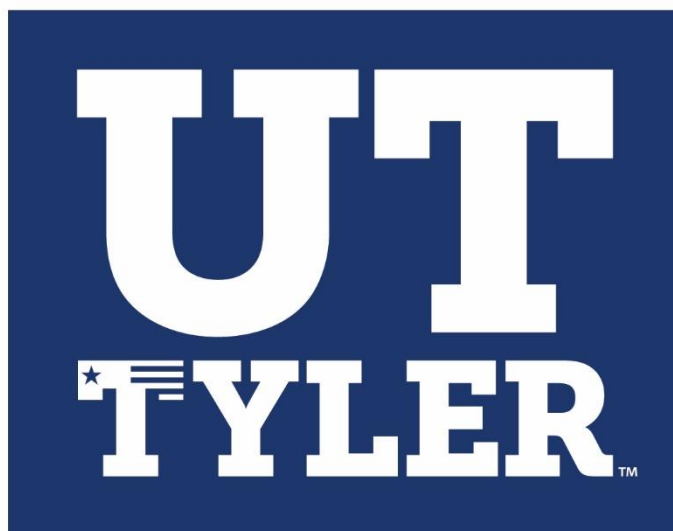


THE UNIVERSITY OF TEXAS AT TYLER



# **Biological Safety Program**

January 2020

## Contents

<b>I.</b>	Overview and Purpose .....	3
<b>II.</b>	Scope .....	3
<b>III.</b>	Responsibilities .....	4
<b>IV.</b>	Biological Laboratory Safety Plan .....	6
<b>V.</b>	Personal protective equipment (PPE) .....	23
<b>VI.</b>	Laboratory equipment .....	25
<b>VII.</b>	Waste management .....	33
<b>VIII.</b>	Emergency procedures and equipment .....	34
<b>IX.</b>	Other Agents .....	37
<b>X.</b>	References .....	37
<b>XI.</b>	Contact Information.....	38

## **I. Overview and Purpose**

The University of Texas at Tyler's (UT Tyler) standing policy is to promote and protect a healthy learning environment for students, employees and UT Tyler properties. This Biological Safety Program outlines basic good laboratory standards (GLSs), special procedures for UT Tyler, federal and state guidelines, and references to other information sources for work in laboratories that handle, use or store biological organisms and/or agents. It is not intended to be a fully comprehensive reference but rather a guidebook to help identify hazards, mitigate or remove their risks and give helpful reference manuals. Information and training on specific instruments and protocols is the responsibility of the principal investigator(s) or laboratory supervisor.

Faculty, staff and students who may be exposed to biological hazards in the laboratory must be informed of the nature of these hazards, know how to contain those hazards, and protect themselves and others who may also be exposed. Everyone in biology laboratories must become reasonably familiar with this program. Safety in the laboratory can be achieved only with proper training, the exercise of sound judgment, proper use of facilities, and everyone ensuring everyone is following these guidelines. You are all safety officers in this respect.

## **II. Scope**

This plan applies to all UT Tyler operated facilities (leased or owned) and equipment including, machinery, instrumentation and vehicles. It also applies to any UT Tyler employee, volunteer or student employee who works directly with, or is in close proximity to, anyone conducting research within a biological laboratory which falls under the purview of federal and state regulations or guidelines.

Research faculty's safety training is under the direct supervision of the Office of Research and Scholarship. Mandatory safety training and guidelines are instituted through this office and should be upheld by every researcher in the UT Tyler. For example, the NIH guidelines are mandatory for all

researchers at institutions receiving NIH funding, even if those researchers are not themselves receiving NIH funding, and therefore would apply to all UT Tyler research. All federal NIH funding can be removed from an institution for violations of the guidelines by any researcher on campus.

### **III. Responsibilities**

#### **A. Environmental Health and Safety will:**

1. Review protocols for work with potentially infectious materials or hazardous biological agents and provide recommendations to the Principal Investigator (PI).
2. Develop safety plans and training programs for work with all risk groups of biological agents, blood borne pathogens, and other potentially infectious materials in use at UT Tyler facilities.
3. Conduct annual certification of biological safety cabinets and fume hoods.
4. Maintain a biological waste disposal program.
5. Supervise decontamination and clean-up activities following spills or exposures.
6. Maintain an EH&S Biological Safety Officer for consultation with students, faculty, and staff concerning safety issues in and around laboratories.
7. Investigate biological exposure incidents.
8. Evaluate laboratories periodically to ensure compliance with institutional, state and federal guidelines and regulations as they pertain to laboratory safety and biosafety.

#### **B. Principal Investigators (PI) or Laboratory Supervisors will:**

1. Ensure compliance with all UT Tyler procedures and policies regarding all risk groups of biological agents.

2. Ensure laboratory personnel have been properly trained to work safely within their laboratory to include how to safely use; any and all equipment in their protocols, chemicals and agents in their protocols, and required safety training provided by EH&S.
3. If necessary develop specific safety procedures or protocols for their laboratory and procedures and ensure EH&S has a copy of those procedures or protocols.
4. Advise EH&S of any significant protocol changes prior to the start of the experiment.
5. Report any exposures, spills, involving biological safety to EH&S as soon as possible via a spill report. You can find the spill report here: <https://www.uttyler.edu/safety/campus-spill-form.php>.
6. Injuries such as sharps sticks, chemical burns, or infection must be reported to EH&S immediately following an incident. The report form mailed to [safety@uttyler.edu](mailto:safety@uttyler.edu). The forms can be found here: <https://www.uttyler.edu/safety/files/Student-Visitor-Injury-Report-Form.pdf> for students and visitors, <https://www.uttyler.edu/human-resources/documents/WorkersCompPPWk.pdf> for employees.
7. Maintain a clean and sanitary workplace.
8. Contact EH&S concerning UT Tyler equipment that is malfunctioning, including but not limited to eyewash stations, safety showers, fume hoods, and biosafety cabinets.
9. Submit non-exempt protocols to IBC if required.

C. Laboratory Staff or Worker will:

1. Observe the established guidelines, protocols and policies for biological safety.

2. Receive all necessary or required training.
3. Report all spills or incidents to their supervisor and to EH&S if necessary.
4. Report to the supervisor or EH&S any unsafe practices or conditions in the laboratory.
5. Properly dispose of all laboratory wastes.
6. Report to their PI any unsafe or malfunctioning UT Tyler equipment.

#### **IV. Biological Laboratory Safety Plan**

##### **A. Biosafety**

Safety is very important in biological laboratories due to the microscopic nature of many of the work, the high concentrations of potentially hazardous substances involved, and the infectious nature of materials and equipment. Pathological exposures have and continue to occur within and near laboratories. These infections are known as laboratory acquired infections (LAI). LAI's may follow a route of infection different from that in nature. Although specific causes of many LAI's are unknown, precautions can be placed to minimize exposure and potential hazards.

Biosafety is the application of combinations of good laboratory standards, laboratory equipment and facilities, and PPE when working with potentially infectious microorganisms in order to mitigate or remove risk. According to the BMBL: The predominant probable routes of transmission in the laboratory are: 1) direct skin, eye or mucosal membrane exposure to an agent; 2) parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors; 3) ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and 4) inhalation of infectious aerosols.

Aerosols are particles of various sizes suspended in air and are of major safety concern. Following biosafety guidelines provide protection for laboratory workers from the potentially hazardous products and procedures within the laboratory working environment. As a note, chemicals used in biological laboratories are also potentially hazard. UT Tyler has created a chemical hygiene plan that strictly deals with chemicals in the laboratory. Please refer to the UT Tyler Chemical Hygiene Plan at <https://www.uttyler.edu/safety/files/lab-safety-manual.pdf> for more safety information about chemicals in the lab.

## B. Good laboratory standards

All laboratories must follow proper procedures while conducting yourself in a laboratory setting. Good laboratory standards must be practiced in all labs on UT Tyler campuses.

1. No mouth pipetting; use only pipetting devices.
2. No food or drink stored or consumed in the laboratory.
3. Wash hands frequently, especially after known contamination. Wash hands after removing gloves, before leaving the work area and after touching common use items such as computer keyboards, telephones, or door handles.
4. Ensure ALL doors in the laboratory are closed.
5. Do not wear gloves while moving or transporting chemicals or microorganisms through hallways and corridors.
6. Remove gloves before touching common use items.
7. Safe handling of syringes, needles and sharps.
  - a. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries.

- 1) Examples of engineering controls include: self-sheathing needles, obvious sharps containers, needleless systems, and blunt tipped equipment.
  - 2) Contact EH&S for guidance about proper sharps usage and waste containers.
  - 3) Examples of work practice controls include: not recapping needles, pointing sharps away from the user (especially when carrying out an injection), and keeping sharps locked at all times while not in use.
- b. Sharps must be placed in an approved sharps container.
- c. Syringes should be handled with great care and only after adequate training.
- 1) After filling, excess fluid and bubbles should be expelled from syringes vertically downward into a plastic test tube containing a sterile cotton pledget to minimize aerosols; or this procedure can be performed in a class II biosafety cabinet.
  - 2) Contaminated needles and syringes will be discarded in the approved sharps container. **DO NOT RECAP CONTAMINATED NEEDLES. ONCE DISCARDED, ITEMS MUST NOT BE REMOVED FROM THE SHARPS CONTAINER.**
- d. **Never bend, shear, break, remove from disposable syringes or otherwise manipulate needles by hand.**
- e. Needles used for blood drawing (phlebotomy) should also be placed in an appropriate sharps disposal container. **DO NOT RECAP CONTAMINATED NEEDLES.**



f. Any sharps incidents and injuries must be reported to EH&S via the applicable injury report (students or employees) form found at <https://www.uttyler.edu/safety/forms.php>.

1) Broken Glass

Clean up broken glass as soon as possible to prevent injuries. Collect broken glass using a broom and dust pan where possible. Tongs or forceps can be used to collect the broken glass in difficult or high hazard environments. NEVER use hands to pick up broken glass even if gloves are worn. Place any broken glass in a sharps or puncture-resistant container. When this container is approximately three quarters full, seal the container and either dispose of it. You can also make arrangements with Service Solutions in order to dispose of it.

g. For more information on sharps or exposure control, please consult UT Tyler's Exposure Control Program at:  
<http://www.uttyler.edu/safety/files/ExposureControlPlan.pdf>

8. Aerosol formation should be avoided at best or minimized as much as possible. Bio-aerosols are formed when the liquid-air interface of a hazardous substance is disturbed, creating airborne particles. Inhalation of bio-aerosols can lead to infections even by organisms not known to be transmitted by the aerosol route. Prevention of bio-aerosols hazards:

a. Perform all bio-aerosol forming procedures inside a Biological Safety Cabinet (BSC) or substitute with other procedures. Some bio-aerosol forming laboratory activities: opening centrifuge tubes, flaming loops, blowing the last drop out of pipets, splashes, bubbles, vortex, operating a cryostat or a cell sorter.

9. Laboratory Acquired Allergies/Laboratory Animal Allergies (LAA)

a. Allergic reactions to animal proteins is a significant occupational hazard when working with animals, with up to 1/3 of workers in an animal laboratory developing symptoms within two years. The

most common source are proteins found in urine, saliva and dander. Animal researchers should be careful to wear the correct PPE and follow handling protocols closely to prevent LAAs.

- b. Ensure you discuss LAAs with your PI or lab professor and follow the appropriate safety guidelines.
- c. Acute signs a symptoms include sinus mucous, irritated skin (erythema) or eyes, sneezing or possibly rash.
- d. Chronic symptoms include coughing, wheezing and possibly asthma.

10. Use of ear buds or headphones

- a. The use of ear buds or headphones is discouraged because their use may impede hearing and be a route of exposure. Headphones are not usually tolerated in industry. Therefore, earbuds and headphones are not allowed in labs deemed hazardous by EH&S.
- b. Exceptions can be made for more trivial lab work to include only wearing one ear bud or have ear buds with an opening to allow outside sound to come into the ear canal.

C. Recombinant DNA

These particular products require extra attention due to the potential for abuse. Recombinant DNA (rDNA) molecules are defined as either: molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from the replication of those described above. Work with rDNA must be approved by the Institutional Biosafety Committee (IBC) unless it is exempt under the NIH guidelines found here: [https://osp.od.nih.gov/wp-content/uploads/2013/06/NIH\\_Guidelines.pdf](https://osp.od.nih.gov/wp-content/uploads/2013/06/NIH_Guidelines.pdf). Please contact the UT Tyler Biological Safety Officer at (903) 566-7011 or the Office of Scholarship and Research (OSR) at (903) 565-5774 for more information. A listing of exempt experiments is listed below. If you have consulted the NIH guidelines and the listing below and still have questions, contact the Biosafety Officer or OSR. Be

aware that some experiments considered exempt by NIH will require a protocol submission to the UT Tyler EH&S.

1. Exempt experiments

- a. The rDNA is never going to be transfected/infected an organism or virus.
- b. The rDNA is solely from a single non-chromosomal or viral source.
- c. The rDNA is solely from a prokaryotic host and propagated in the same host or transferred to another host by well-established physiological means or protocols.
- d. The rDNA is from a eukaryotic host and is propagated in the same host.
- e. The rDNA is from a species that naturally exchanges DNA by known physiological processes. A list of species are also available within the NIH guidelines from the website above.
- f. The rDNA is of a type which does not present a significant risk to health or the environment, as determined by the NIH Director\*.

\*The NIH has determined that rDNA from infectious agents of BSL-2 or above is not exempt and must receive IBC approval. Additionally, certain cloning vectors, such as Adeno or Sindbis based vectors, or amphotrophic MMLV based vectors, are some examples that are nonexempt. For a complete list consult the BMBL and NIH at <https://www.cdc.gov/labs/BMBL.html> and <https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/>, respectively.

2. Experiments Requiring Prior Approval

The following experiments require prior approval from the NIH, Recombinant DNA Advisory Committee (RAC), Food and Drug Administration, and/or the IBC.

- a. Gene transfer experiments in humans;
  - b. Genes for toxins lethal for vertebrates;
  - c. Release of genetically engineered organisms to the environment;
  - d. Those using human or animal pathogens (biosafety level 2 and higher) as host-vector systems, including adenovirus vectors and murine retroviruses that infect human cells;
  - e. Cloning DNA from human or animal pathogens (biosafety level 2 and higher) into a non-pathogen host-vector system;
  - f. Cultures of rDNA modified organisms of more than 10 liters; and,
  - g. Experiments involving whole plants or animals, including transgenic organisms.
3. Experiments Requiring IBC Notice Simultaneous with Initiation. Some recombinant DNA work requires IBC review and approval, but prior approval is not required for some experiments, and may be conducted at BSL-1 containment. Examples include:
- a. Recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus (with some restrictions) propagated and maintained in cells in tissue culture. It must be demonstrated that the cells lack helper virus for the specific families of defective viruses being used. Many, but not all, experiments involving whole plants.\*

\*Some plant experiments do not require prior approval. Work with recombinant DNA in plants or any work with plant pathogens must also comply with USDA and EPA regulations.

#### D. Working with Potentially Infectious Agents

Infectious agents are viable microorganisms, or their toxins, which cause or may cause disease in humans or animals. Examples of infectious agents include bacteria, viruses, fungi, parasites and prions. Infectious agents are classified

into four risk groups by the NIH according to the severity of their effects on human health.

1. Risk Groups:
  - a. Risk Group 1- Agents that are not associated with disease in healthy adults.
  - b. Risk Group 2 – Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
  - c. Risk Group 3 – Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk, low community risk)
  - d. Risk Group 4 – Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)
  
2. Biological Safety Levels: Biological Safety Level (BSL) refers to the actions, precautions, and equipment needed to protect people and the environment from biological agents being worked with in the laboratory. The criteria cover four topics including: infectivity, severity of disease, transmissibility, and the nature of work. They roughly follow work with risk groups describes in section D.1. BSLs are defined through four risk levels :
  - a. BSL-1 involves agents which are not known to cause disease in healthy adults. This is the level that teaching laboratories work at for the most part. Few special precautions are necessary when working at this level, but eye protection is mandatory. However, glove usage is recommended.
  - b. BSL-2 involves agents that are known to cause disease in humans. The diseases are not known to result in major illnesses in healthy adults. Some precautions are necessary when working at this level. BSL-1 precautions must be followed. Gloves must be worn

and laboratory coats are strongly suggested. Any work that can result in the creation of bio-aerosols, should be carried out in the appropriate class of biological safety cabinet (BSC).

- c. BSL-3 involves agents that are known to cause disease through the aerosol route. These diseases are usually not communicable, but they do result in serious diseases. They have a high morbidity rate, but the mortality rate is not high. UT Tyler does not have any BSL-3 facilities.
- d. BSL-4 involves agents that cause serious diseases which have high mortality rates and for which there is no known cure. There are very few laboratories in the U.S. that operate at this level. UT Tyler does not have any BSL-4 facilities.
- e. Biosafety cabinets are research specific. For more information on the correct biosafety cabinet consult the PI, OSR, the Biological Safety Officer at EH&S or consult the definite definitions of the BMBL at <https://www.cdc.gov/labs/BMBL.html>.

3. Requirements for facilities and practices at BSL-1.

- a. Standard Microbiological Practices
  - 1) Eye protection must be worn at all times while performing an experiment.
  - 2) Limited or restricted access to the laboratory when work is in progress.
  - 3) Biohazard warning signs must be in place.
  - 3) **No eating, drinking or smoking in laboratory.**
  - 4) No mouth pipetting.
  - 5) Hands must be washed after handling viable materials and when leaving the laboratory.

- 6) Efforts to minimize splashes and aerosols must be made.
  - 7) Work surfaces must be decontaminated daily and immediately after spills.
  - 8) Wastes must be decontaminated through appropriate methods depending on the risk level; see section J.
  - 9) An insect and rodent control program must be maintained.
  - 10) Personal protective equipment will be required at the discretion of the laboratory supervisor or principle investigator.
- b. No Special Practices Required
  - c. Secondary Barrier – Facility Requirements: Doors for the laboratory should remain closed, hand washing sink available, emergency eyewash and safety shower available, work surfaces made from easily cleanable material, bench tops made from material impervious to water, sturdy furniture, and all windows are fitted with fly screens.
  - d. No special facilities required
4. BSL-2: Standard Microbiological Practices
- a. All BSL-1 Practices Plus:
    - Use of Class II Biosafety Cabinets (BSC) for work with agents involving aerosols and splashes, large volumes or high concentrations.
  - b. BSL-2: Special Practices
    - 1) Policies and procedures must be in place for workers to gain entry.

- 2) Documentation of protocols, training and annual updates must be provided to EH&S for laboratory specific issues and SOP's.
  - 3) Leak-proof transport containers are necessary with secondary containment.
  - 4) Immunizations against agents being worked with must be available to UT Tyler full time employees employed as laboratory staff.
  - 5) Work surfaces must be cleaned and decontaminated regularly.
  - 6) Spills and accidents must be reported to EH&S ([safety@uttyler.edu](mailto:safety@uttyler.edu) or (903) 566-7011).
  - 7) Equipment should be cleaned with an autoclave.
  - 9) No animals or plants are allowed in the laboratory that are not part of the research.
  - 10) Sharps precautions must be followed including, dispose of sharps in an approved sharps container. Never recap, bend, break or reuse needles or syringes and never use hands to pick up broken glass.
- c. Secondary Barrier – Facility Requirements: adequate illumination, eyewash inside the laboratory, laboratory pressure negative to the hallway, air from the laboratory cannot be recirculated within the building (negative pressure inside laboratories), the doors must remain closed to maintain ventilation requirements, the door must be lockable to limit access when work is in progress, an autoclave must be available and the lab must be separated from public areas of the building.

5. BSL-3: Standard Microbiological Practices



- a. UT Tyler has no BSL-3
- b. All BSL-1 and/or BSL-2 Practices Plus: All manipulations of infectious agents must be carried out inside a class II or III BSC.

Personal Protective Equipment for BSL-1 and BSL-2 and respiratory protective equipment as indicated.

- c. BSL-3: Special Practices
  - 1) BSL-2 Special Practices Plus: When carrying out bio-aerosol forming procedures, bio-aerosol containing equipment must be used.
  - 2) All spills must be promptly decontaminated.
- d. Secondary Barrier -- Facility Requirements: All requirements for BSL-1 and BSL-2 Plus; the laboratory should be in a separate building or in an isolated zone, double door entry into the laboratory, single pass air with 10-12 air changes per hour, room penetrations must be sealed, walls, floors and ceilings must be water resistant and vacuum lines must be protected by traps or HEPA filters.

6. BSL-4: Standard Microbiological Practices

- a. UT Tyler has no BSL-4
- b. BSL-4: Special Practices
  - 1) All BSL-1, 2 and 3 PPE Plus: A Class A positive pressure suit must be worn for entry into the laboratory.
  - 2) BSL-3 Plus: All liquid effluent and solid waste must be decontaminated prior to disposal.
  - 3) Personnel must enter through a changing room and must change into laboratory clothes to wear underneath the positive pressure suit.

- 4) Supplies must enter the laboratory through double door autoclaves or fumigation chambers.
- c. Secondary Barrier – Facility Requirements: All BSL1, 2 and 3 plus: a dedicated supply, exhaust, vacuum and decontamination system, double door autoclaves, the walls, ceilings and floors must be sealed, the doors must be interlocked, a communication system between the laboratory and the outside is needed, and there must be emergency breathing air, an emergency generator and an emergency exit.

7. Animal Biosafety Levels:

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals infected with agents that cause, or may cause, human infection. These four combinations, designated Animal Biosafety Levels (ABSL) 1-4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to Biosafety Levels 1-4, respectively.

- a. Animal Biosafety Level 1 (ABSL-1) is suitable for work involving well-characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.
- b. Animal Biosafety Level 2 involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.
- c. Animal Biosafety Level 3 involves practices suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or

potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

- d. Animal Biosafety Level 4 involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening disease, aerosol transmission, or related agents with unknown risk of transmission. ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. Procedures must be developed locally to address specific operations of the Class III cabinet line or the suit laboratory.

8. ABSL's mimic BSL's except for:

- a. ABSL-1 must have self-closing and lockable doors, the interior surfaces must be water resistant, windows are not recommended, the floor drain traps must be filled with water and disinfectant, the air cannot be recirculated and the laboratory air pressure must be negative to the hallway.
- b. ABSL-2 should have a mechanical cage washer which operates at 180°F, an autoclave must be available within the facility, and a hand washing sink must be in the room.
- c. ABSL-3 must be physically separated from access corridors, with self-closing double-door access, preferably interlocked or alarmed, and windows and penetrations must be sealed.
- d. ABSL-4 should have two workers in the laboratory when working with infected animals and the cages must be autoclaved or decontaminated before being cleaned.

9. There are also 4 biosafety levels for plants (BL1-4-P) listed in Appendix P of the NIH Guidelines. The requirements for practices and facilities are divided into Greenhouse Access levels (BL1-4-P) and standard laboratory plant biosafety levels (BL1-4-P).

- a. Greenhouse Access Level 1 (BL1-P) is a standard greenhouse with open windows and gravel walks permitted.

- b. Greenhouse Access Level 2 (BL2-P) is GAL-1 (BL1-P) plus screens over the openings and an autoclave available.
- c. Greenhouse Access Level 3 (BL3-P) is GAL-2 (BL2-P) plus an anteroom or head house, impervious bench tops and work surfaces, an autoclave inside the facility, an independent air supply with negative pressure, the exhaust is HEPA-filtered and a security fence or an equivalent form of security is present.
- d. Greenhouse Access Level 4 (BL4-P) is GAL-3 (BL3-P) plus the area is accessed through an airlock, there is a shower facility at all entrances and a dunk tank or fumigation chamber.
- e. Standard Laboratory Plant Biosafety Level 1 (BL1-P) has limited access, an entry log is maintained, a standard procedures manual must be used, experimental organisms must be inactivated, and a pest, rodent and weed control program must be in place.
- f. BL2-P is BL1-P plus biohazard signs in place where applicable, cages for small animals and procedures to minimize the escape of motile organisms.
- g. BL3-P is BL2-P plus access restricted to trained workers, equipment and supplies must be decontaminated, biohazards signs in place, efforts to minimize the formation of aerosols must be made, the surfaces of secondary containers used to take live organisms out of the laboratory must be decontaminated and a written record of accidents must be maintained. Special clothing must be worn in the laboratory and the clothing must be decontaminated prior to laundering.
- h. BL4-P is BL3-P plus an entry/exit log must be strictly maintained, personnel must shower and change into special clothing upon entry and exit, all experimental materials and clothing must be decontaminated prior to removal and all accidents must be reported immediately.

10. There are BSL's for large scale work. Large scale work is defined as any work involving ten or more liters. Prior to working at large scale at UT Tyler the Biosafety Officer must be consulted and the IBC must approve the protocols.

E. Hazards of tissue culture—human and primate tissue:

1. Work with human and non-human primate tissues and cell lines must be carried out at BSL-2 level or above. These tissues and cell lines can harbor human pathogens and required precautions when working with them. They can also become contaminated with pathogens in the laboratory. Questions about individual cases should be addressed to the Biosafety Officer or ORS.
2. Tissue cultures from other animals can contain infectious agents, such as oncogenic viruses. Precautions should always be taken when working with tissue culture.
3. Fixed tissue is much less likely to contain infectious agents but as an example, prions can remain active after the fixation process.

F. IBC—Institutional Biosafety Committee

UT Tyler does not currently work with rDNA and therefore the IBC is currently inactive.

1. Committee Charge

The charge of the Committee is to formulate and implement procedures to assure a University's compliance with all federal regulations for the construction, handling and disposal of recombinant molecules, organisms and viruses containing recombinant DNA molecules and other biologically hazardous organisms and toxins; to review and exercise approval authority of all proposals for grants and contracts that involve recombinant DNA molecules and other biologically hazardous organisms and toxins; to monitor all projects that involve recombinant DNA molecules and other biologically hazardous organisms and toxins; and to maintain the required records of the review, approval and monitoring of

the use and disposal of recombinant DNA molecules and other biologically hazardous organisms and toxins.

## 2. Application to IBC for Approval

For more information contact the UT Tyler Office of Research and Scholarship at (903) 565-5774, also at <http://www.uttyler.edu/research/compliance/ibc/> or the UT Tyler Biological Safety Officer at (903) 566-5774.

## G. Select Agents

Select agents are biological agents or toxins that could pose a severe threat: to public health and safety, to animal or plant health, or animal or plant products. A current listing of select agents and toxins can be found at <https://www.selectagents.gov/SelectAgentsandToxinsList.html>.

It is illegal to possess select agents without registration with the federal government. If you have any select agents which have not been registered, contact the Biological Safety Officer. If you plan to begin work with select agents, the application process should be started as early as possible. The process for approval for select agent work can take several months and requires additional security clearance procedures and approval of UT Tyler EH&S.

## H. Blood Borne Pathogens Policies and Procedures

### 1. Laws and Regulations

Work with materials known or suspected to contain Bloodborne Pathogens (BBP's) is regulated by both the federal and state governments. In the state of Texas the Texas Department of State Health Services regulates work through 25 TAC Part 1 Chapter 96, Bloodborne Pathogen Control, which became final September 1, 2000. Disposal of

waste which contains or could contain BBP's is regulated by the Texas Commission on Environmental Quality through 30 TAC Chapter 330, 1201-1221, the Regulated Medical Waste Rule. Both the federal and state regulations require annual training on BBP's and a BBP Exposure Control Plan. UT Tyler's Blood Borne Pathogens Exposure Control Plan can be found at the EH&S website at <https://www.uttyler.edu/safety/files/bbp-exposure-control-plan.pdf>.

EH&S also has training for BBP for those who regularly work with common routes of infection. Contact EH&S to inquire about training.

#### I. Housekeeping methods

There are different levels of cleanliness used in a laboratory setting.

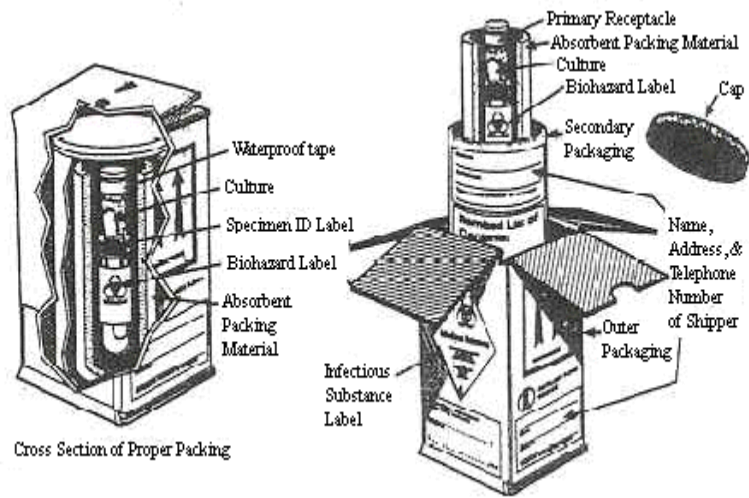
1. **Sterilization** is a method which destroys all microbial life, including bacterial spores. Autoclaving (dry or steam) is one common method of sterilization.
2. **Disinfection** is a method which reduces all forms of disease causing organisms on inanimate surfaces.
3. **Decontamination** is a method which reduces the numbers of organisms to acceptable levels.
4. **Antisepsis** is a method which reduces the number of organisms on living tissues.
5. The different decontamination methods which can be used for cleaning biological spills, work areas and equipment or glassware are heat, UV radiation and chemicals. Heating involves dry or steam autoclaving which is sterilization method. Radiation in the form of ultraviolet light (UV) has limited effectiveness and should not be used as the only decontamination method. Chemicals have varying degrees of effectiveness according to the biological agent involved and may be hazardous. Phenols, aldehydes, halogens such as chlorine and iodine present health hazards and/or are unstable. Contact the Biological Safety Officer for more information

## J. Transportation and Shipping

Biological agents which are shipped fall into two categories: category A – Infectious Substances (UN 2814 or UN 2900) and category B - Biological Substances (UN 3373). There are various agencies and regulations to comply with when shipping. Entities involved in shipping include the USDA Animal and Plant Health and Inspection Service (APHIS), the Department of Transportation (DOT), the United States Postal Service (USPS), the Federal Aviation Administration (FAA), the International Air Transport Association (IATA) and the International Civil Aviation Organization (ICAO). Import/export permits are sometimes required, even within the US for specific agents. Specific shipping training with periodic re-training is required every 2 years for IATA member regulated air shipments and 3 years for DOT regulated ground shipments. Shipping without training can result in high fines and additional sanctions against the University.

1. Packaging for shipping biological agents requires a primary container with a positive seal surrounded by enough absorbent material to completely contain a spill. Secondary packaging which is watertight and leak proof holds the primary container. An outer container which must pass specific performance tests completes the containers. Packaging labels are necessary and between the secondary and outer packaging there must be a list of contents, the shipper's label, including name, address and phone. The shipper's label must also be on the outer container.
2. EH&S can advise and aid on any packaging. Please contact the Biological Safety Officer for more information.
3. Example Packaging label for a category A – Infectious Substance





## V. Personal protective equipment (PPE)

Personal protective equipment (PPE) is a device or clothing worn to help protect you from direct exposure to hazardous materials. Examples include safety glasses or goggles, laboratory coats or aprons, gloves, face shields and respirators. Remember, PPE only protects you if you use it properly.

## A. Eye and face protection

Eye protection must be worn at all times while conducting experimentation and should meet standards for impact resistance and provide splash protection. Safety glasses with side shields usually provide adequate impact resistance with limited splash protection. Chemical splash goggles (with no perforations around the goggles) provide adequate impact resistance and splash protection and limited vapor protection and therefore provide the best all-around eye protection. Vapor resistant goggles are available if needed. In addition to protective eyewear, face shields or freestanding shields should be used in situations where implosion or explosion may occur. Follow these guidelines for effective eye and face protection:

1. Wear protective eyewear at all times in the laboratory.
2. Wear chemical splash goggles for maximum protection, especially if you wear corrective lenses (glasses or contacts).

## B. Hand protection

Gloves protect your skin from the biological agents you work with. Use gloves when your work contains some hazard (chemical or biological). Disposable latex gloves protect against water, dirt and microorganisms. Due to latex allergies or the possibility of developing such allergies, gloves made for other materials should be available in the laboratory. Information is available online, from the manufacturer or from EH&S on the different types of gloves available and under which circumstances they should be used. Follow these guidelines for effective hand protection.

1. Wear gloves that provide the greatest protection from the biological agents with which you are working.
2. Wash your hands promptly after removing protective gloves to avoid exposure due to microscopic holes, tears, or accidental contact with the outside of the gloves when removing them.

3. Remove gloves when handling common laboratory items (telephones, doorknobs, etc.) to prevent their contamination.

#### C. Body protection

Wear body protection when your work contains some hazard. The most common form of body protection in the laboratory is the laboratory coat. Laboratory coats protect your skin and clothes in the event of a spill or a splash. Follow these guidelines for effective body protection.

1. Protective clothing should be easily removable and free from rips or tears.
  - a. Proper attire includes pants, short or long-sleeve shirt, close-toed shoes.
  - b. Improper attire includes shorts, v-necks, sandals or woven shoes, miniskirts, excessive jewelry, or flip-flops/sandals.
2. Wear your laboratory coat or apron only in the lab to prevent the potential spread of contamination.
3. Laboratory clothes should not be taken home to launder.

#### D. Respiratory protection

Respiratory protection in the laboratory is normally provided as engineering controls such as the ventilation system and the biological safety cabinet(s). When a higher level of respiratory protection is required, an N-95 or PAPR (Powered Air Purifying Respirator) can be used. Contact EH&S for assistance in selecting the correct respirator. Medical assessment, fit testing and training on proper use and storage are necessary prior to using a respirator. Follow these guidelines for effective respiratory protection:

1. Do not use a respirator unless you have been trained to do so and have undergone a medical evaluation.
2. If you are wearing a respirator, be sure appropriate fit-testing has been performed by a medical facility.
3. Properly store a respirator to prevent continued contamination.

## VI. Laboratory equipment

A general understanding of laboratory equipment and how it works is essential to working safely in the laboratory.

### A. Biological safety cabinets.

Biological safety cabinets (BSCs) are among the most effective and most commonly used primary containment devices in laboratories working with infectious agents. The BSC is designed to capture and contain infectious particulates or aerosols generated within the BSC and exhaust them through a HEPA filter. HEPA filters are ineffective against volatile chemicals; therefore, volatile chemicals should not be used in BSCs.

Most BSCs recirculate 30 or 70 % of their air within the cabinet. During this recirculation, the air passes over motors and wiring which are incompatible with a flammable atmosphere. Open flames can be problematic as well. The accidental release of flammable gas into the ventilation system of a BSC can result in a fire. Heat buildup from the use of a flame in a BSC can damage the HEPA filter, releasing infectious agents into the laboratory or the environment. Also important in some work is the fact that flames in BSCs will disrupt the laminar flow of the air and can cause contamination problems within the cabinet or in the laboratory. **For this reason flammables are not for use in BSCs.**

For information on alternatives to flames in BSC's contact the Biosafety Officer. NEVER have a BSC connected to natural gas lines without first gaining contacting Biosafety Officer.

BSC's must be installed and certified (annually) by a certified professional. EH&S arranges the annual certification. BSC's which have been moved must be recertified before use. Contact EH&S to arrange recertification.

1. Classes of BSC's

a. Class I BSCs

These offer HEPA-filtered exhaust air; however, the supply air is not HEPA-filtered (—dirty room air|| drawn inside), thus offering minimal protection to the user's hands and arms and vulnerable research materials inside the BSC. The Class I BSC is designed for general microbiological research with low- and moderate-risk agents (biosafety level 1 and 2 agents) and is useful for the containment of mixers, blenders and other equipment. Class I BSC's are not routinely made.

b. Class II BSCs

There are different types of Class II BSCs, but they all offer HEPA-filtered supply and exhaust air. This BSC protects the user, environment and research material and is suitable for work with moderate to high-risk agents (biosafety level 2 and 3 agents). Class II BSCs are the most commonly used.

Class II Type A1 cabinets were previously called Type A. This type of BSC recirculates 70% of the air and exhausts 30%. It has a positive pressure plenum. The Type A2 resembles the Type A1, but the positive pressure plenum is surrounded by negative pressure plenum. This cabinet was previously known as the Type B3. It can be connected to building exhaust with a thimble unit.

The Class II Type B BSC is hard-ducted to an external exhaust system. The Type B1 recirculates 30% of the air and exhausts 70%.

The cabinet has a positive pressure plenum with all contaminated air contained within a negative pressure plenum. The supply blower is interlocked to prevent it from operating when the exhaust is insufficient. Emergency power can be connected to the exhaust blower to prevent the blower from shutting down.

The Class II Type B2 BSC is a total exhaust cabinet. Its blower is interlocked. Contaminated air is contained in positive pressure plenums enclosed in a negative pressure plenum. This is the only BSC in which small amounts of toxic chemicals, volatiles, and radionuclides (may need charcoal filters) may be used.

c. Class III BSCs

Often referred to as glove boxes, these gas-tight BSCs are under negative pressure. All work in the cabinet is done through rubber gloves attached to entry portals. The exhaust is double HEPA filtered and/or incinerated. Materials must enter through a sealed airlock and exit through an autoclave or dunk tank. Class III BSCs offer the highest level of protection and are suitable for work with extremely high risk agents (biosafety level 4).

2. Cabinets known as clean air centers, laminar flow clean air stations, laminar flow benches, aseptic work stations or horizontal/vertical flow benches function differently from BSC's. They deliver HEPA filtered air across a bench top. This provides product protection and can be used with sterile equipment, for the assembly of equipment and preparation of sterile media. The air blows towards the worker. This can cause exposure to workers if improperly used with infectious materials. Reverse-flow cabinets of this type pull air from the front of cabinet through a pre-filter and HEPA filter at the rear. It can be used for cage changes, but PPE must be worn. These cabinets are not for work with biohazards since there is no containment.

3. Proper techniques for working in BSC's:

a. Always enter straight into cabinet with no sweeping motions.

- b. Place materials well within cabinet.
- c. Place the discard pan within cabinet.
- d. Watch for disruptions of the laminar flow.
- e. Decontaminate materials before removal from the cabinet.
- f. Protect vacuum lines with HEPA filters or traps.

## B. Compressed Gas Cylinders

Compressed gas cylinders can present a dual hazard in the laboratory because the contents are under pressure and may contain hazardous materials, such as flammables, corrosives or toxics. Follow these guidelines for proper use of compressed gas cylinders:

1. Compressed gas cylinders, empty or full, must be secured at all times.
2. Cylinder caps must be in place except when the cylinder is in use.
3. Do not transport gas cylinders without the cylinder cap in place and an appropriate dolly with a securing strap.
4. Cylinder and delivery valves should be closed when not in use (especially true for toxic, flammable or corrosive gases).
5. Highly toxic, corrosive, and reactive gases present greater degrees of hazard. Work with these gases might require special containment, PPE, ventilation, piping systems, or alarm systems. Prior to ordering or working with these types of gases contact EH&S for a risk assessment and determination of requirements.
6. Liquid nitrogen or any other liquefied gas can present additional hazards for handling and storage. Details on proper handling and storage can be found in the Chemical Safety Plan.

### C. Centrifuges

Improper centrifuge use can result in the generation and release of hazardous aerosols. Centrifuges present a contamination problem when tubes break and the contents are released. Follow these guidelines for proper centrifuge use:

1. Make sure the lid is on and secured before operating the centrifuge and remains secured until the centrifuge has come to a complete stop.
2. Always balance the load in the centrifuge. If you are not filling the entire centrifuge rack, position the tubes opposite one another. If you have an odd number of samples, use an empty tube with enough water to be of equivalent weight.
3. If vibration occurs, stop the centrifuge and check the load balances. Never operate an unbalanced centrifuge; this could result in breaking the centrifuge tube(s) and generating hazardous aerosols. Also, unbalanced rotors have the potential to become projectiles.
4. Keep the rotors and buckets clean, and promptly clean breakages or spills.
5. Ensure that the proper rotor is used for the centrifuge or the conditions of centrifugation.

### D. Refrigerators

Follow these guidelines for proper laboratory refrigerator use:

1. Never place food or beverages in a refrigerator where biohazardous materials are stored. Signage must be placed on the refrigerator indicating no food/drink contents should be stored.
2. Refrigerators containing biohazardous materials must be labeled, "biohazard," with the appropriate biohazard symbol in black on an



orange-red background along with other necessary signage (flammability, NFPA 704, no food, etc...).

#### E. Autoclaves

Autoclaves operate at high temperature and pressure and can present a physical hazard if not operated properly. Follow these guidelines for proper autoclave use:

1. Users must be trained on operation prior to operating any autoclave.
2. Ensure cycles are completed using autoclave tape. The autoclave must be set correctly for the agent or sterilization will not be achieved. Check with protocol information for specific agents.
3. Functionality tests must be performed periodically and recorded to confirm the autoclave is functioning properly.
4. Logs must be kept for each use of the autoclave.
5. If using an autoclave for waste management, monthly tests must be conducted to ensure all life and equipment is destroyed in the process. Contact the biological safety officer for more information.

#### VII. Waste management

For information on biological waste, including reporting waste pickup and extra waste bins, please contact EH&S at (903) 566-7011 or [safety@uttyler.edu](mailto:safety@uttyler.edu).

- A. All biohazardous material must be put in a red biohazard bag contained inside a red biohazard bin for secondary containment. Contact department lab managers for proper disposal.
- B. All sharps, including needles and broken glass, should be put in an approved red sharps container.

- C. Chemical waste should be labeled with the chemical name, name of the waste generator, building and room number, and date of the first aliquot. The waste must remain in the lab until EH&S has properly picked up the waste for disposal. Contact your department or EH&S directly to schedule pickups.
- D. EH&S disposes of all hazardous waste. If you require waste disposal fill out and submit the following form: <https://www.utt Tyler.edu/safety/waste-pickup-request-form.php>

## **VIII. Emergency procedures and equipment**

For immediate help with emergencies, call *911*.

### **A. Biological Emergencies**

Response to biological emergencies should be planned before they occur. The plan must be thorough to prevent further injury or contamination.

Each laboratory should design its own response plan based on its unique hazards in a separate binder available to all lab personnel and visitors. The binder must include contact information, the location of the laboratory, laboratory self-evaluation form, and protocols for each hazardous experiment to include laboratory risk assessment template (SOP) and laboratory experiment/demonstration hazard assessment form (all forms can be found at the EH&S website at <https://www.utt Tyler.edu/safety/forms.php>), and contain the following general guidelines:

1. Instructions on how to evacuate the laboratory. The decision to evacuate is a judgment call based on the properties and hazards of the spilled biological agent. If biological aerosols result from the spill, evacuation should follow. Contact EH&S immediately at (903) 566-7011;

2. Always attend to injured people before attending to the spill. Skin areas splashed by chemicals or biologicals should be rinsed with water for at least 15 minutes in an, emergency shower or eyewash as appropriate. After thorough rinsing, seek medical help. Be sure to have the identity of the biological agent and other information, if possible, available for the medical help.
3. Try to contain spills to keep it from spreading. Contact EH&S to advise or assist in the containment, air handling, disinfection and cleanup of the spilled biological agent. Do not attempt to clean the spill without proper spill control supplies or equipment.
4. If the emergency is likely to affect other facilities within the building or campus, contact the UT Tyler Police Department at (903) 566-7300.

## B. Emergency equipment

Laboratory emergency equipment includes emergency showers, eyewashes and fire extinguishers. Staff in laboratories that do not have their own emergency shower and eyewash station should know where the closest one is located.

### 1. Showers

An emergency shower is used to decontaminate someone who has been exposed to biological agents or chemicals.

- a. Remove clothing, jewelry and shoes while standing under the shower. These items trap agents against the skin and will prevent proper cleaning if not removed.
- b. Remain under the shower for at least 15 minutes to ensure adequate flushing of exposed areas.
- c. Seek medical attention.

- d. If the shower does not have a drain, promptly clean up the water to prevent slip hazards adding the appropriate decontamination agent.
- e. Always keep the area under an emergency shower unobstructed.

## 2. Eyewashes

- a. If biological agents are splashed into your eyes, locate the nearest eyewash station and hold your top and bottom eyelids open and flush with water continuously for at least 15 minutes. Move the eye up and down and sideways to wash thoroughly behind the eyeball where agents could be trapped.
- b. Seek medical attention.
- c. Always flush your eyes immediately if biological agents are splashed into them. Immediate action may prevent an infection.
- d. Continuous-flow eyewashes are preferred over the portable, squeeze-bottle type, whose disadvantages include an insufficient supply of water (not 15 minutes' worth) and easy contamination with microorganisms. Only temperature controlled eyewashes are allowed at UT Tyler.
- e. To ensure a clean supply of water in the eyewash, operate it weekly to flush out any impurities.

## 3. Spill Kits contents:

- a. Neutralizing agents for acids and bases
- b. Absorbent material

## **IX. Other Agents**

- A. Some agents not classified as infectious or select pose a danger in biological laboratories.
  - 1. Formaldehyde

Formaldehyde is a chemical used in the preservation of biological organisms. UT Tyler still uses formaldehyde for some preservation. It is important to note that formaldehyde is a skin and respiratory irritant. Prolonged contact could be extremely hazardous to your health. Use all formaldehyde products according to the SDS provided by the manufacturer. If the SDS cannot be found contact EH&S at [safety@uttyler.edu](mailto:safety@uttyler.edu) or (903) 566-6168.

**X. References**

- A. The University of Texas at San Antonio. Biological Safety Manual. 2011.
- B. Chapter 502 of the Health and Safety Code. Texas Hazard Communication Act, Revised 1993. Texas Department of State Health Services, Division of Regulatory Services, Enforcement Unit.
- C. Handbook of Laboratory Safety. Chemical Rubber Company, Third Edition, 1990.
- D. Biosafety in Microbiological and Biomedical Laboratories. The Centers for Disease Control and Prevention and the National Institutes of Health, Fifth Edition, Revised December 2009.
  - 1. Appendix A - Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.
- E. Biological Safety in the Laboratory: A Guide for Biological Safety and Handling Biological Agents. The University of Texas Health Science Center at San Antonio, Department of Institutional Safety, 1995.
- F. Guidelines for Research involving Recombinant DNA Molecules. National Institutes of Health, January 2011.

**XI. Contact Information**

Emergency.....911  
Central Dispatch.....903-566-7300  
EH&S General Contact:..... 903-566-7011  
Laboratory Safety:.....903-566-6168

Facilities Operations:.....X-7291

See: <http://www.uttyler.edu/safety/> for more information.