD MICROBIOLOGICAL PRACTICES

Standard microbiological practices represent the minimum biosafety practices acceptable at UTA for BSL-1 and BSL-2 laboratories. The following is the exact wording from the CDC/NIH publication BMBL, accompanied by the EH&S interpretation for the purpose of routine laboratory safety inspections:

BMBL GUIDELINES	EH&S INSPECTION CRITERIA
1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.	The room must have a closable door to corridors or adjacent rooms. The door must remain closed during normal working hours. Rooms without doors cannot be approved as laboratories. BSL-2 rooms must have lockable doors. Research laboratories where work involves handling human blood / OPIM / tissue/cells shall have card-reader(s) to limit access to only those who have completed the appropriate requirements.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.	Frequent hand washing is required. Gloves, dirty laboratory coats, or other PPE may not be worn outside of the laboratory. Non-disposable laboratory coats/gowns must be autoclaved before taking them home to be laundered.
3. Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.	Any sign of food/drinks in laboratories (coffee cups, lunch bags in refrigerators, food items in the trash, etc.) indicates non-compliance with BSL-1 and BSL-2 practices.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.	Mouth pipetting is not allowed in UTA laboratories.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.	In laboratories that produce sharps, sharps containers must be properly maintained and readily accessible. They need to be marked with biohazard sign, if applicable. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.	When aerosol generating equipment is used in a room, tube lids and equipment covers must be securely in place. Face splashguards or other face protection must also be available in the room and used when applicable.

BMBL GUIDELINES	EH&S INSPECTION CRITERIA
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.	Work surfaces must be clutter free. Proper disinfectant must be available.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. 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.	All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Biohazard (Stericycle) boxes with contaminated items must not be overfilled or used for liquids. Biohazard boxes are picked-up by EH&S and waste autoclaved offsite. See: SOP How to Properly Package Biohazard (Stericycle) Boxes. Liquid wastes may be decontaminated by appropriate cold disinfectant with sufficient exposure time, and can then be poured down the sink with copious amount of water.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.	All areas where BSL-2 agents are handled must have a sign with the universal biohazard symbol and biological agent list on the door(s) exiting to the corridor(s). Laboratory door sign must indicate the name(s) and phone number(s) of the investigator(s) overseeing the laboratory operations, and emergency contact information, including name and phone number.
10. An effective integrated pest management program is required.	Rodent or insect infestations must be promptly reported to facility services for them to start extermination.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.	Please, refer to sections 9 “Safety Training”, 5.6.1.1 “Hepatitis B Vaccination Program” and 5.8 “Occupational Health and Safety in the Animal Care and Use Program.

10.2 HAND WASHING AND HAND DECONTAMINATION

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling biohazardous materials and/or animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate hands, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 20 seconds, rinsed in clean water and dried. Foot- or elbow-operated faucets are recommended. If these are not available, a paper towel should be used to turn off the faucet handles to avoid re-contaminating washed hands.

Alcohol-based hand-rubs may be used to decontaminate lightly soiled hands when proper hand-washing is not available. The use of hand-rubs should be followed up with a soap and water wash as soon as possible.

10.3 EATING, DRINKING, AND SIMILAR ACTIVITIES IN THE LABORATORY

Eating, drinking, chewing gum, applying cosmetics or lip balm, handling contact lenses, taking medications, or similar activities in biological laboratories may result in an accidental ingestion of hazardous materials (biological as well as chemical and/or radiological) and therefore these activities are strictly prohibited in all UTA biological laboratory spaces. This requirement helps to prevent the ingestion of hazardous materials, which can occur by touching one's mouth with contaminated hands, eating from a container that is contaminated, eating food that has come into contact with hazardous materials accidentally.

Food and drinks shall not be kept in laboratory refrigerators, freezers, ice machines, shelves, cabinets, or on countertops/bench tops where potentially infectious materials are present.

In addition to prohibiting eating, drinking, and other similar activities, here are some other actions that can be taken to prevent exposure to hazardous materials by ingestion:

- Remove gloves and wash your hands before leaving the laboratory.
- Wash your hands before handling anything (chewing gum, food) which goes into your mouth.
- Use the water fountains for a drink, never a laboratory faucet.
- Never use chemicals (salt, sugar, etc.) from the laboratory or stockroom on food.
- Never use laboratory glassware as a food or drink container.
- Never consume ice from a laboratory ice machine.

10.4 LIQUID NITROGEN SAFETY

Liquid nitrogen presents a unique hazard since at -321ºF (-196°C) it can cause severe freezer burns. Cryo-aprons, elbow length cryo-gloves, and face shields should be worn to prevent burns when adding or removing samples from liquid nitrogen freezers and adding liquid nitrogen to freezers and dewars. Dimethyl sulfoxide (DMSO) is often used prior to freezing cells in liquid nitrogen. While DMSO is not known to be acutely hazardous, it is volatile and enhances percutaneous penetration of water insoluble organic molecules through the skin, eyes and lungs. DMSO has a strange smell resulting in a bitter taste in the mouth after exposure. It is best to add DMSO to cell suspensions inside of an exhausted BSC or fume hood. To avoid DMSO exposure, non-volatile glycerin can often be substituted for DMSO when freezing cells.

10.5 CRYOGENIC VIAL SAFETY

A common problem with cryogenic vial removal from liquid nitrogen freezers is a "pop and spew" that happens when the temperature is quickly raised and liquid nitrogen trapped inside the vial rapidly expands to the gas phase causing the vial to explode, destroying the sample and possibly causing injury. Several types of vials are commercially available and the following precautions will help you avoid exposures from spewing vials upon removal from liquid nitrogen freezers.

CRYOGENIC VIAL SAFETY PRECAUTIONS
Always keep apron, hard plastic face shield, and cold resistant gloves beside each liquid nitrogen station so that they are easy to reach when adding or removing samples.
Never use old or dated vials (plastic or glass). Inspect each vial closely before filling.

CRYOGENIC VIAL SAFETY PRECAUTIONS
Lift ampoules very slowly from the dewar to atmospheric pressure. If an ampoule is going to spew, it will do so early in the warm-up process and while still inside the freezer.
It is advisable when removing vials from a liquid nitrogen freezer, to ask others to leave the room, to put on a particulate respirator, a face shield, heavy gloves, and a rubber apron over a disposable laboratory gown before beginning the task.
Place high hazard sample vials (concentrated human pathogens) inside boxes before placing them in the freezer.
Always disinfect the outside of cryogenic vials and ampoules before touching them with bare hands or place them on a laboratory counter. They may have been contaminated with pathogens released from previous spewing vials.
Be sure to decontaminate the inside of liquid nitrogen freezers with an appropriate disinfectant before performing maintenance.

10.6 MOLECULAR BIOLOGY SAFETY

10.6.1 ETHIDIUM BROMIDE

Ethidium bromide (EtBr) is the most commonly used stain for detecting DNA/RNA in molecular biology laboratories. EtBr is highly toxic, mutagenic, and potentially carcinogenic. EtBr is available in a powdered or liquid form.

Wear gloves and a dust mask for protection when handling this chemical in powdered form. To avoid creating aerosols, weigh the powder into vial inside of a chemical fume hood. Clean any equipment that was used to handle the chemical, so that others do not spread it by touch. It is best to order the liquid form rather than the powdered form to avoid inhaling the dust when weighing.

Dispose of EtBr solutions, gels, or other materials contaminated with EtBr (or EtBr alternatives like SYBR Safe™, GelRed™, GelGreen™, or MegaFluor™) as stated in the Ethidium Bromide Use and Disposal SOP and use CEMS to request a chemical waste pick-up.

10.6.2 OTHER MOLECULAR BIOLOGY CHEMICALS

Some chemicals are volatile and should only be used in the chemical fume hood to avoid inhaling vapors. Many nonvolatile powdered chemicals are dangerous upon inhalation of dust, skin contact, or ingestion. Always check the SDS before using chemicals. It is best to get powdered chemicals into a liquid form (stock solution) for routine work, if possible. Select the type of glove appropriate for the chemical because some chemicals can be absorbed through latex gloves. Double gloving can be helpful to avoid percutaneous absorption. Always clean the area after use so others will not be exposed and discard gloves when you are finished to prevent cross-contamination of pagers, bench tops, etc.

10.6.3 ULTRAVIOLET TRANS-ILLUMINATORS

Only purchase and use UV illuminating equipment that comes equipped with UV-blocking safety covers. Leave covers in place while working with the trans-illuminator. When photographing, only use viewing eyepieces that have built in UV absorbing windows. If using handheld cameras that sit directly on the gel, keep the hinged UV blocking cover between you and the UV light source at all times. Set up your item to be photographed with the UV lamp off. You can mask off areas with cardboard if necessary to capture stray UV light. Always wear UV safety glasses or a UV face shield and cover all exposed skin areas using gloves and long sleeve laboratory coats for maximum protection before

turning on the UV lamp. Place UV warning signs on all equipment emitting UV light in the laboratory area and warn others in the vicinity that could receive an unintentional dose of UV light before you turn on the lamp.

10.7 GERMICIDAL LAMPS

UV radiation can be used to kill viruses, mycoplasma, bacteria, and fungi in air and water. UV light has limited ability to kill these agents on surfaces because of UV light's lack of penetrating power and shadow casting. UV light is a carcinogen, and its use must be strictly controlled. Skin and eye burns caused by UV exposure are common acute hazards in research laboratories and enough exposure can destroy eyesight. Reflective glare from UV germicidal lamps within a room can add to the UV exposure burden of people in that area. Do not rely on UV light as the only method to decontaminate BSCs. Chemical disinfection of cabinet surfaces is effective and less hazardous than the use of UV germicidal lamps.

INFORMATION REGARDING UV LAMP USE INSIDE BSC FOR DECONTAMINATION OF SURFACES
UV lamps are ineffective if the lamp is not wiped weekly with 70% alcohol and the lamp output decreases below 40 microwatts per square centimeter of incidental light surface area.
Lamps fail to render adequate germicidal activity if the light frequency falls below 220 nm. The UV light spectrum range is 15 to 330 nm – the blue light range. The peak germicidal wavelength is 265 nm. Most new lamps are rated at 253.7 nm, but they may quickly diminish in power and drift in frequency.
UV lamps burn blue long after they become ineffective at killing microorganisms, so you cannot assume a UV lamp is working properly unless you test it regularly.
Laboratories must have the equipment to test UV lamp performance every six months or replace UV lamp bulbs every six months.
A "UV lamp change out schedule" should be posted on the BSC. The schedule should require change of lamps when 80% of the manufacturer's anticipated bulb life expectancy has expired or every 6 months, whichever comes first.
UV lamp testing is not cost effective when compared with chemical disinfecting methods.
Older BSCs do not have automatic controls to turn off UV lamps when the sash is open, thus increasing the exposure potential.

10.8 FLAME USE IN BIOLOGICAL SAFETY CABINET

Open flames (i.e., Bunsen burners) should not be used in the near microbe-free environment of a BSC and can be considered as artifact left over from usage of Class I cabinets several decades ago. An open flame in a BSC creates turbulence, which disrupts the pattern of air supplied to the work surface.

Volatile gases such as natural gas should never be used inside of Class II A BSCs or other type cabinets that recirculate the air back into the work surface. A gas leak could build up unnoticed inside of the working environment and ignite.

Small electric furnaces are available for decontaminating inoculating loops and needles. Electric furnaces are preferable to an open flame inside of a BSC. Disposable sterile inoculating loops are recommended.

Methods for decontaminating dropped media bottle lids, 'touched' flask lips, or other items, should be devised to be substituted for 'flaming' when working inside of BSCs. These methods could be the following:

- A sterile gauze pad soaked with 70% alcohol will kill any vegetative microorganisms that may have been transferred after an accidental 'touch'.

- Spare sterile bottle lids and other small sterile supplies can be prepackaged and used to replace dropped lids or other items that may have been contaminated during culture manipulations.

A word of warning: Never place both alcohol and a gas flame inside of any cabinet. This is a very common cause of fire in laboratories.

10.9 MAINTENANCE WORKER SAFETY IN BSL-2 LABORATORIES

All persons entering a BSL-2 laboratory must be advised of the potential hazards present in that area. This task is accomplished by door signage that informs personnel about biological hazards present in a BSL-2 area, what are the PPE requirements, and also lists emergency contacts. Only persons whose presence in the facility or individual laboratory rooms is required for support purposes are authorized to enter. Entry into a BSL-2 facility must be limited by means of secure, locked doors.

In order to minimize the risk of exposure to facilities maintenance staff (heating, ventilation, and air-conditioning (HVAC) workers, electricians, welders, plumbers, etc.) who may come into contact with biological hazards present in an area / biohazardous waste generated in a BSL-2 laboratory, the laboratory supervisor/PI of the laboratory should make sure that all areas where facilities maintenance staff will be working are thoroughly decontaminated before the worker arrives and starts onsite repairs. Pieces of equipment scheduled for maintenance must be cleaned and decontaminated, if applicable, before the equipment can be opened or moved from the laboratory for repair. The laboratory supervisor/PI of the laboratory should be present when the worker arrives and inform them that entry is only allowed when employees/students are not at work in a BSC or working with biohazardous materials, and that maintenance workers should never touch any ongoing experiments.

10.10 SPECIAL PLUMBER SAFETY

All plumbers working in laboratories should have completed online Bloodborne Pathogens (Non-Research personnel) Training that can be accessed at https://uta-ehs.org/. This course provides detailed information on Universal Precautions and the Exposure Control Plan. It also covers how to prevent becoming exposed to bloodborne pathogens in the work place.

Plumbers working in laboratories should be offered the hepatitis B vaccination series, that they can officially decline Hepatitis B Vaccination / Hepatitis B vaccine Waiver / Exemption (CO-LS-F12).

Plumbers working in laboratories should wear appropriate PPE, e.g. tear resistant gloves (that need to be removed and hands washed before leaving the laboratory), face shield or goggles, disposable coverage to protect clothing.

10.11 EXPOSURES TO POTENTIALLY INFECTIOUS MATERIALS

Potentially infectious materials in the laboratories include items such as cell cultures, serum, environmental specimens that may contain pathogens, or any items contaminated with such material.

A potentially infectious material exposure incident occurs when this type of material:

- Comes into contact with a worker's mucous membranes (eyes, nose, or mouth)
- Enters the body through possible breaks in the skin
- Is accidentally ingested

Example incidents include:

- Splashing cell culture waste into eyes
- Spilling liquids that may contain pathogens onto an open wound on hand
- Piercing mucous membranes or the skin barrier through such events as needle sticks, human bites, cuts, and abrasions

10.11.1 POTENTIAL EXPOSURE INCIDENT TO BLOODBORNE PATHOGENS

If an employee of UTA incurs a potential exposure incident to bloodborne pathogens, the supervisor and EH&S should be notified immediately, no matter how minor the incident may seem. A student of UTA should notify their supervisor or PI of the incident immediately. Potential exposure incident to bloodborne pathogens means skin, eye, mucous membrane, or parenteral contact with blood or OPIM that may result from the performance of an employee's duties or during student's coursework/research. OPIM include various contaminated human body fluids, unfixed human tissues or organs (other than skin), and other materials known or reasonably likely to be infected with HBV and/or HIV through cells, tissues, blood, organs, culture mediums, or solutions.

Please, refer to Employee Blood and Body Fluid Exposure and Student Blood and Body Fluid Exposure documents for more information.

10.11.2 WHAT TO DO IN THE EVENT OF AN EXPOSURE

When an exposure incident has occurred, an immediate response is the key to reducing the risk of getting a LAI. These actions should be taken:

EMERGENCY RESPONSE TO EXPOSURES
Flush the exposed area with water. If eyes, nose, or mouth were exposed to blood or OPIM, flush these areas for 15 minutes. If skin was exposed, thoroughly wash these areas with soap and water. Do not scrub the area; instead bathe the area in water and soap. Bandage the affected area if needed to control the bleeding.
Employees of UTA: Notify the supervisor or PI and EH&S immediately. Students: Notify your supervisor/PI.
Seek medical help/advice from a healthcare professional.
Employees of UTA: When checking in at the clinic, the individual should indicate that he/she is an employee of UTA and present the Notification of a Work-Related Injury or Occupational Disease Form (CO-CS-F16) along with the Workers' Compensation Pharmacy Information.
Reporting: Employee The affected employee is required to complete the Employee's Report of Work-Related Injury or Occupational Disease (CO-CS-F1) and the Workers' Compensation Network Acknowledgement (CO-CS-F32). The affected employee's supervisor must complete the Supervisor's Report of Employee Work-Related Injury or Occupational Disease (CO-CS-F2) and give a copy of the Notice of Network Requirements to the employee. These forms must be submitted within 24 hours of reporting the incident exposure to the EH&S via fax at 817-272-0273. The originals should be sent to the EH&S via campus mail at Box 19257.

EMERGENCY RESPONSE TO EXPOSURES
Student The supervisor or PI overseeing the student's coursework or research should submit an Injury/Illness Reporting Form for Students and Visitors (CO-CS-F12) to EH&S via electronic mail to ehsafety@uta.edu or via fax 817-272-2144 within 24 hours of notification of the incident exposure. The original should be mailed to EH&S via campus mail at Box 19257. The student is required to complete the Injury/Illness Reporting Form for Students and Visitors (CO-CS-F12) within 24 hours of reporting the incident exposure. The original should be mailed to EH&S via campus mail at Box 19257.
Call EH&S at 817-272-2185 for guidance. Refer to section 6 "Emergency Procedures for Biohazardous Spills".

11 BIOSECURITY / ACCOUNTABILITY

Laboratory biosecurity includes the protection, control, and accountability of valuable biological materials (VBM) within laboratories. In order to prevent unauthorized access, loss, theft, misuse, diversion, or intentional release of VBM, administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories are required. VBM may include pathogens and toxins, as well as non-pathogenic organisms, vaccine strains, foods, GMOs, cell components, genetic elements, and extraterrestrial samples. Laboratory biosecurity aims to protect VBM economic and historical (archival) value, and/or the population from VBM potential to cause harm.

A written or computerized inventory log must be kept. CEMS can be utilized for this task. The inventory should be complete enough so that the PI would know:

- If materials are missing
- What those materials are
- Potential hazards of the materials
- Quantity of the materials

Accountability does not necessarily imply the identification of exact quantities of biological materials. Living replicating organisms may vary in quantity and quality over the course of laboratory activities and time, and knowing the exact quantity of organisms at any given time is generally not realistic. Biological materials that are confined to particular containers should be tracked as separate items. For example, it is possible to maintain an inventory of frozen stocks and an access log to many forms of stored materials. These forms of records should be secured and easily identified, legible, and traceable to the activities described. Records will be useful as a means of knowing permanently where VBM are located and who has responsibility of them. Accountability also means ensuring that materials are properly safeguarded. A person(s) with expert knowledge of the material in use and its storage should be accountable. Unauthorized access is the result of inappropriate or insufficient control measures to guarantee selective access. Losses of VBM often result from poor laboratory practices and poor administrative controls to protect and account for these materials. Any anomalies seen by the laboratory personnel should be promptly reported to the PI.

The objective of specific accountability procedures for VBM is to know which materials exist in a laboratory, where they are located, and who has responsibility for them at any given point in time. To achieve this, the following points should be defined:

- Which materials (or forms of materials) are subject to material accountability measures

- Which records should be kept, by whom, where, in what form and for how long
- Who has access to the records and how access is documented
- How to manage the materials (e.g. where they can be stored and used, how they are identified, how inventory is maintained and regularly reviewed, and how destruction is confirmed and documented)
- Which accountability procedures will be used (e.g. manual logbook, electronic tables, etc.)
- Which documentation/reports are required
- Who has responsibility for keeping track of VBM
- Who should approve and clear the planned experiments and the procedures to be followed
- Who should be informed of and review the planned transfer of VBMs to another laboratory

12 TRANSPORTING BIOLOGICAL MATERIALS ON CAMPUS / OFF CAMPUS

Biological materials can be safely transported between buildings on the UTA campus when they are appropriately packaged, labeled, and transported in a manner that minimizes the potential for environmental release. The following procedure for preparing and transporting biological materials between University buildings should be used:

- Primary container (innermost container): Infectious materials and recombinant/synthetic nucleic acid molecules need to be packaged in a sealed, leak-proof, **primary container** (e.g., plastic screw cap tube). Food containers or other containers not originally designed for laboratory storage purposes should not be used. Clearly identify the contents and avoid abbreviations (e.g. write out *E. coli* K-12 - DH1 rather than just DH1). Wrap the primary container tightly using Parafilm to ensure that there will be no leakage.

- Secondary container: Primary sample containers need to be positioned securely inside a closable **secondary leak proof container** (e.g., Ziploc bag) for transport. If sample material is liquid or may release liquids, enough absorbent material (i.e. paper towels) needs to be placed in the secondary container to absorb all free liquids in the event that primary containers rupture or break during transport. Primary containers need to be packaged in the secondary container in a manner that will reduce shock, rupture, and/or breakage. Bubble wrap or similar shock-absorbing materials may be used to minimize the potential for primary container rupture. A list of contents as well as emergency information (i.e., PI's name and phone number) should accompany the material.

- Transporting container: Use a container made of sufficient strength to protect the transported specimen(s), e.g., a cooler or a shipping box. The **transporting container** needs to be marked with the PI's name and telephone number. Containers used for transporting biohazardous agents/human blood or OPIM must also be labeled with the biohazard symbol.

Note: Dry ice should never be placed in a sealed container!

Please, refer to Guidelines for Shipping/Transporting Non-Infectious Biological Materials and Non-Pathogenic Biological Cultures.

Contact EH&S if biological materials need to be transported off UTA campus locations.

13 SHIPPING OF BIOLOGICAL MATERIALS TO OFF CAMPUS DESTINATIONS

Each person who offers hazardous materials (dangerous goods) for transportation must be trained to properly classify, package, mark, label, placard, and document the shipment. All persons coming in contact with and directly affecting the safe transporting of the shipment must also be trained in their specific job function.

EH&S offers assistance to campus personnel who plan to ship biological materials. If materials to be shipped include infectious substances or biological materials with dry ice, these materials are regulated for transportation and will require specific packaging, labeling, and documentation. The shipper must have documented training relative to the tasks associated with the shipment. As a shipper, it is essential to assure that materials are properly classified and that all applicable regulatory provisions for shipment are met. Shipping Class 6 Division 6.2 Dangerous Goods Compliance Training is obligatory when planning to ship dangerous goods nationally or internationally.

EH&S is UTA's designated shipper of biological materials, infectious agents, genetically modified organisms/microorganisms, patient specimens, and also shipments that include dry ice because dry ice is Class 9 "miscellaneous" hazard. Researchers are responsible for packing the shipment and contacting EH&S (Link to: Request to Ship Biologicals with Dry Ice, Form #.....) to inspect/pick up the shipment when it is ready for shipment but still open. EH&S performs all shipping of biological materials with dry ice via FedEx using eShipGlobal.

The primary agencies and regulations dealing with the transportation of infectious substances include, but are not limited to:

- Code of Federal Regulations, Title 49 (49 CFR) – all modes of transport in the United States
- The ICAO Technical Instructions for the Safe Transport of Dangerous Goods by Air – international requirements for air transport
- The IATA Dangerous Goods Regulations – commercial requirements for international air transport
- The International Maritime Dangerous Goods Code (IMDG Code) – international requirements for ocean transport
- Transport of Dangerous Goods Regulations (TDG) – all modes of transport in Canada

For more information on biological materials shipping requirements, please contact EH&S at 817-272-2185.

14 GUIDELINES FOR TISSUE CULTURE / CELL CULTURE WORK

The CDC and OSHA recommend that all cell lines of human origin be handled at BSL-2 containment. Universal Precautions and BSL-2 practices need to be followed when working with contact-transmitted microorganisms, and when handling biological materials of human origin. All personnel at UTA working with or handling these materials need to complete online Bloodborne Pathogens for Laboratory Research Personnel Training **annually** (https://uta-ehs.org/). Please, refer to the UTA Exposure Control Plan for Bloodborne Pathogens for additional information. Research laboratories where work involves handling human tissue/cells shall have card-reader(s) to limit access to only those who have completed the appropriate requirements.

The hepatitis B vaccine is strongly recommended for all laboratory personnel conducting research involving human materials. Tissue culture procedures and animal inoculations with cells should always be performed inside a Class II

BSC, not on the open bench top, and never inside a vertical flow clean bench. Layout of operations inside the Class II BSC should be performed in a clean to dirty direction (see section 5.11.4.3.1.6 “Working in a Class II BSC”. Rapid movements should be minimized to avoid airflow disruptions within the cabinet, and the outside of culture vessels and other materials (such as pipettes) should be disinfected or bagged prior to removing them from the BSC. When a BSC is not available or a procedure cannot be performed within a BSC, a combination of suitable PPE and other containment devices (e.g., face shield, safety shield) must be used.

Special care needs to be taken with primary tissues and cell lines of human origin since the potential laboratory hazards associated with tissue/cells include bloodborne pathogens, as well as contaminates that may be present in human tissues such as mycoplasmas and viruses. *Mycoplasma* species are often found in research laboratories as contaminants in cell cultures. *Mycoplasma* cell culture contamination occurs due to contamination from individuals or contaminated cell culture medium ingredients. *Mycoplasma* microbes are tiny bacteria and they are therefore difficult to detect with a conventional microscope. Severe *Mycoplasma* infections may destroy a cell line. Detection techniques include DNA probe, enzyme immunoassays, Polymerase Chain Reaction, or plating on sensitive agar and staining with a fluorescent DNA stain. Cells transformed with viral agents such as Simian virus 40, Epstein-Barr virus, HBV as well as cells carrying viral genomic material, present potential hazards. Each new cell line purchased or established within the laboratory needs to be investigated for potential contaminants. Not all human pathogens are as yet discovered and it is always possible that a “clean cell line” may contain an unknown agent with the possibility to cause the laboratory worker or environment harm. Tumorigenic human cells can also be hazardous when accidental self-inoculation is possible.

While a negative pressure tissue culture room is required for tissue/cell culture work in all newly constructed buildings, older laboratory buildings may not be able to meet this stringent engineering control. Recent changes and clarifications to CDC and NIH safety guidelines strongly encourage designing directional negative airflow for all newly constructed BSL-2 laboratories and that the air needs to be 100% exhausted from the room to the outside of the building. It is also prudent to design laboratory suites with directional airflow so that fresh room air (supply air) passes first across the least hazardous operations within the suite, then migrates towards the more hazardous ones before exiting the room. Doors to tissue culture rooms must remain closed during operations. These points need to be kept in mind when arranging equipment, procedural workflow, and when planning a remodel of an older facility.

Aspiration of tissue culture media from tissue cultures and of supernatants from centrifuged samples into collection flasks is a common laboratory procedure. To prevent accidental contamination of in-house vacuum lines, protection must be in place against pulling biohazardous aerosols or overflow fluid into the vacuum system. The collection flask(s) (containing 10% bleach) should be placed inside of the BSC. If interior cabinet space is a concern, an acceptable alternative is to place the flask(s) on the floor outside of the BSC, but only if the connections are very secure and the flask(s) are placed within a secondary containment tray located outside of traffic areas. An air filter needs to be used in the line immediately leading into the in-house vacuum line and an overflow flask should be installed between the collection flask and the air filter. A cartridge-type filter (capacity to remove airborne particles 0.22 micron in size) provides an effective barrier to passage of contaminated aerosols into the house vacuum system (see Figure 4 below). Use flexible tubing of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. When the filter gets wet or requires changing, the filter and flask can be safely removed by clamping the line between the filter and the vacuum source. Wet filters do not adequately remove airborne particles.

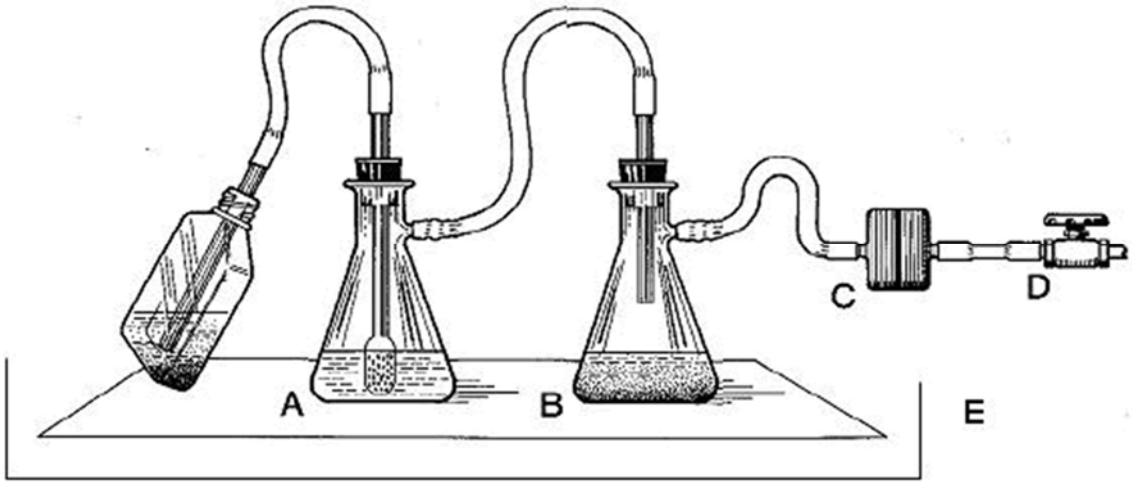


Figure 4: One method to protect an in-house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. Flask B is used to minimize splatter. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms. A spill tray (E) should be used when the flasks are outside the BSC.

Source: adapted from BMBL, fifth edition, Appendix A.

All residual liquid from tissue cultures must be decontaminated by autoclaving or cold liquid disinfecting before discarding into the sanitary sewer system. All disposable equipment and solid wastes used in culturing should be wiped or doused with disinfectant while still inside the BSC, prior to placing them in biohazard boxes.

15 STANDARD OPERATING PROCEDURES

SOP: Proper Use of Gloves
SOP: Biological Waste Management and Disposal Instructions
SOP: How to Package Biohazard (Stericycle) Boxes
SOP: Request for Biological Waste Removal
SOP: Steam Autoclaves
SOP: Performance Verification of Steam Autoclave Kill Cycle
SOP: Steam Chemical Integrators/ Sterilization Assurance/ Steam Autoclave Kill Cycle
SOP: Ethidium Bromide Use and Disposal
SOP: Proper Use of Bleach (Sodium Hypochlorite) as a Chemical Disinfectant in Biolaboratories