

# BIOLOGICAL SAFETY MANUAL



THE UNIVERSITY OF  
TENNESSEE  
HEALTH SCIENCE CENTER.

MEMPHIS CAMPUS

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# THE BIOSAFETY PROGRAM

The University of Tennessee Health Science Center, Memphis, hereafter referred to as the UT Health Science Center; is committed to protecting faculty, staff, students, visitors, the general public and the environment from exposures (or potential exposures) to biological hazards, and to ensuring that all activities involving biological hazards and the facilities used to conduct such work are following applicable U.S. Federal, State and Local laws, regulations and guidelines.

## COMPOSITION OF THE BIOSAFETY PROGRAM

The Biosafety Program is composed of the Institutional Biosafety Committee and the UT Health Science Center Office of Research Safety Affairs. The UT Health Science Center Institutional Biosafety Committee (IBC) oversees the use of infectious materials, recombinant DNA and synthetic nucleic acids at UT Health Science Center. Information about IBC's mandate and objectives are provided in the UT Health Science Center **IBC Charter**.

The IBC considers the following biological hazards which require review and approval:

1. **Recombinant or synthetic nucleic acid molecules** as defined in the **National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules** (Hereafter NIH Guidelines), including transgenic plants and animals (see details below).
2. **Biological agents (bacteria, viruses, fungi, protozoa, parasites, and prions)** which: 1) cause or are reasonably expected to cause disease in immunocompetent humans; or 2) cause or are reasonably expected to cause significant disease in local livestock (including poultry), agricultural crops,

or indigenous wildlife; 3) are covered by the **Federal Select Agent Program**; or 4) otherwise require enhanced safety and/or containment procedures.

3. **Acute biological toxins** having an LD50 < 100 ng/kg in mammals and/or those listed as select toxins by the Federal Select Agent Program.
4. **Human or nonhuman primate blood, blood products, tissues, secretions, excretions, or cell lines** unless documented to be free of bloodborne pathogens (BBP) or are otherwise low risk as per written risk assessment.
5. **Venomous animals** housed and/or manipulated in laboratories or other indoor facilities.
6. **Insects or arthropods** harboring pathogens with the potential to cause disease in humans and animals.
7. **Novel nanoparticles** conjugated to biologically active or cell-modifying molecules.
8. **Diagnostic specimens or environmental samples** likely to contain any of the above and posing a significant risk to humans or local livestock (including poultry), agricultural crops, or indigenous wildlife as per documented risk assessment.

The IBC mandates the use of biosafety precautions that effectively mitigate the risk of exposure to potentially hazardous agents. These precautions are established by regulations, guidelines, and institutional policy:

1. **At the FEDERAL level:**
  - a. Occupational Safety and Health Administration (OSHA) **Bloodborne Pathogens (BBP) Standard and Lab Safety Standard**
  - b. **NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules** (see details below)

- c. **USDA Animal & Plant Health Inspection Service (APHIS)**
  - d. **US Federal Select Agent Program**
  - e. **Center for Disease Control (CDC)/NIH published Biosafety in Microbiological and Biomedical Laboratories, 6th Edition (BMBL)**
  - f. **Department of Transportation** and the **International Air Transport Association (DOT/IATA)** Hazardous Materials/Dangerous Goods Regulations.
2. **At the STATE level:**
    - a. **Tennessee Occupational Safety and Health Administration (TOSHA)** oversight of federal OSHA requirements (Bloodborne Pathogens Standard and Lab Safety Standard).
    - b. **Tennessee Department of Environment and Conservation (TDEC)** oversight of regulated medical waste.
  3. **At the INSTITUTIONAL level:**
    - a. **UT System Policy SA0450-Biological Safety**
    - b. **Institutional Biosafety Committee (IBC)**
    - c. **Institutional Animal Care & Use Committee (IACUC)**
    - d. **Office of Sponsored Programs**
    - e. **Institutional Review Board**

## The IBC and the NIH Guidelines

The *NIH Guidelines* mandate the establishment of an IBC for the review and approval of research involving recombinant and synthetic nucleic molecules (rsNA). To familiarize you with the NIH Guidelines:

- The *NIH Guidelines* cover rsNA, defined as two or more adjoined molecules that can base pair and replicate, and which are not found contemporaneously in nature, and/or any synthetic counterparts meeting the same definition. rsNA that does not encode proteins or functional elements are not excluded from coverage.
- All research use of rsNA must be communicated to the Biosafety Program for determination of review/approval requirements. This includes the creation/use of transgenic plants or animals and any project involving genome editing (CRISPR/Cas9, TALEs/TALENs, zinc fingers, directed mutagenesis, etc.).
- Some projects involving rsNA require IBC (and sometimes NIH) approval prior to commencement.
- The IBC can only approve projects in containment. Environmental releases of rsNA-containing organisms is subject to review and approval by an applicable federal agency (USDA, FDA or EPA)
- Compliance with the *NIH Guidelines* is not optional. These are a term/condition of NIH funding, which all UT Health Science Center campuses receive.
- The *NIH Guidelines* apply to all institutional research involving recombinant or synthetic nucleic acids regardless of funding status or funding agency (i.e., they are not exclusive to NIH-funded projects).
- The *NIH Guidelines* outline roles/responsibilities for the institution, IBC, biosafety officer, and PIs (outlined in the following module).

- As required by the *NIH Guidelines*, the IBC is staffed with expertise to competently review the safety and containment of recombinant/synthetic nucleic acids. Additionally, at least two local non-affiliated members are appointed to represent the safety interests of the community.

## The Office of Research Safety Affairs

The UT Health Science Center Office of **Research Safety Affairs** supports the IBC and research community by:

- Assisting faculty with registration of research projects and keeping project information current (amendments and updates).
- Performing risk assessments.
- Monitoring and maintaining institutional records.
- Providing training for faculty, staff and students.
- Conducting laboratory inspections and post-approval monitoring.
- Investigating spills/accidents/exposures involving biological hazards and recommending remedial actions.
- Monitoring for updates or changes to Federal, State and local standards/regulations.
- Assisting with biological hazard and dry ice shipping.
- Providing guidance and support for federal/state permits.

Guidance and support for federal/state permits includes:

1. United States Department of Health and Human Services/Center for Disease Control (U.S. DHHS/CDC) permits.

The **CDC Import Permit Program**, or IPP, regulates the importation of infectious biological materials that could cause disease in humans in order to prevent their introduction and spread into the U.S. Materials requiring import permits include:

- a. Infectious biological agents capable of causing illness in humans.
- b. Materials known or reasonably expected to contain an infectious biological agent.
- c. Vectors of human disease (such as insects or bats).

2. United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits - these permits are issued for the import, transit and release of regulated animals, animal products, infectious agents/vectors affecting poultry/livestock, veterinary biologics, plants, plant products, plant pests, plant pathogens, soil, and genetically engineered organisms.

3. **United States Fish and Wildlife Service (USFWS)** permits - these permits are issued under various wildlife laws and treaties at different offices at the national regional and/or wildlife port levels. These permits cover a wide range of import/export regulations including the trapping, buying, selling, and trading of live animals (non-agricultural), preserved animal trophies, animal hides, and animal tissues.

4. **Department of Commerce (DOC)** export of biological agents - The DOC has developed a list of biological agents (among other products and technologies) that are restricted by licensing requirements for export.

Additional information on the Biosafety Program can be found at [uthsc.edu/research/safety](http://uthsc.edu/research/safety).

# INTRODUCTION TO THE BIOLOGICAL SAFETY MANUAL

## THE BIOSAFETY MANUAL

This Biological Safety Manual is a repository of information offered as a representation of the purposes and services of the UT Health Science Center Biosafety Program. To take advantage of the Internet, this document is formatted to be a “front door” to other resources, including useful web links. Where appropriate, web links are provided that can be clicked to view the webpage. This Biological Safety Manual should be considered a living document and will be reviewed periodically and updated as needed.

This manual focuses on Biosafety Levels 1 and 2 as most UT Health Science Center research laboratories fall within these designations. A separate manual is available for researchers working in the BSL-3/ABSL-3 laboratory in RBL. *No work with Biosafety Level 4 agents may be conducted at the University of Tennessee Health Science Center.*

The Biosafety Program and the requirements for researchers are outlined in this manual. Registration and training information is provided along with details on work practices and safety equipment. It is the responsibility of the Principal Investigator or Supervisor to ensure that his/her laboratory is complying in compliance. That responsibility includes identification of the risk or hazards associated with their research and the application of the appropriate safety procedures. Please read the section on responsibilities for additional information.

The Office of Research Safety Affairs (RSA) is available for consultation if you have any question or concern with any aspect of the Biosafety Program at the UT Health Science Center. If you are unsure of a requirement or biosafety practice, please contact the RSA at 901-448-6114 for assistance. Any feedback or comments that will improve this document or fortify the service provided by the Office of Research Safety Affairs are welcomed.

## 1 RESPONSIBILITIES

### 1.1 UT Health Science Center Campus-wide laboratory safety responsibilities

#### 1.1.1 University President

The President of the University of Tennessee assumes overall responsibility for promoting a culture of safety and creating a safety plan to protect the well-being of faculty, staff, students, and the university.

#### 1.1.2 Chancellors, Vice-Chancellors, and Deans

- Promote a culture of safety and enforce the health and safety plan at the campus level.
- Designate campus Safety Officers with responsibility for implementation, management, and enforcement of environmental health and safety programs on campus.

#### 1.1.3 Designated Official for the Biosafety Program

The UT Health Science Center Vice-Chancellor for Research is the Designated Official (DO) for the Biosafety Program. The DO is responsible for the following:

- Coordinating with applicable University administrators to ensure that research, teaching, diagnostic testing, clinical trials, or other activities involving biological hazards are conducted safely and in compliance with applicable regulations, standards and guidelines.
- Establishing and implementing policies/procedures that provide for the safe conduct of research, teaching and diagnostic testing involving biological hazards.
- Maintaining an active IBC; appointing the IBC Chair, Vice-Chair, and committee members in accordance with the NIH Guidelines and other University requirements as applicable.
- Granting the IBC and Institutional Biological Safety Officer (IBO) authority to oversee the safe and responsible use of biological hazards at the UT Health Science Center-area campuses.
- Verifying that all IBC-approved projects include the necessary resources for the construction and operation of safe research and for the implementation of the Biosafety Program.
- Providing adequate resources for the dissemination of information on biosafety policies and procedures, including training programs and workshops.
- Coordinating or providing resources for medical surveillance measures or occupational health programs to protect the health and safety of faculty, staff, students, and visitors.
- Imposing or upholding disciplinary actions or sanctions on principal investigators or laboratory supervisors who fail to comply with established regulations, standards, guidelines, or University policies.
- Reporting any significant problems, or violations to U.S. Federal, State, or ILocal agencies as applicable. If appropriate, agency reporting may be delegated to the IBC or BSO.
- Representing the IBC as needed.

#### 1.1.4 Responsible Official

The RO is the University delegate with the legal authority and responsibility to oversee possession, use, and transfer of select agents, or those infectious agents and toxins that are considered by the Department of Health and Human Services (DHHS) or the United States Department of Agriculture (USDA) as having the potential to pose substantial harm or a severe threat to human, animal, or plant health. The RO is appointed by the DO, approved by the applicable federal agencies, and charged with the legal authority and responsibility to oversee the possession, use and transfer of select agents. The RO is responsible for the following:

- Possessing a detailed knowledge of the Select Agent Regulations to the extent that he/she can ensure the University is compliant with the programmatic requirements.

- Conducting annual inspections for each laboratory and all other registered areas where select agents are stored and/or used to determine compliance with the requirements of the Select Agent Regulations. The results of each inspection must be documented, and any deficiencies identified during an inspection must be corrected by a specified date.
- Conducting annual emergency preparedness, spill response, and/or security drills as required.
- Having a physical presence at UT Health Science Center to maintain compliance with the Select Agent Regulations and be able to respond in a timely manner to onsite incidents involving select agents in accordance with the incident response plans.
- Be granted sufficient authority to speak and act on behalf of the UT Health Science Center.

#### 1.1.5 Institutional Biosafety Committee (IBC)

The IBC establishes, recommends, and approves policies ensuring the prudent management of biological hazards, including recombinant and synthetic nucleic acid molecules. Policy objectives are to protect faculty, staff, students, research subjects, the public, and the environment from biological hazards used in university research, teaching, diagnostic testing, or other specified activities. The IBC is responsible for the following:

- Establishing, communicating, and monitoring policy, practices, and procedures covering biological hazards which are in accordance with applicable regulatory standards and guidelines.
- Reviewing biological hazard registrations to ensure compliance with regulations, guidelines, and adopted policies. Review will include an independent assessment of the risk(s), required safety practices, biological/physical containment and associated facilities, and training and expertise of affiliated personnel. The IBC will communicate registration review outcomes and necessary actions to the principal investigator or laboratory supervisor in a timely manner.
- Regularly assessing safety practices and containment facilities to ensure they are appropriate for the proposed biological hazards and affiliated procedures. The IBC will use the biosafety levels (BSL) published by the CDC, NIH, and USDA as the usual standards of containment to be set for work with a given biological agent. To the extent allowed by Federal law and regulation, the IBC may, at its discretion, increase or reduce the BSL depending on the circumstances presented by a specific project.
- Investigating and recommending corrective actions for accidents, exposures, illnesses, environmental release and/or other adverse events involving biological hazards. The NIH Office of Science Policy (OSP), CDC, USDA, or other regulatory or funding agencies will be notified if required.
- Investigating and setting corrective actions for violations of policies, practices, or procedures. The IBC, at its discretion, may deny, suspend, or terminate approval for use of biological hazards if such use poses a risk to personnel or public health and safety, or for issues of noncompliance. If necessary, recommendations for additional disciplinary actions may be made to UT

Health Science Center administration. The NIH OSP, CDC, USDA, or other regulatory or funding agencies will be notified if required.

Additionally, the IBC, in conjunction with the Institutional Biological Safety Officer (IBO) and/or Select Agents RO has a role in the following:

- Reviewing and approving design specifications and certification criteria for high-containment laboratories (BSL-3/ABSL-3).
- Reviewing and approving policies and procedures related to select agents (or others categorized as Risk Group 3), including security/access, inventory management, laboratory protocols and emergency response plans.
- Reviewing and assessing compliance with permit or license-related requirements for biological materials subject to USDA APHIS, FDA, and/or EPA regulations.
- Consulting with the IBO on biological hazards used in teaching or diagnostic testing laboratories, as necessary.
- Establishing a framework for the identification, management, and reporting of dual use research of concern as defined in the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern as applicable. If dual use research of concern is identified, an IBC-selected panel will serve as the Institutional Review Entity (IRE) as described in the policy.
- Performing other functions as delegated by UT Health Science Center administration.

#### IBC Chair responsibilities include:

- Setting meeting agendas and establishing meeting dates.
- Conducting meetings.
- Prescreening submitted registrations as assigned.
- Approving registrations.
- Reviewing and approving amendments, updates, and forms as necessary.
- Ensuring member training; this task may be designated to the IBO or another qualified individual.

#### IBC Vice-Chair responsibilities include:

- Substituting for the Chair, as necessary.
- Coordinating the periodic review and revision of the IBC Charter.
- Prescreening submitted registrations as assigned.
- Approving registrations, amendments, or updates in the absence of the Chair.

#### IBC member (including non-affiliated members) responsibilities include:

- Completing biosafety regulatory awareness training (IBC training) before participating in voting activities of the committee; completing annual retraining covering IBC-related topics, as necessary.
- Attending monthly meetings; notifying the IBC Chair if attendance is not possible.
- Prescreening submitted registrations as assigned.
- Reviewing registrations and providing feedback to the IBC, as necessary.

### 1.1.6 Institutional Biosafety Officer

The IBO is the primary intermediary between the IBC and principal investigators (PIs) and/or laboratory supervisors. The IBO and Research Safety Affairs staff are responsible for the following:

- Managing the administrative tasks of the Biosafety Program and supporting implementation of IBC policies and procedures.
- At the discretion of the IBC, establishing/implementing a framework for oversight of biological hazards and associated procedures in teaching, research, and diagnostic testing/clinical labs.
- Performing risk assessments, confirming NIH Guidelines review categories, and providing technical advice to the IBC, DO, RO, PIs, or other stakeholders as required or requested.
- Performing annual inspections of facilities where biological hazards are being used or stored to ensure safety and containment measures as outlined in the NIH Guidelines, the BMBL, the OSHA BBP Standard, and/or other standards as applicable.
- Reporting any significant problems, violations, or research, teaching, or diagnostic testing-related accidents or illnesses to the IBC, DO, RO, or other campus administrators as applicable.
- Assisting PIs, laboratory supervisors, staff, and students in conforming to applicable regulations, standards, guidelines, and IBC policies by communicating expectations, providing training, technical advice, conducting facility inspections, and providing hands-on assistance as necessary (e.g., shipping biological materials).
- Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving biological hazards.
- Developing and implementing an exposure control plan for those research and teaching programs handling human derived materials as stipulated by the OSHA BBP Standard.
- Administratively reviewing proposals and protocols submitted by PIs and laboratory supervisors and making recommendations to the IBC Chair.
- Screening protocols submitted to the IACUC for identification of occupational hazards, consulting with animal facility management, veterinarians and/or PIs regarding appropriate containment procedures for biological hazards. Employing a similar mechanism of review, assessment, and implementation for other UT Health Science Center compliance committees (e.g., human subjects research involving biological hazards and/or recombinant nucleic acid submitted to the Institutional Review Board (IRB)).
- Serving as a member of the IBC. If the university conducts research involving recombinant or synthetic nucleic acid molecules requiring BSL3 or BSL4 containment or engages in large-scale research or production involving viable organisms containing recombinant or synthetic nucleic acid molecules, then the IBO shall become a member of the IBC sub-committee as per requirements of the NIH Guidelines.

### 1.1.7 Principal Investigator (PI)

The PI is defined as the faculty member or other University employee in whose assigned space research activity is conducted. *The PI is primarily responsible for the prudent management of biological hazards, the safety and health of laboratory staff, students and visitors, and full compliance with applicable regulatory standards, guidelines, and policies/procedures. Although the PI may choose to delegate these aspects to other laboratory personnel or faculty, this does not absolve the PI of his/her ultimate responsibility.*

The PI is responsible for the following:

- Creating and fostering an environment in the laboratory that encourages open discussion of biosafety issues, problems, and violations of procedure. The PI will not discipline or take any adverse action against any person for reporting problems or violations to the IBC, IBO, DO, Risk Management, or State or Federal agencies.
- Complying with all regulations, standards, guidelines, and University policies/procedures involving handling, storage, disposal, inventory, security/access, and transportation of biological hazards.
- Developing, implementing, and maintaining written laboratory-specific biosafety procedures appropriate for the biological hazards used in the laboratory. The PI shall ensure that all laboratory staff, students, and visitors understand and comply with these laboratory-specific biosafety procedures.
- Delaying initiation of research involving biological hazards (*including recombinant and synthetic nucleic acids*) until the research protocol has been submitted to, reviewed, and approved by the IBC.
- Maintaining an inventory of all biohazards used or stored in the laboratory. Logs should include species/strain-specific details, approximate quantity on hand, and where they are stored in the laboratory.
- Restricting access to the laboratory and stored biological hazards to authorized personnel only.
- Ensuring that all laboratory personnel, maintenance personnel and visitors who may be exposed to any biological hazards are informed in advance of the potential risk and of the practices required to minimize that risk.
- Adding staff, students, and visitors working on IBC-approved projects to the respective IBC registration(s). Regularly reviewing listed personnel and providing updates to the IBC or IBO as applicable.
- Completing training as required by the IBC or any other oversight agency, ensuring that all staff, students, and visitors participating in biohazard-related activities have completed appropriate training, and maintaining documentation of training. The PI or designee must provide protocol-, agent- and laboratory-specific training. The PI should contact the IBO for assistance with all biosafety training needs.
- Supervising the performance of the laboratory staff to ensure that required safety practices are employed. Work errors and conditions that may result in accidental releases or exposures are to be corrected immediately.

- Providing and maintaining all personal protective equipment (PPE) designated by risk assessment, including routine cleaning and/or replacement of dirty or contaminated PPE as appropriate. Additionally, the PI shall ensure that all affected laboratory faculty, staff, students, and visitors wear PPE as prescribed.
- Ensuring that all safety and containment equipment is maintained in good condition and functionally verified, as necessary. Maintenance work in, on or around contaminated equipment is to be conducted only after that equipment is thoroughly decontaminated by the laboratory staff or PI.
- Properly segregating and decontaminating biohazardous wastes before final disposal. All laboratory faculty, staff, and students are to be familiar with the appropriate methods of waste disposal.
- Coordinating with the IBO to develop emergency plans for accidental spills and exposures.
- Immediately notifying the IBO of laboratory spills, accidents, containment failure or violations of biosafety practice which result in the release of biological hazards and/or the exposure of laboratory personnel (or the public). The IBC may be consulted by the IBO, as necessary.
- Informing affected personnel of signs/symptoms that may result from accidental exposures and ensuring that they are informed of and receive medical surveillance or occupational health reviews, as necessary.
- Notifying the IBO immediately if a laboratory-acquired infection is known or suspected, or if a spill of any quantity involving an agent infectious to humans, plants, or animals occurs in a public area.
- Complying with shipping and permit regulations for biological hazards. The Research Safety office conducts shipping training for PIs and personnel, as necessary. The PI should contact the IBO to ensure that all applicable transportation safety regulations have been met prior to shipping microbiological cultures, tissues (human or animal) or body fluids.
- Immediately notifying the IBO if a select agent/toxin or other high-consequence pathogen (i.e., Risk Group 3 or 4) has been isolated and confirmed from environmental and/or diagnostic specimens.

*Note: The Principal Investigator is responsible for biosafety in the laboratories. The effectiveness of the information contained in this Biosafety Manual is dependent upon strong laboratory leadership that embraces a culture of safety in the lab.*

### 1.1.8 Laboratory Supervisor

The laboratory supervisor is the individual (faculty member or departmental designate) with primary responsibility for the use of biological hazards in the University's teaching/research or diagnostic testing laboratories. The laboratory supervisor is responsible for the following:

- Complying with all regulations, standards, guidelines, and University policies/procedures involving handling, storage, disposal, inventory, security/access, and transportation of biological hazards.
- Registering biological hazards with the IBC or IBO (as designated).

- Completing training and ensuring that all teaching or diagnostic testing staff have completed training, as required by the IBC or any other oversight agency, as applicable. Comparable information or training materials should be provided to students and trainees. Training and/or distribution of training materials should be documented by the laboratory supervisor.
- As necessary, communicating to teaching/research or diagnostic testing staff and students/trainees the signs and symptoms which may result from accidental exposures to the biological hazards in use.
- Stipulating the safety precautions to be followed by teaching or diagnostic testing staff, students, and trainees and ensuring that these are followed. Work errors and conditions that may result in accidental releases or exposures are to be corrected immediately.
- Determining the proper PPE to be worn for designated procedures. The laboratory supervisor (or designate) shall ensure that PPE is worn as directed and cleaned/replaced as appropriate.
- Immediately notifying the IBO of any laboratory spills, accidents, containment failures, or violations of biosafety practice which result in the release of biological hazards and/or the exposure of laboratory personnel or students/trainees. The IBC may be consulted by the IBO if necessary.
- Notifying the IBO immediately if a laboratory-acquired infection is known or suspected, or if a spill of any quantity involving an agent infectious to humans, plants, or animals occurs in a public area.

### 1.1.9 Department Chairs

The Department Chairs are responsible for the following:

- Reviewing and approving IBC registrations submitted by the departmental faculty members.
- Verifying that appropriate facilities are available to control biological hazards.
- Verifying that the PI or teaching lab supervisor has competency commensurate with the proposed project and/or assigned laboratory course(s).
- Ensuring that any reported safety deficiencies or compliance concerns/violations are corrected by the PI or teaching supervisor in a timely fashion.
- Notifying the IBO or new faculty hires, preferably before the new PI arrives on campus, if he/she plans to work with biological hazards.
- Notifying the IBO /IBC if a PI can no longer carry out his/her responsibilities (e.g., leaves the University, retires, etc.). If this is the case, the Department Head must select one of the following actions within 30 days (about 4 and a half weeks) of the PI's departure:
  1. Assume responsibility for PI's registration(s) and manage under his/her own program. This change in proprietorship must be reported to the IBO/IBC, and the Department Head must comply with all IBC provisions; or
  2. Assign the registration(s) to another investigator. This change in proprietorship must be reported to the IBO/IBC, and the designated PI must comply with all IBC provisions; or
  3. Terminate the registration.
- Ensuring that the PI and/or designated staff follow laboratory close-out procedures, including the appropriate disposal of biological hazards and disinfection of affected surfaces and equipment.

### 1.1.10 Laboratory Personnel

Laboratory personnel (staff, students, volunteers) are responsible for the following:

- Participating in biosafety orientation and training programs offered by the University.
- Becoming familiar with the work practices for lab-specific biological hazards approved by the IBC.
- Abiding by all biosafety precautions that are relevant to the assigned duties.
- Using prescribed personal protective equipment directed by the supervisor and in accordance with proper biosafety precautions.
- Exercising good judgment and asking questions regarding lab practices where you question the level of risk.
- Looking out for the safety of others in the lab and lab facilities.
- Reporting any observed unsafe conditions and unsafe practices to the University administration.
- Maintaining a positive attitude toward safety promotes safe working behavior.

## 2 PRINCIPLES OF BIOSAFETY

### 2.1 BIOHAZARD AWARENESS AND RISK ASSESSMENT

Biological laboratories are special work environments that can pose infectious disease or toxin exposure risks to persons working or entering these laboratories. In fact, there is a clear historical record of infections having been acquired in laboratory settings. More than 4,000 laboratory-acquired infections (LAIs) have been reported since the 1920s and many others have occurred. Some LAIs have been associated with morbidity and mortality, expensive remediation costs, and/or damaging publicity.

There may be instances where agent or procedural characteristics create unique hazards such as the potential for aerosolization of pathogens cultured in concentrations higher than found in nature, inadvertent contamination of surfaces, etc. Given the microscopic nature of most biological hazards, it is difficult-to-impossible to directly evaluate the risk in real time. Consequently, only ~15% of total LAIs are attributable to a known or identifiable accident or exposure. Therefore, understanding the risk assessment (potential risks and proactive/preventative measures) is essential.

This section outlines the biosafety principles related to risk assessment, agent risk groups, and prevention paradigms (biosafety levels).

#### 2.1.1 BIOLOGICAL HAZARDS

Biological hazards are any agents, materials, or conditions that pose a threat to human, animal, plant, or environmental health.

### Biological hazards include the following:

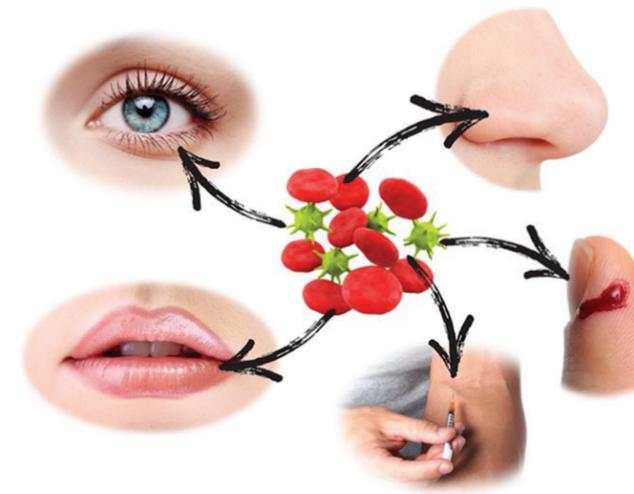
- **Biological agents**, including bacteria, viruses, fungi, protozoa, helminths, and prions. These are also referred to as infectious agents, etiological agents, or pathogens. Biological agents are propagative, cause a broad range of diseases (asymptomatic-to-fatal), and may take hours-to-years to manifest as disease in the host.
- **Recombinant or synthetic nucleic acid molecules.** While nucleic acids do not pose an explicit risk, the macromolecules they encode (or interact with) and resulting phenotypes may. Recombinant or synthetic constructs that encode toxins, viruses, oncogenes, antibiotic resistance (of clinical relevance), or any other molecule that contributes to disease are of particular concern. Host cells/systems, method/control of gene expression, potential for horizontal/vertical transfer, and/or research procedures may contribute to the risk of recombinant/synthetic nucleic acid molecules.
- **Biological toxins**, venoms or other molecules derived from biological systems that may cause or contribute to disease. These are non-propagative, but often have acute and serious-to-fatal effects.
- **Blood, blood products, tissues, secretions, excretions, or cell lines derived from humans or animals.** The risk profile is generally: human > primate/simian > other mammals > avian > reptile/amphibian > arthropods (other invertebrates)
- **Novel nanoparticles conjugated to biologically active or cell-modifying molecules** (e.g., siRNA, antibodies, effector proteins, etc.).
- **Environmental specimens**, particularly plant, soil, or water samples, are reservoirs of high-risk biological agents or toxins.

#### 2.1.2 ROUTES OF TRANSMISSION

One of the unfortunate consequences of working with biological hazards is the potential for acquiring an infection. History has shown that such infections occur and that laboratory workers are clearly at higher risk for infection with certain agents, such as the hepatitis B virus, than the general population.

Although work-related infections can occur via routes that differ from those in naturally occurring disease, there are limited routes of exposure and modes of entry into the body. A worker exposed to an infectious aerosol could inhale respirable particles. Larger droplets of that aerosol could fall on skin, mucous membranes, or environmental surfaces. The worker could then inadvertently inhale or ingest the agent without experiencing an overt accident. On the other hand, a needle stick or an animal bite would usually be noticed. Providing awareness and barriers for these routes of infection is a preventative approach to biosafety. The following are the primary routes of transmission that can result in laboratory acquired infections.

1. Injection (percutaneous)
  - a. Contaminated sharp objects (e.g., needle, scalpel)
  - b. Animal bites, scratches
  - c. Through broken or abraded skin (including rashes, eczema, split cuticles, etc.)
2. Absorption (mucous membrane contact)
  - a. Splashes to the eyes, nose, mouth
  - b. Hand-to-face movements, (i.e., applying cosmetics, cell phone usage, etc.)
3. Ingestion
  - a. Eating/drinking
  - b. Applying cosmetics
  - c. Contact with tear ducts
4. Inhalation (aerosols)
  - d. Liquid disturbance
  - e. Syringe preparation
  - f. Dried animal excretions
  - g. Leakage from injection site



#### 2.1.3 HOST FACTORS

In addition to the biohazard and route of transmission, host factors play an important role in the outcome of an exposure/infection. Factors that can increase susceptibility include:

- Underlying diseases, particularly those affecting the immune system
- Age (children and elderly are at higher risk)
- Treatment with antimicrobials, steroids, or anticancer drugs
- Vaccination status
- Type of pathogen/agent exposure
  - Opportunistic pathogens can cause disease only when introduced into an unusual location or an immunocompromised host (e.g., normal flora, most

environmental yeasts/molds, etc.).

- A primary pathogen (also known as true or frank pathogen) can cause disease in an otherwise healthy individual (e.g., Staphylococcus aureus, Streptococcus pyogenes, hepatitis B virus, influenza virus, etc.).

### 2.1.4 THE BIOLOGICAL RISK ASSESSMENT

A thorough biological risk assessment determines the proper safety and containment precautions given the intrinsic risk of the biohazard(s), procedures, and health of laboratory workers.

The risk assessment is a guide for the selection of appropriate controls and microbiological practices, safety equipment, and facility safeguards. The risk assessment will be used to alert others to the hazards of working in the lab and to the need for developing proficiency in the use of safe practices and containment equipment. Successful control of hazards in the laboratory also protects people not directly associated with the laboratory, such as other occupants in the building, infrequent visitors (e.g., facilities services), and the public.

Risk assessments begin with the question, "Is the biological material capable of causing human disease or environmental harm?" If so, the PI should work with the IBO to determine the best safety practices and level of containment to reduce the chance of accidental exposure or release of infectious agents, recombinant agents/organisms, or relevant biohazard. If you are unsure, contact RSA for guidance.

Basic considerations for the risk assessment are listed in the table below:

AGENT	PROCEDURE
Host range	Concentration of organisms
Pathogenicity/virulence	Scale of procedures
Availability of prophylaxis	Use of animals
Route of transmission	Use of sharps
Viability in the environment	Potential for the generation of aerosols
Origin of the source	Field procedures
For recombinant DNA: 1) Nature of insert: 2) Method of delivery: 3) Recombinant host: and 4) Safety/environmental impact	Experience level of personnel

### 2.1.5 RISK GROUPS

Infectious agents are grouped according to their intrinsic biological properties, particularly their pathogenicity and virulence in humans. Similar principles are used to categorize the risk of recombinant materials, which could impact human (or animal or plant) health and/or the environment if released.

Risk groups consider the following:

- Pathogenicity of the organism
- Virulence of the organism
- Mode of transmission and host range
- Availability of effective preventive measures (e.g., vaccines)
- Availability of effective treatment (e.g., antibiotics, antivirals)
- For recombinant materials: type/pathogenicity of host, gene/transgene products, gene expression, targeted effects (and consideration of off-target effects), phenotypes, biological/environmental impacts

Risk groups range from 1-4, with RG1 posing minimal risk to healthy individuals and public health and RG 4 posing a profoundly serious risks to individuals and the public. The Research Safety Affairs follows the World Health Organization's (WHO) *Laboratory Biosafety Manual, 3rd ed., NIH Guidelines*, and BMBL categorization of risk groups as follows:

- **RG1** – Are rarely associated with disease in healthy adult humans or animals and pose no-to-little public health risk (e.g., *Saccharomyces cerevisiae*, *E. coli* K-12 strain derivatives often used for recombinant/molecular biology, many environmental organisms).
- **RG2** – Are associated with mild to moderate disease for which preventative measures or post exposure treatments are often available; public health impact is limited (e.g., *Streptococcus pyogenes*, *Salmonella* spp., hepatitis B virus)
- **RG3** – Are associated with serious to lethal human disease for which preventative vaccines or post exposure therapies may be available. Public health impact maybe limited-to-moderate (e.g., *Mycobacterium tuberculosis*, hantaviruses, West Nile virus) or have wide reaching impacts e.g., SARS-CoV-2 referred to as Covid19.
- **RG4** – Are associated with serious to lethal human disease for which preventative vaccines or post exposure therapies are not available. Public health impact is high (e.g., Ebola virus, Marburg virus, smallpox virus, etc.).

**RG4 agents are not permitted at the UT Health Science Center!**

### 2.1.6 RISK REDUCTION FOR BIOHAZARDOUS AGENTS

Once the RG and other procedural factors are determined, basic considerations for risk reduction include:

#### Risk awareness

- Risk assessments are performed and communicated.
- Materials and procedures are reviewed by the IBC as required.
- Containment recommendations are implemented.
- Standard Operating Procedures (SOPs) are developed and communicated.

#### Control of materials

- Containment requirements per the risk assessment are followed.
- Materials are labeled and securely stored per risk assessment.
- Biohazard materials inventory is documented, maintained, and controlled.
- Secondary containment is used for storage and transport of biohazards.

#### Good Practices

- Follow prescribed personal and lab hygiene principles, also known as Standard Microbiological Practices (SMP; see Module 4 for details).
- Wear and maintain personal protective equipment (PPE) per the risk assessment and manufacturers recommendations.
- Communicate hazards via door placard, biohazard labels, etc.
- Segregate and dispose of biohazardous waste per RSA Office guidelines.
- Decontamination must be routine and effective based on the risk assessment and prudent practices.
- Communicate and follow emergency response procedures (spills, personal exposures, injuries, etc.).

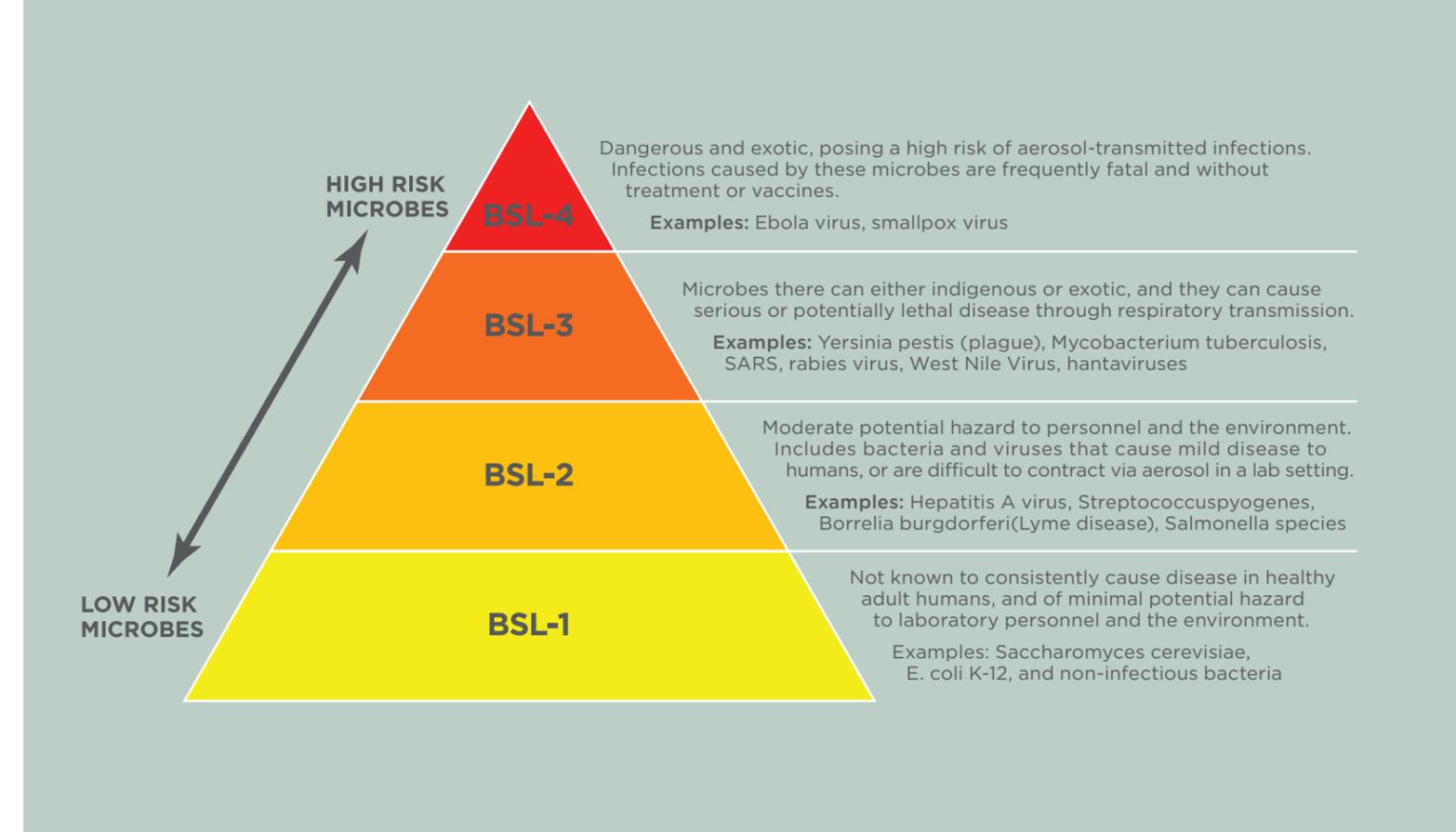
#### Restriction of Access

- Follow prescribed laboratory security procedures per institutional policy.
- Secure storage equipment in shared areas and placard with content owners name and contact information.

### 2.1.7 BIOSAFETY LEVELS

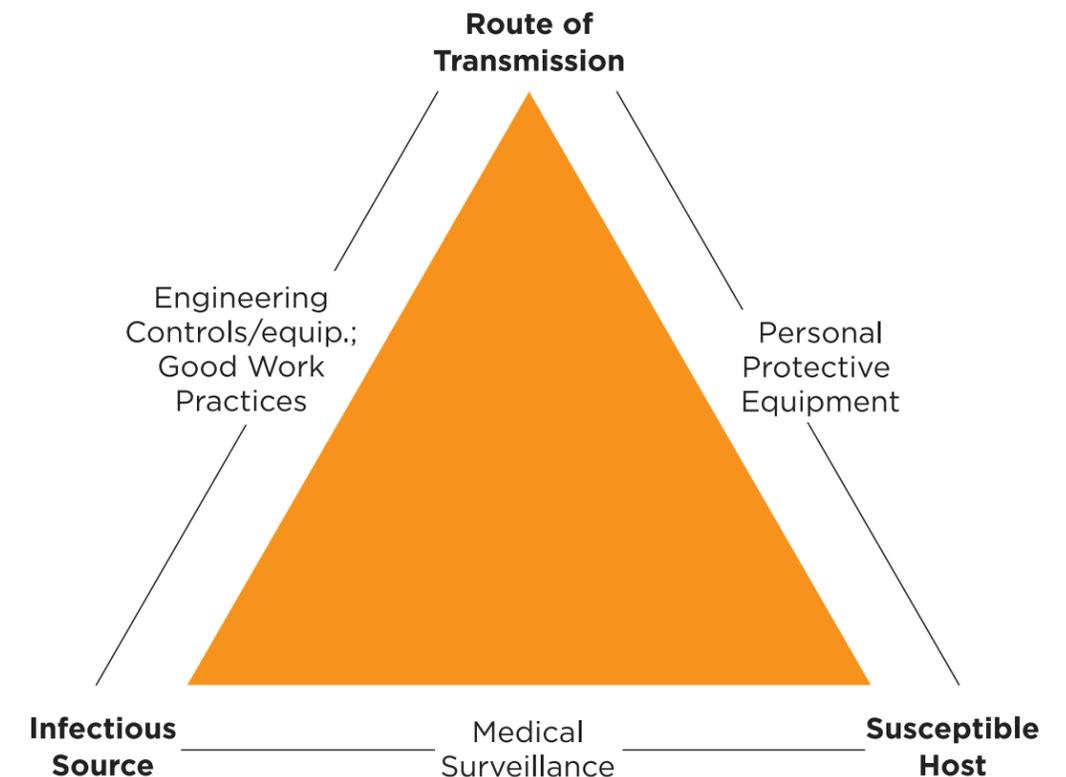
Biosafety Levels (BSL) prescribe procedures and levels of containment for the microorganism or material (including research involving recombinant or synthetic nucleic acid molecules) and associated procedures. In addition to the risk reduction strategies highlighted above, BSLs also consider primary barriers (e.g., biosafety cabinets), secondary barriers, facility design, air handling, laboratory security, etc. BSLs are graded from 1 - 4; as the BSL increases so does the relative risk of the agent/procedures as well the stringency of procedures and facility design. Generally, these correlate with RGs (e.g., a RG2 agent is worked with at BSL-2), but there are exceptions (e.g., production volumes, high-risk procedures, etc.).

The work at UT Health Science Center involves Biosafety Level 1,2 and 3 practices.



#### Biosafety Levels: Breaking the Chain

Another way to think about biosafety levels is that they are a systematic approach to disrupting the chain of infection (or release). By disrupting or eliminating the pathways between an infectious source and a susceptible host (or environment), safety, containment, and security can be assured. Examples are provided in the diagram below.





### BSL-1

Used for work with biological agents and materials that pose minimal risk to people or the environment.

**Features:**

- Work on open bench
- Lab coat and gloves recommended
- Decontamination procedures in place



### BSL-2

Used for work with biological agents or materials that pose moderate risk to people or the environment.

**Features:**

- Aerosol-generating procedures performed in a biosafety cabinet (BSC)
- Lab coat and gloves required
- Biosafety manual with lab-specific procedures/training and restricted access



### BSL-3

Used with indigenous or exotic biological agents with potential for airborne transmission or for procedures involving aerosolization, concentration or large quantities of moderate risk materials.

**Features:**

- Lab designed to contain airborne hazard (i.e., double door entry, negative airflow relative to surrounding areas, no recirculation of air)
- All open manipulations of materials in BSC
- Respiratory protection usually required
- Facility design and operational procedures documented; annual functional verification

#### 2.1.8 RISK ASSESSMENT RESOURCES

Resources that are helpful in determining risk group and biosafety levels include:

- Agent summary statements in the CDC/NIH document [Biosafety in Microbiological and Biomedical Research Laboratories \(BMBL\)](#).
- Appendix B [Classification of Human Etiologic Agents on the Basis of Hazard](#) from the NIH Guidelines.
- The [OSHA Bloodborne Pathogen Standard interpretation letter regarding risk for all human](#)

[cell lines](#) (must use BSL-2 work practices).

- The [American Biological Safety Association's \(ABSA\) database of Risk Group Classification of Infectious Agents](#).
- The [Public Health Agency of Canada's PSDS for Infectious Substances](#).
- The [World Health Organization \(WHO\)](#).
- Research publications (various)
- UT Health Science Center Biosafety Manual

## 3 BIOSAFETY PRACTICES AND PROCEDURES

This module covers the laboratory practices and procedures associated with Biosafety Levels 1 and 2.

**Biosafety Level-1** represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. BSL-1 practices, safety equipment, and facility design and construction are appropriate for most undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and well-characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans or negatively impact the environment. However, many agents not ordinarily associated with disease processes in humans can be opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Additionally, vaccine strains that have undergone multiple in vivo passages should not be considered avirulent.

Examples of agents that can work at BSL-1 include *Bacillus subtilis*, *Saccharomyces cerevisiae* (baker's yeast), adeno-associated virus (AAV), most *E. coli* strains used for cloning/molecular biology (e.g., DH5a, DH10B), and many environmental microorganisms.

**Biosafety Level-2** containment and safety practices are suitable for work with a broad spectrum of indigenous moderate-risk agents that typically cause mild-to-moderate human disease. Additionally, BSL-2 is usually the default biosafety level when working with diagnostic specimens from humans and/or animals.

Examples of agents that can be worked with at BSL-2 include hepatitis A virus, herpes simplex virus, *Toxoplasma gondii*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella* species and many other foodborne pathogens. Additionally, human and nonhuman primate specimens can typically be manipulated using BSL-2 practices and containment. Finally, BSL-2 practices are often employed while handling acute biological toxins.

Risk group and Biosafety containment level are summarized below. With elevated risk, comes elevated containment.

#### Risk group and biosafety containment level

RISK GROUP (NIH GUIDELINES)	BIOSAFETY CONTAINMENT LEVEL	EXAMPLES
<p><b>RISK GROUP 1:</b> Experiments that do not pose a risk to the environment - release would not result in surviving in the environment. <i>(Low risk)</i></p>	<p><b>BSL-1</b></p> <ul style="list-style-type: none"> <li>• Work is done on open bench tops and special containment equipment is not required</li> <li>• Standard microbiological practices are observed</li> </ul>	<p><i>Escherichia coli</i>; KI2 derivatives (DH5a, JH 1 09, pBluescript, psi2); All EXEMPT rDNA work; <i>Rhizobium</i>, <i>Agrobacterium</i></p>
<p><b>RISK GROUP 2:</b> Experiments that involve work agents or transgenics that if released would be viable in the environment but would have a negligible impact or could be readily managed. <i>(Moderate risk)</i></p>	<p><b>BSL-2</b></p> <p>All BSL-1 containment and practices plus the following:</p> <ul style="list-style-type: none"> <li>• Laboratory/Facility/Greenhouse access is restricted when experimental work is in progress</li> <li>• Personnel have specific training in handling of agents</li> <li>• Biological safety cabinets (BSC) or other physical containment devices are used for potential aerosol generation procedures</li> <li>• Biohazard signs must be posted</li> <li>• Specific PPE (personnel protective equipment) and entrance requirements</li> </ul>	<ul style="list-style-type: none"> <li>• rDNA work on plants that could become established if released</li> <li>• Potentially harmful microorganisms to other animals or plants but that are manageable</li> <li>• Exotics that pose no potential harm to managed or natural ecosystems</li> <li>• Herpes Simplex Virus</li> <li>• Rabies Virus</li> <li>• Pseudorabies Virus</li> </ul>
<p><b>RISK GROUP 3:</b> Experiments that a release outside the lab would have significant detrimental impact on the environment. <i>(High risk)</i></p>	<p><b>BSL-3</b></p> <ul style="list-style-type: none"> <li>• All BSL-2 containment and practices plus the following:</li> <li>• Specific facility design parameters must be followed, including requirements for location, ventilation, room integrity and security</li> </ul>	<ul style="list-style-type: none"> <li>• Exotic infectious agents capable of causing serious environmental harm</li> <li>• Plants containing genes from exotic infectious agents</li> <li>• <i>Mycobacterium bovis</i></li> </ul>

### 3.1 STANDARD MICROBIOLOGICAL PRACTICES

The foundation of each biosafety level is standard microbiological practices (SMP). BSL-1 labs employ SMP as the baseline criteria for biological safety and containment. Subsequent BSLs build upon SMP with more specialized design, equipment, and practices.

#### 3.1.1 Basic elements of SMP at BSL-1

- Restricting access to laboratories using biological hazards per the BMBL and the UT Health Science Center laboratory safety procedure. The PI is responsible for restricting access to approved personnel or visitors.
- Hand washing after handling biological and potentially hazardous materials, after taking off gloves and before leaving the lab.
- Avoiding hand-to-face (or mouth) contact. No eating, drinking, smoking, or applying cosmetics in the lab.
- Disinfecting work surfaces daily and decontaminating after spills.
- Prudent handling, management, and disposal of sharps.
- Using procedures that minimize the formation of aerosols and splashes; engineering controls for aerosol release may be required.
- Wearing appropriate personal protective equipment (PPE), e.g., lab coats, gloves, safety glasses, safety goggles, etc.
- Using primary and secondary containment when transporting materials.
- Disposal of all non-sharps solid biological waste in a proper container lined with an autoclavable bag for disposal. Liquid biological waste must be disinfected or autoclaved before sink disposal.

#### 3.1.2 SMP and BSL-2 Practices

Other SMP to be used in laboratories handling biological materials include:

- Keeping personal items such as cell phones or tablets outside of the work area.
- Disallowing outside pets, plants, or other personal effects/decorations from the work area.
- Minimizing exposed skin, and thoroughly covering broken or abraded skin or skin with rashes.
- Restricted open toed shoes, sandals, flip flops or any footwear with exposed skin or holes while working in the laboratory.
- Knowing the risks of lab activities, and donning/doffing the appropriate PPE.
- Restraining long hair when operating equipment with exposed moving components or when working with chemicals or biologicals.

Biosafety Level 2 practices (BSL-2) consist of SMP plus:

- Hazard communication through door signs/placards and biohazard labelling.

- More stringent PPE requirements, typically gloves, long-sleeved lab coat/smock, and protective eyewear.
- Aerosol control and containment.
- Enhanced engineering controls:
- Biological safety cabinets
- Emergency eyewashes
- Integrated pest management program.
- Medical surveillance programs as indicated by risk assessment.

### 3.2 Hazard Communication

#### 3.2.1 Placards

Labs at UT Health Science Center must have a lab hazard placard posted at the entrance. This placard is shown here.

BSL-2 and BSL-3 labs are differentiated from BSL-1 labs through the posting of a biohazard placard prominently featuring the biohazard symbol and identifying the biosafety level of that lab. At UT Health Science Center, the universal biohazard symbol and the word "BIOHAZARD" are used to indicate higher risk areas where there is a risk to human health. Therefore, BSL-2 door placards feature the universal biohazard symbol which alerts lab workers, visitors, maintenance and housekeeping personnel, vendors, and others who may need to enter the laboratory.

Placards also communicate entry considerations, e.g.:

- Entry requirements such as PI authorization.
- Health: immunocompromised individuals are advised of the increased risk and an individual risk- assessment can be conducted for these individuals; vaccine requirements may also be posted.
- Practices: PPE requirements and SMP highlights.
- Doors should be kept closed during experiments when BSL-2 agents are in use.

Lab Hazard Placard

Biosafety Level 2 Placard

#### 3.2.2 Hazard Communication - Equipment Labels

Equipment used to process or store biohazards that may impact human health must be labeled with the universal biohazard symbol. For example, hoods, freezers, incubators, and centrifuges, etc. are labeled if they are used with risk group 2 (RG2) agents or higher. Also, transport containers and biohazard waste containers must display a biohazard label on the outermost part.



#### 3.3 Hand Washing

One of the simplest measures you can take to protect yourself (and others) inside and outside of the laboratory is washing your hands. This simple procedure has been thoroughly demonstrated to be the primary means of infection and contamination control in laboratory and clinical settings. In other words, hand washing is extremely important in preventing lab acquired infections (LAIs) and minimizing the spread of infectious materials.

*Upon entering the lab, you should familiarize yourself with critical lab infrastructure and where it is located: fire alarms/extinguishers, emergency shower, first-aid kits, emergency eyewash stations and hand washing sinks.*

Hands must be washed:

- Anytime they may have become contaminated with infection-risk material.
- After glove removal.
- Before leaving the work area.

Procedure:

- Use cold-to-tepid water and soap (does not need to be antibacterial).
- Lather for 20-30 seconds, paying attention to palms, backs of hands, finger webbing, cuticle beds, and wrists.
- Rinse and dry. Use a paper towel to turn off the water faucet drying your hands.

Hand Hygiene - A word about hand sanitizers:

In case of limited access to soap and water (e.g., field procedures), waterless hand sanitizers may be used as a temporary means of reducing contamination until

a source of running water and soap can be reached. However, because of the variety of products available and organisms used in UT Health Science Center labs, Research Safety Affairs cannot validate waterless hand sanitizers for efficacy in all cases. Therefore, these products are NOT a replacement for hand washing with soap and water and should not be used as such.

When using a waterless sanitizer:

- Ensure that the active ingredients include > 60% alcohol (ethanol or isopropanol).
- Apply quantity recommended by manufacturer.
- Thoroughly distribute as if you were washing your hands (paying attention to palms, backs of hands, finger webbing, cuticle beds, and wrists).
- Rub until completely dry.
- Wash your hands with soap and water (as described on previous slide) once available.

#### 3.4 Emergency Eyewashes



Eyewashes are required for labs operating a BSL-2, though they are often found in BSL-1 labs as well. Eyewashes must be maintained in good working order, activated weekly and tested according to **GS5115: UT Health Science Center Emergency**

**Eyewash and Showers.** The eyewash is in place for your protection. Weekly flushes keep sediment and bacteria from accumulating in the lines which could potentially cause harm to your eyes in case of emergency use.

The following items are important to know for eyewash care/use:

- A functional eyewash should project streams of clear, tepid water at a pressure that is strong and even enough to easily reach the eyes of the user in a way that is comfortable enough for the user to maintain hands-free flushing for 15 minutes.
- Handles should be clear of obstruction and located so that they are easily found.
- The interface between the spray-head and the cap should be cleaned routinely with 70% ethanol or isopropanol.
- If the pressure isn't high enough to remove the protective caps without manual intervention, particulates are present in the stream, or the water is discolored, then the eyewash isn't working properly. Contact the RSA or Facilities if the eyewash isn't functioning.

#### 3.5 Eliminate Routes of Entry

### 3.5.1 Laboratory Clothing



Proper attire is a prudent practice in any research laboratory, including those where biological materials are handled. Prior to working in the laboratory, ensure that you are wearing clothing that covers exposed skin and close toed shoes made of substantial, non-porous materials. Proper attire minimizes potential routes of entry and is particularly important at BSL-2.

### 3.5.2 Personal Protective Equipment

#### What is personal protective equipment?

Personal protective equipment, commonly referred to as "PPE", is equipment worn to minimize exposure to hazards that cause serious workplace injuries and

illnesses. These injuries and illnesses may result from contact with biological, chemical, radiological, physical, electrical, mechanical, or other workplace hazards. Personal protective equipment may include items such as gloves, safety glasses, chemical splash goggles, lab coats, and respiratory protection.

#### What can be done to ensure proper use of personal protective equipment?

All personal protective equipment should be safely designed and constructed and should be maintained in a clean and reliable fashion. It should fit comfortably, encouraging worker use. If the personal protective equipment does not fit properly, it can make the difference between being safely covered or dangerously exposed. When engineering, work practice, and administrative controls are not feasible or do not provide sufficient protection, employers (e.g., PI or lab supervisor) must provide personal protective equipment to their workers and ensure its proper use.

Employers are also required to train each worker required to use personal protective equipment to know:

- When it is necessary.
- What kind is necessary.
- How to properly put it on, adjust, and take it off.
- The limitations of the equipment.
- Proper care, maintenance, useful life, and disposal of the equipment.

#### Donning and Doffing Gloves



### 3.5.2.1 Personal Protective Equipment - Hands

#### Gloves

Gloves are required for all manipulations of RG2 organisms unless alternative protection is established in an SOP.

Gloves must be:

- Fluid resistant (a non-latex material, e.g., nitrile, must be available)
- Powder-free
- Disposable - do not reuse gloves unless they are utility gloves that are intended to be reused; dispose when compromised.
- Free of defects, tears, or breaks.
- The right size for your hands.
- Removed before leaving the lab, touching phones, doorknobs, or other common contact surfaces or objects (fomites, see below).

### 3.5.2.2 Personal Protective Equipment - Body

#### Lab Coats



Protective lab coats are recommended to prevent contamination of personal clothing at BSL-1. At BSL-2, they must be worn when working with hazardous materials.

Lab coats must be:

- Long sleeved - preferably with a gathered cuff
- Removed before leaving for non-laboratory areas
- Laundered appropriately - lab coats must be laundered routinely and when contaminated. **Lab coats should never be cleaned in personal or public washers and dryers.** UT Health Science Center departments must provide accommodations for the laundering of lab coats for or by personnel. Contact your department chair or RSA for additional information about practices for laundering lab coats in your department.

### 3.5.2.3 Personal Protective Equipment - Eyes

#### Eye Protection

Wear protective eye wear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.



Eye protection must be:

- Worn according to the laboratory hazard assessment.
- Cleaned, stored, and replaced - according to manufacturer's recommendations and prudent laboratory practices.
- Appropriate for the task:
  - Safety glasses with side shields must be worn when there is a risk of particulate projectiles (broken glass shards).
  - Indirectly vented chemical splash goggles must be worn when there is a risk of aerosols, splashes, sprays, or splatters of infectious or hazardous liquid material encountering the eyes or the tissues surrounding the eyes (bleach, liquid cultures, etc.).

### 3.5.2.4 Personal Protective Equipment - Face

#### Face Protection

Face protection must be used if splashes or sprays of infectious or other hazardous materials are anticipated.

Face protection must be:

- Worn according to the laboratory hazard assessment.
- Cleaned, stored, and replaced - according to the manufacturer's recommendations and prudent laboratory practices.
- Appropriate for the task:
  - o UV exposure requires a specially rated type of material for the face shield.
  - o Liquid exposure requires protection against potential splashes or sprays of infectious or hazardous liquid.



### 3.5.2.5 Personal Protective Equipment - Respiratory Protection

#### Respiratory Protection

Additional PPE such as surgical masks may be worn for procedures with high probability for splashes, spray, splatter and/or droplets. Work in rooms with infected animals or high-risk inhalation hazards (infectious to humans) may require respiratory protection.

If respiratory protection is used voluntarily or as required PPE, the use must comply with the requirements of **GS5105: Respiratory Protection**. When use of respiratory protection is required, this includes medical approval from University Health Services, fit testing (RSA) and training (RSA).



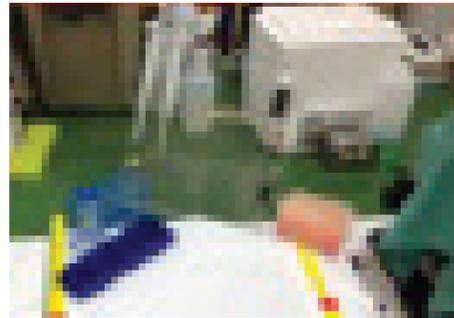
### 3.6 Fomite Transmission

Besides direct contact with contaminated fluids, infectious agents may also be transmitted by fomites. Fomites are inanimate objects or materials that are likely to harbor and spread infectious agents through contact. Examples include equipment, utensils, doorknobs, keyboards, furniture, PPE, and cell phones. Practices to minimize fomite transmission are outlined below:



#### EXAMPLES OF FOMITES

- Work surfaces
- Doorknobs
- Tools such as pipettes
- Telephones
- Lab equipment
- Storage containers



#### WAYS TO PROTECT YOURSELF

- Set up a “contamination zone”
- Routine surface disinfection
- Wear gloves when handling tools or equipment
- Disinfect storage containers before and after sample retrieval



#### WAYS TO PROTECT OTHERS

- Hazard communication
- Routine surface decontamination
- Don't touch common surfaces (phones, doorknobs, etc.) with gloved hands
- Remove PPE before leaving the lab space

### 3.7 Aerosol Risk

In 2012, the CDC estimated approximately 80% of laboratory acquired infections are caused by aerosols of pathogenic microorganisms. Laboratory personnel who work with agents infectious to humans must ask themselves:

- 1) Am I working with concentrated stocks?
- 2) Will my procedures concentrate the risk group 2 pathogens (e.g., high-speed centrifugation)?
- 3) Does my procedure generate aerosols?

#### What are aerosols?

Aerosols are solid or liquid particles suspended in the air (1-100  $\mu\text{m}$ ). The fate of these particles is determined by relative size:

- Larger particles settle more rapidly becoming a risk for surface contact.
- Smaller particles can remain airborne for a long period of time, dehydrating and becoming “droplet nuclei” and spreading wide distances.
- Smaller particulates (1 to 10  $\mu\text{m}$ ) are also more easily inhaled.

#### Aerosol Control: Aerosolizing Procedures

Common laboratory procedures that may produce aerosols include:

- Blending, macerating, or sonicating infectious material.
- Stirring or vortexing liquids.
- Opening lyophilized cultures, culture plates, ampoules, tubes, and bottles.
- Removing stoppers.
- Pouring liquids.
- Pipetting and blowing out pipettes.
- Dropping culture containers.
- Flaming inoculating needles, slides, or loops.
- Streaking or spreading inoculum.
- Freeze-drying specimens.
- Inserting a hot loop into a culture.
- Breakage of culture containers.
- Cage cleaning and changing animal bedding.
- Intranasal inoculation of animals.
- Animal or human necropsy.
- Harvesting infected materials.
- Carelessly removing protective gloves or PPE.

#### Aerosol Control: Aerosolizing Equipment

##### Devices that have the potential to create aerosols:

- Blenders and vortexes
- Bottles and flasks
- Cell sorters
- Centrifuges
- French press
- Homogenizers
- Needles and syringes
- Pipettes
- Pressurized vessels
- Rubber stoppers
- Shakers
- Sonicators
- Vacuum and aspirating equipment

##### Aerosol Control

Aerosol droplets are formed with virtually any activity that disrupts the surface tension of a liquid or applies mechanical force to a solid. As liquid particles bearing infectious agents may remain in the air for extended periods, it is important to consider methods of aerosol control.

##### Safe Work Practices to Minimize the Creation of and Exposure to Aerosols:

Using a combination of the appropriate safety equipment and safe procedures is the primary method to minimize the creation of and exposure to aerosols.

Lab safety equipment to protect personnel from aerosols:

- The certified biological safety cabinet (class I or II) is the primary barrier to protect workers from aerosols. Other safety devices include safety centrifuges with automatic locking mechanisms or solid lids, bioseal rotors, safety centrifuge cups, safety blenders, safety sonicators.
- If aerosol production cannot be prevented or contained, respiratory protection may be required. **Contact RSA at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu)** to determine if the use of a respirator is appropriate.
- Vacuum line trap and filter systems are used to protect the vacuum system from aerosols.

##### Safe work practices for centrifugation of biohazards:

- Confining work with viable biological agents to a designated workspace.
- Wear appropriate personal protective equipment according to the lab's assigned biosafety level or documented risk assessment. If respiratory protection is indicated, special training and a medical evaluation may be required. **Contact RSA at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu)** for more information.
- Routinely inspect centrifuge to ensure that leakage is not occurring.
- Do not overfill centrifuge tubes. Wipe the outside of the tubes with disinfectant after they are filled and sealed.
- Centrifugation may be performed in a centrifuge that is contained within a specially designed biological safety cabinet or other physical containment device.

- If a centrifuge containment device is not available, internal aerosol containment devices (e.g., sealed canisters, safety cups or buckets with covers, heat sealed tubes or sealed rotors, etc.) may be indicated.
- Aerosol containment devices should be removed from the centrifuge and opened in a biological safety cabinet. If a biological safety cabinet is unavailable, a settling time based should be allowed. If there is no known settling time specific to the materials being centrifuged, an industry standard of 10 minutes is recommended.

##### Safe work practices for blending, sonicating, grinding, and lyophilizing of biohazards:

- Operate blender, sonicator, and grinder in a biological safety cabinet if required for the biosafety level of the lab, or place a towel moistened with disinfectant over the top of blender, grinder, or sonicator.
- Segregate the sonication procedure to a specific area of the lab, keeping the sonication vessel covered to the extent possible.
- Thoroughly disinfect the area when the process has been completed.
- Use safety blenders designed to prevent leakage.
- If leak-proof blender is not available, regularly inspect the bottom of the blender for leakage.
- Avoid glass blenders.
- Open equipment in a biosafety cabinet if required for the biosafety level of the lab. If not, allow aerosols to settle for at least 10 minutes before opening the equipment.
- Filter lyophilizer vacuum pump exhaust through HEPA filters or vent into a biological safety cabinet.
- Autoclave or disinfect all equipment promptly after use.

##### Safe work practices for pipetting of biohazards:

- Pipette all biohazardous materials in a biological safety cabinet if possible.
- Drain a pipette with tip against the inner wall of the receiving vessel. Never forcibly expel any hazardous material from a pipette.
- Place reusable pipettes horizontally in a pan filled with enough liquid disinfectant to completely cover them.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.

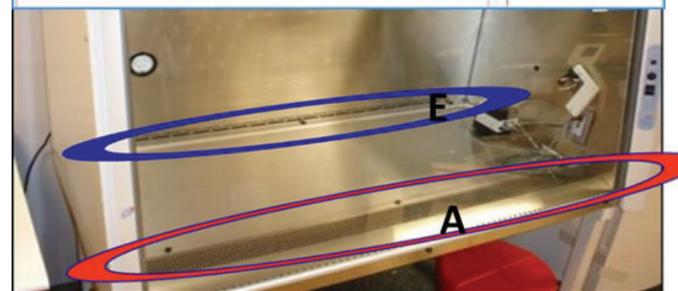
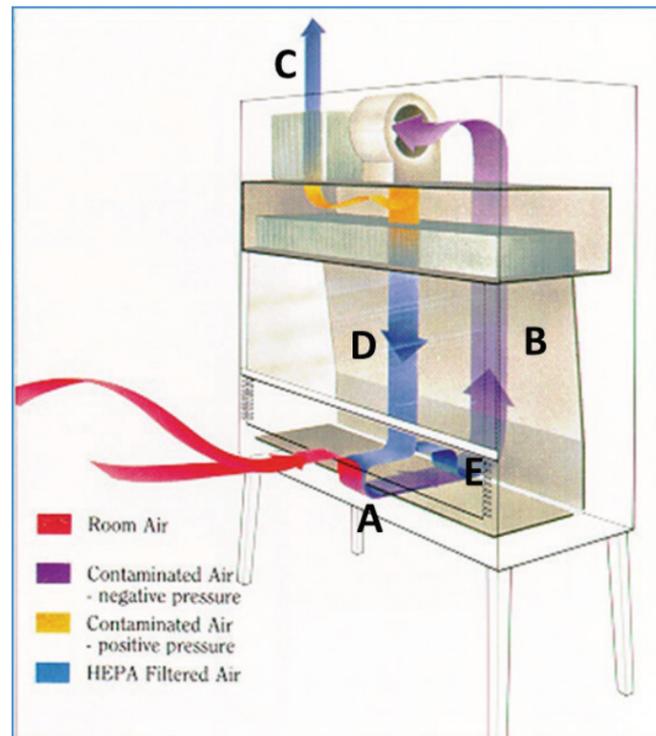
##### Other safety precautions:

- Minimize air bubbles when filling a syringe. Place a pad moistened with disinfectant over the tip of the needle when expelling air. Perform work in a biological safety cabinet whenever possible.
- Use a shielded electric incinerator or hot bead sterilizer to sterilize inoculating loops. Disposable plastic loops and culture needles are good alternatives to open flames.
- If a spill occurs that may generate aerosols, follow the lab specific spill response plan. Ensure that all lab personnel are aware of the lab's spill response plan.
- Wear gloves when handling infectious materials, or infected animals.

### 3.8 Biosafety Cabinets (BSC)

BSCs use HEPA (high-efficiency particulate air) filters to filter infectious particles from an airstream. BSCs may be used at BSL-1 to maintain an environment of sterility that is not achieved on the open bench. At BSL-2, the BSC is relied upon to protect the worker and the environment from procedures that are likely to generate aerosols. However, BSCs are only effective if used properly. This requires an understanding of how they function as well as proper technique. *NOTE: Class A2 BSCs remove particles, not vapors. Volatile hazardous chemicals are not to be used in a BSC unless approved by the Research Safety Affairs Office.*

#### Air Flow within a Class II (Type A2) Biosafety Cabinet



BSCs offer protection by drawing the air in at the face opening (A) and immediately drawing the air through the front grille and under the work surface. The air is then blown through the rear air plenum (B) to the top of the cabinet where it is divided into two chambers. Thirty percent of the air is exhausted out of the cabinet (C) through a high efficiency particulate air (HEPA) filter into the laboratory room. The remaining (70%) of the air is directed through another HEPA filter down onto the work surface (D) in a laminar flow directional air pattern. Air then splits to either the front grille or back grille (E). Most BSCs at UT Health Science Center operate in this manner. Use discretion when handling hazardous chemicals in a BSC.

### Certification

The Office of Research Safety Affairs requires that BSCs used for BSL-2 containment be certified in accordance with the NSF 49 standard:

- Upon initial installation.
- If physically moved or relocated.
- At least annually.

Certification verifies HEPA filter efficiency and calibration of the airflow that provided that protective inward airflow at the face of the cabinet. Certification is performed by a contracted field certifier in accordance with prescribed parameters. Once certified, the BSC will be tagged by the contractor as illustrated here.

While biosafety cabinets are not required for worker protection in BSL-1 containment, if they are used, annual certification will help ensure that the intention of use is met, regardless of the operating biosafety level.

If your BSC is due for certification, contact RSA at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu).



#### Prior to use

1. Turn on blowers and allow cabinet to run for 5-10 minutes to cycle air.
2. Confirm that the BSC is within its annual certification.
3. Check to ensure UV light source is turned off. Many BSCs use UV (germicidal) bulbs as a means of decontamination. UV bulbs may cause severe burns to exposed areas such as the skin and eyes. While newer models have safety interlocks in place to prevent operation of UV sources while the cabinet is in use, this is not always the case. **Speak to senior lab personnel before you use the BSC to ensure you understand the equipment.**

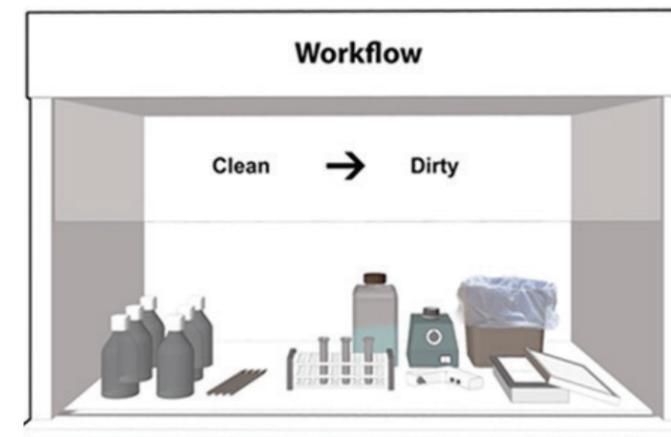


4. Disinfect all internal surfaces with an effective disinfectant. Allow the disinfectant to remain wet for the time recommended by the manufacturer (contact time).
5. Ensure that your BSC is not overloaded; avoid clutter or have materials in the cabinet that are not required for your procedure. Overloading the BSC disrupts the laminar flow that is integral to providing a sterile work environment inside the cabinet. BSCs are not engineered to provide storage. Placing items on the grate at the front of the cabinet allows non-HEPA filtered air to enter the work surface inside the cabinet. This could result in contaminated cultures or the escape of concentrated pathogens. *Note: If BSC is in alarm and you do not know why contact RSA. Do not work in a BSC that is alarming.*



#### During use

- Set up the cabinet in clean, transfer, and disposal zones. Work from clean to dirty and keep all materials inside the BSC until completion of procedures. If necessary, set up a small biohazard bag or pipet disinfectant tray inside the cabinet to discard contaminated wastes.



- To minimize infectious aerosol leaks:
  - Use deliberate motions when placing or removing hands and objects in the BSC. Move hands straight in and straight out. Avoid rapid side-to-side radial arm motions.
  - Keep front and rear air intake grilles clear of obstructions.
  - Avoid excessively disrupting the air curtain by keeping items and waste inside the cabinet to the extent that is reasonably allowable.

- **Avoid open flames in the BSC.** According to the World Health Organization (WHO), open flames should be avoided in the near microbe-free environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are also used. To sterilize bacteriological loops, microburners or electric "furnaces" are available and are preferable to open flames.
- If a spill occurs, leave the cabinet running and follow spill procedures.
- **Under no circumstances should your head be inside the BSC while in use!!**

#### After use

- Allow the cabinet to continue to run.
- Close all primary containers. Wipe down exterior surfaces of containers with appropriate disinfectant (soaked paper towel, gauze, or wipes).
- Close waste containers, wipe with disinfectant and discard as biohazardous waste.
- Thoroughly wet all interior surfaces with appropriate disinfectant. Allow to remain wet for contact time and clean. Note that bleach solutions are corrosive and will "pit" and rust stainless steel over time. If bleach is used, a follow-up rinse/wipe with alcohol or mild detergent is recommended.
- Allow the cabinet to run for 5-10 minutes after disinfection.
- Turn off cabinet and activate germicidal lamp (optional). Germicidal lamps should be inactivated after 15-20 minutes.

For additional resources on the care and use of BSCs, see:

- [CDC training video](#)
- [NIH training video](#)
- [ESCO Global training video](#)

#### Beware of BSC Lookalikes

The Laminar Flow Bench:

- Also referred to as a "clean bench".
- Typically, these benches are used for mixing sterile solutions, prepping agarose plates, etc.



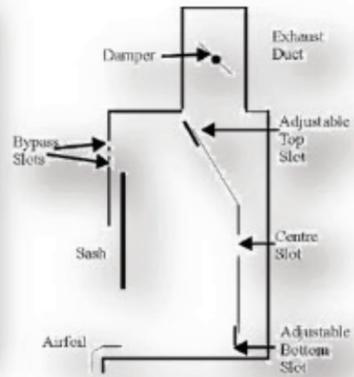
- Pulls room air using a blower mounted below a HEPA filter.
- Filtered air is passed across bench space to produce a "clean zone".
- These cabinets provide product protection, but no personnel protection.

#### Do not use in conjunction with infectious agents, human/nhp materials or hazardous chemicals

The Chemical Fume Hood:

- Chemical fume hoods use directional air flow to remove harmful vapors and gases from the cabinet and are hard ducted to the building exhaust system.

- Cabinets are typically not equipped with HEPA filters and are not compatible with viable infectious agents.
- Incoming air is not filtered, so product sterility is not feasible.
- Chemical fume hoods are useful for applications such as formalin fixing of cells, as the chemical hazard greatly outweighs the biological. **Use discretion.**



### 3.9.1.2 During centrifugation

- Keep the lid always closed during operation. Never open a centrifuge until the rotor has stopped.
- Do not exceed safe rotor speed.
- The operator should not leave the centrifuge until full operating speed is attained and the machine appears to be running safely without vibration.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins and check load balances.

### 3.9.1.3 After centrifugation

- Allow the centrifuge to come to a complete stop before opening.
- Wear gloves to remove the rotor and samples.
- Check inside of centrifuge for spills and leaks, clean centrifuge, and rotor thoroughly if necessary.
- Wash hands after removing gloves.

### 3.9.1.4 Centrifuging Infectious Materials or Human Samples

Follow the safety procedures above, plus:

- Place a biohazard label on the centrifuge.
- Always wear gloves when handling tubes or rotors.
- Avoid the use of celluloid tubes with biohazards. If celluloid tubes must be used, an appropriate disinfectant must be used to decontaminate them.
- Always use sealed safety cups, safety buckets, or sealed rotors with O-ring as secondary containment if available.
- Fill centrifuge tubes, load into rotors, remove from rotors, and open tubes within a biological safety cabinet if biological safety cabinet is available.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or bucket. Seal rotor or bucket, remove outer gloves, and transport to the centrifuge.
- If possible, wait approximately 10 minutes after the run to allow aerosols to settle before opening the centrifuge. Check for spills or leaks. For spills of infectious materials, see centrifuge emergency procedures below.
- Decontaminate centrifuge interior, safety cups or buckets, and rotors if tube breakage occurs.
- Include centrifugation procedure and decontamination plan in lab SOPs.

### 3.9.1.5 Centrifuge Emergency Procedures

#### 3.9.1.5.1 Emergency Situations:

The following events are considered an emergency:

- If there is a spill in the centrifuge.
- If centrifuge malfunctions.
- If there is a rotor failure.
- If there is a tube breakage.

### 3.9.1.5.2 Emergency Procedures

For emergencies with RG2 organisms or hazardous materials

- Turn centrifuge off immediately and keep centrifuge lid closed.
- Notify others.
- Evacuate the lab if necessary.
- Close the lab door.
- Post a biohazard spill sign at the lab door.
- Leave for 30 minutes to reduce the risk of aerosols.
- For spill cleanup, the operator should wear proper PPE, remove debris, clean, and disinfect centrifuge interior, rotors, safety cups or buckets following the manufacturer's instructions.
- Place any contaminated protective clothing, gloves, and all cleanup materials in a biohazard bag.
- Wash hands and any exposed skin surfaces with soap and water.
- Seek medical attention as necessary.
- Report incidents to P.I. or lab supervisor and RSA if the incident involves chemical hazards. Call Research Safety Affairs (901.448.6114) for assistance.

### 3.10 Decontamination and Disinfection

The university requires that all individuals that work in a laboratory are informed about the chemical, physical, and health hazards present in the laboratory, the known risks, and what to do if an accident occurs.

Proper approaches to disinfection and decontamination are important for the protection of lab personnel and for the prevention of environmental release of organisms. Removal of infectious agents by surface cleaning is a critical component of biological safety in the lab.

While the immediate workspace is an obvious area requiring regular decontamination procedures, there remain other critical items which might not be as apparent.

These can include:

- Outer surfaces of storage and sample containers.
- Vortexes.
- Centrifuges (housing and rotor).
- Outer and interior surfaces of incubators.
- Interior surfaces of the BSC before and after procedures.
- Common contact surfaces (doorknobs, keyboards, etc.).

Any surface you may have touched with gloved hands.

Additionally, **surfaces should be disinfected:**

- When retrieving sample containers.
- After removing from water baths.



- Upon completion of procedures involving infectious agents.
- Any time a spill occurs.

When selecting a disinfectant, it is important to understand the compound that you are using:

1. Is the product appropriate for the organisms that are being targeted?
2. Is it safe for the given surface?
3. Are there personal hazards to consider that would warrant the use of PPE?

**Remember** that disinfectants typically work by targeting basic cellular structures/macromolecules (dissolve lipids, denature proteins, induce nucleic acid breaks, etc.). Our cells share this biology; thus, disinfectants can act as hazardous chemicals. Depending on the type/class, these may cause skin/eye burns, sensitization of the skin (rashes) and/or respiratory tract (wheezing/asthmatic symptoms), or cancer (the aldehydes and ethylene oxide are recognized carcinogens).

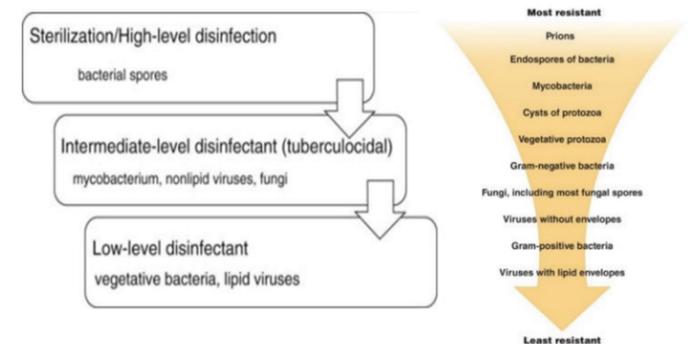
4. Are there special instructions for the preparation of the mixture?
5. Does it need to be diluted?
6. What is the minimum time it must be in contact with contaminated surfaces to reduce the infectious potential to acceptable levels (also called the contact time).

Currently, there are a wide variety of disinfectant options available. The products fall into the following categories (listed from low-level to high-level disinfectants):

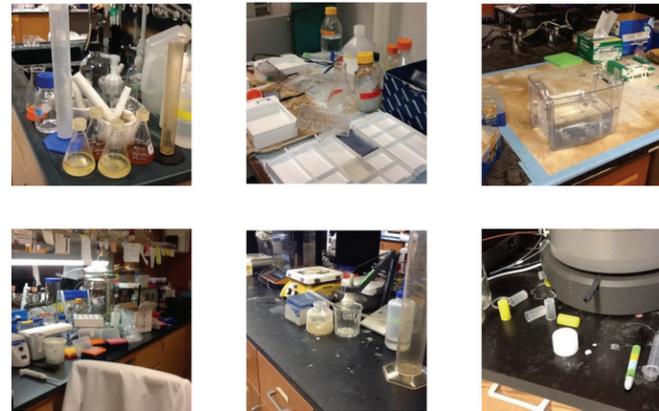
1. Quaternary Ammonium (Cavicide; Lysol).
2. Hydrogen peroxide based products (Spor Klenz).
3. Ethyl and isopropyl alcohol\*.
4. Phenols (Clorox bleach-free, Vesphene).
5. Chlorine compounds and hypochlorite/bleach (Clorox).
6. Iodophors (Povidone-iodine solution).
7. Aldehydes: Glutaraldehyde (Cidex); formaldehyde; paraformaldehyde .
8. Ethylene oxide.

**Disinfectants must be selected in accordance with UT Health Science Center IBC and IACUC requirements. The UT Health Science Center IBC does not permit the use of alcohols for disinfection on protocols approved for work at A/BSL-2 or A/BSL-3.**

#### Surface Disinfection



- Keep bench clutter to a minimum! From a disinfection/decontamination standpoint, poor housekeeping is problematic because:
  - Cross contamination from surface contact with infectious materials (higher risk).
  - Spill cleanup becomes more complicated, more time consuming, less effective, and poses a higher risk of exposure to infectious agents.
  - Surface disinfection is hindered by clutter.



### 3.11 Sharps Management

In the lab, a sharp refers to any object that is contaminated with a biologically hazardous agent and is sharp enough to puncture the skin without excessive applied pressure of force. While needles and scalpels could be considered the most apparent objects in this category, other items may also meet this definition.

Some examples include:

- Broken glassware.
- Serological pipettes (especially if broken or damaged).
- Pasteur pipettes.
- Metal edges.
- Unpolished glass (slides and cover slips).

**Penetration of the skin with a biologically contaminated sharp device is one of the most efficient means of transmitting infection.**

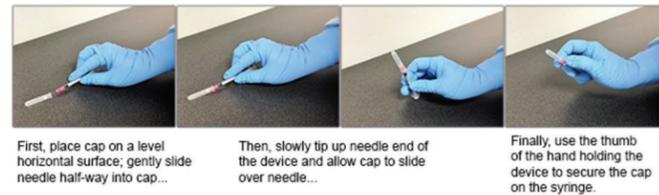
*To minimize the risk of a sharps injury the following guidelines must be followed:*

- Use disposable sharps when possible and have a sharps container readily available within arm's reach for disposal of sharps immediately after use (do not manipulate (recap) by hand before disposal).
- Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Do not pass a sharp to another person.
- When cleaning and processing reusable sharps, use cleaning tools that limit the potential for contact between your hands and the sharp surface.
- Don't leave sharps unattended.
- Do not leave sharp devices in your pockets.

- Broken glassware is a risk of injury in BSL-1 labs and a risk of injury and infection in BSL-2 labs. It is prudent to substitute plastic for glass in BSL-2 labs whenever possible. If you have large biologically contaminated broken glass items, they must be treated as sharps. Always wear gloves and use tongs or a brush and dustpan to collect broken glassware.

#### 3.11.1 Recapping Needles

Needles are not to be recapped at BSL-2/ABSL-2 unless specifically exempted/approved. Needle recapping is discouraged at BSL-1, but, if necessary, the one-handed scoop technique may be used. In rare instances where re-capping is permissible, utilize the one-handed scoop method. First, place the cap in a flat surface. Second, using one hand, scoop the cap up with the needle. Third, using the same hand, "click" the cap in place by pressing with your thumb at the base of the needle cap.



### 3.12 Biohazardous Waste Management

#### 3.12.1 Overview:

#### Infectious Waste Collection, Disinfection, and Disposal



#### 3.12.2 Biohazardous Waste Streams:

There are four general categories of biohazardous wastes based on the physical form of the waste. Each form must be segregated, identified, decontaminated and disposed of in an appropriate manner for the form in order to minimize occupational exposure and environmental release risks.

Biohazardous waste in any form should not be left unsecured in areas that are accessible to the public (i.e., left in hallways). Only lab personnel should remove biohazardous waste from the lab area and transport it to waste holding areas for final disposal.

#### 3.12.2.1 Solid Biohazardous Waste (Non-Sharps):

In the research lab or field environment, this includes any non-sharp item that is contaminated with human or animal diagnostic specimen material (i.e., body fluids, tissue debris), any microbiological culture material (including recombinant DNA).

Examples include but are not limited to:

- Gloves and other disposable PPE contaminated with specimen or culture material.
- Plasticware such as pipettes or pipette tips, culture plates, specimen vials, etc. that are contaminated
- Towels and bench paper that are biologically contaminated (note: Bench paper that is used in areas where samples or cultures are opened and manipulated must be regarded as biologically-contaminated and therefore removed and managed as solid biohazardous waste).
- All culture or sample containers that are contaminated with biological materials.
- Tubes of blood (note: glass blood vials that could break easily upon disposal should be segregated as sharps waste; see below).

## Packaging Infectious Waste for Transport

#### Step 1:

Get Stericycle box and tape the bottom closed (Make sure arrows are pointing upwards)



#### Step 2:

Place red liner bag into the box



#### Step 3:

Place closed biohazards waste bag or closed sharp container into liner inside the box



#### Step 4:

Once box is full close the box liner and tape the box closed



#### Step 5:

Place the shipping label on the appropriate area on the box



#### Step 6:

Place the box in the designated area for Stericycle to pickup



#### Note:

- All waste bags and sharps containers must have the PI name and room number written on them.
- Do not leave sharps containers on the floor.
- Do not obstruct the door, stack full boxes.

#### Storage:

Non-sharps solid biohazardous waste must be collected for final treatment and disposal in a leak-proof container lined with an autoclavable bag of moderate thickness to prevent punctures. The collection container must have a lid or other means of closure and the container must be labeled with the biohazard symbol regardless of the lab's operating biosafety level. For BSL-2 labs, bags must be red, orange, or embossed with the biohazard symbol.



Bench top containers should be used for collection of small quantities of contaminated dry goods (i.e., pipette tips, centrifuge tubes, etc.). Small plastic containers or wire bag racks lined with a biohazard bag are suitable for bench top collection. These containers do not need to have a lid (unless waste is contaminated with a pathogen) but daily disposal of the secured bag into a larger collection container such as the one shown to the right is strongly recommended.

#### 3.12.2.1.1 "Breakable" biohazardous wastes:

Tubes of blood and other "breakable" biohazardous waste can be troublesome to manage properly and safely for treatment and disposal. For small amounts of "breakable" biohazardous waste, these items may be placed in sharps containers for disposal. However, if your lab generates a large amount of "breakable" biohazardous waste, please contact the Institutional Biosafety Officer at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu) for assistance with finding solutions for safer waste management.

### 3.12.2.1.2 Serological Pipettes:

Pipette wastes may require creative approaches for accumulation prior to disposal. Serological pipettes and micropipette tips are good examples, as they may not fit some biohazardous waste bins or may present a sharps hazard combined with heavier wastes (e.g., agar plates).

#### Suggested practices:

- Line a cardboard box, 5-gallon bucket, or similar container with a biohazard bag and collect the pipettes with the tips oriented in the same direction.
- Ensure the outside of the container has a biohazard label.
- When the bag is full, treat the pipettes by autoclave or disposal through the medical waste contractor as indicated above.
- Pipette tips may be placed in the sharps container or other puncture resistant collection device.



### 3.12.2.1.3 Pathological Waste:

Animal tissues, tissue trimmings, organs, or carcasses must be:

- Collected in leak proof, sealed bags. Bags must be red, orange, or embossed with the universal biohazard symbol if the pathological material contains an infectious agent, recombinant/synthetic nucleic acid, or biological toxin.
- Frozen and stored (tissues) for disposal through the respective lab animal facility or satellite facility.
- Unless otherwise indicated by the RSA, pathological waste is not to be autoclaved. Rather it will be shipped offsite for incineration or submitted to the College of Veterinary Medicine's necropsy unit for alkaline hydrolysis.



**NOTE: Never discard pathological wastes into the trash!**

### 3.12.2.1.4 Soil Collection, Treatment, and Disposal:

The collection of autoclaved soil, plants, and plant materials must be conducted in a manner that follows the correct waste stream and does not create ergonomic risks for facilities personnel. This guidance applies to the collection, treatment and disposal of all soil and plant material as defined below. This shall apply to all students, staff and faculty responsible for handling and disposing of soil, plants, and plant materials.

### 3.12.2.1.5 Field Generation:

Waste should be collected and stored as previously outlined. Contact the UT Health Science Center Biosafety Officer for assistance with identifying disposal options. If you plan to transport waste for treatment and/or disposal, please refer to guidelines at the end of this document for this activity.

### 3.12.2.2 Liquid Biohazardous Waste:

This includes bulk quantities of blood, blood products, body fluids from human and animal research origin and culture media. Note: Disposable primary containers or sample containers containing small quantities of liquids (less than 10 ml) should be managed as solid biohazardous waste.

#### 3.12.2.2.1 Storage:

These liquids must be stored in closed, leakproof containers while awaiting treatment and disposal. Collection vessels should be secured so that they cannot be tipped over. Secondary containment is strongly recommended and can be achieved by placing the vessel in a bucket or deep tray.

Storage vessels or the secondary container must be labeled with the biohazard label if the liquids will not be treated and disposed of within the shift. If disinfectant is added to the vessel, provide labeling so that the chemical hazard is identified as well. For instance, if your collection flask contains waste cell media and bleach, place a biohazard label on the flask (or secondary container) as well as the words "bleach-treated cell culture materials" to properly identify both the chemical and biological hazard.



#### 3.12.2.2.2 Treatment and disposal:

Liquid wastes may be treated and disposed of by either one or the other of the following methods:

- **Chemical treatment of liquids with disinfectant; disposal via lab sink:** Disinfectants may be used for "treatment" of liquid biological waste to prohibit growth of microorganisms. Here is an example for the use of household bleach.

Add household bleach to the collection vessel so that the bleach makes 10% to 15% of the final volume. Allow a contact time of at least 30 minutes. Carefully discharge the mixture to the sanitary sewer by way of the lab sink, then thoroughly rinse down the sink with water. Remember to wear splash goggles, gloves, and a lab coat for handling of bleach and bleach-treated liquids.

**NOTE: Diluted bleach solutions may go down the drain in most cases. However, many chemicals used for disinfection cannot be discarded down the drain. Contact Research Safety at 448-6115 to determine if sink disposal of disinfectants other than diluted bleach solutions is acceptable.**

### • Autoclave treatment of liquids; disposal via lab sink

Place the closed collection vessel in a secondary container and transport by cart to the autoclave facilities. Treat by autoclave using the liquids cycle. (Remember to loosen or remove the closure on the vessel before placing in autoclave.) Discharge cooled, treated liquids to the sanitary sewer by way of the lab sink. Note: Only personnel who have received training regarding the operation of the autoclave should use this device.

**Safety Note: PLEASE do not autoclave liquids containing chemical disinfectants!**

### 3.12.2.3 Sharps:

Biohazardous sharps waste must be disposed in an FDA-approved container that is manufactured for the disposal of biohazardous sharps waste: 1) puncture resistant; 2) restricted opening disallowing retrieval of sharps; 3) a lid that can be securely closed once full; and 4) labeled with the universal biohazard symbol.

Additionally:

1. All sharps containers must be permanently closed and disposed of when 2/3 to 3/4 full or whenever items do not freely fall into the container. Never pack, tamp, or shake a sharps container to fit additional items.
2. Wipe down the exterior surface of the container with disinfectant prior to submission for disposal.
3. Clean sharps may also be placed in the red sharps containers as necessary.



### 3.12.3 Disposal for non-sharps and sharps biohazardous waste:

Basic procedure:

1. Collect biowastes at the laboratory level in designated biohazard-labeled cans per current biowaste collection procedures. Segregate serological pipettes and pipette tips to prevent bag punctures or tears. Collect biohazardous sharps in sharps containers as required.
  - a. The following are **acceptable wastes**: stock/propagated cultures of infectious agents; materials that have been used for the collection/processing/storage of human or animal blood or body fluids (including cell lines); recombinant/synthetic nucleic acids; or lab consumables contaminated with any of these materials.
  - b. The following are **unacceptable wastes**: hazardous chemicals (e.g. phenol, chloroform, agarose gels with ethidium bromide, etc.); radioactive wastes; bulk liquid wastes (>25 ml/container); pathological waste such as human/animal bulk blood, tissues, or animal

carcasses (contact Biosafety for guidance); human fetal remains, limbs, or cadavers; compressed gas cylinders; loose sharps; controlled substances; etc.

2. Once the bag is ~2/3 full, close the bag by gathering the top, twisting, and closing with a single overhand knot. This method of closure minimizes the risk of leaks and spills and is required by DOT (since materials will be transported in commerce). Do not tie bag closed by crossing tabs ('bunny or dog-eared' method).

3. Once the bag is properly closed, double bag it using the same closure technique listed above. The bag can then be deposited directly into a medical waste contractor-provided receptacle, a ~90-gallon bin emblazoned with the company's name, the universal biohazard symbol, the UN shipping identifier (UN3291) and proper shipping name (Regulated Medical Waste, n.o.s.). Again, autoclaving bags prior to disposal will not be required, **except for the following circumstances:**

a. Any infectious agent listed on the DOT **Category A Infectious Substances list:**

- Bacillus anthracis (cultures only)
- Brucella abortus (cultures only)
- Brucella melitensis (cultures only)
- Brucella suis (cultures only)
- Burkholderia mallei (cultures only)
- Burkholderia pseudomallei (cultures only)
- Chlamydia psittaci (avian) (cultures only)
- Clostridium botulinum (cultures only)
- Coccidioides immitis (cultures only)
- Coxiella burnetii (cultures only)
- Crimean-Congo hemorrhagic fever virus
- Dengue virus (cultures only)
- Eastern equine encephalitis virus (culture only)
- Escherichia coli, verotoxigenic (cultures only)
- Ebola virus
- Francisella tularensis (cultures only)
- Hantavirus causing hemorrhagic fever with renal syndrome
- Hantaan virus
- Hepatitis B virus (cultures only)
- Herpes B virus (cultures only)
- Human immunodeficiency virus (cultures only)
- Lassa virus
- Marburg virus
- Monkeypox virus
- Mycobacterium tuberculosis (cultures only)
- Poliovirus virus (cultures only)
- Rabies virus (cultures only)
- Rickettsia rickettsii (cultures only)
- Rift Valley fever virus (cultures only)
- Shigella dysenteriae type 1 (cultures only)

- Variola virus
- Venezuelan equine encephalitis virus (cultures only)
- West Nile virus (cultures only)
- Yellow fever virus (cultures only)
- Yersinia pestis (cultures only)
- Classical swine fever virus (cultures only)
- Foot and mouth disease virus (cultures only)
- Goat pox virus (cultures only)
- Lumpy skin disease virus (cultures only)
- Newcastle disease virus (cultures only)
- Sheep pox virus (cultures only)
- Swine vesicular disease virus (cultures only)
- Vesicular stomatitis virus (cultures only)

For UT Health Science Center, this means that stock or propagated cultures of **verotoxigenic E. coli, Shigella dysenteriae, and hepatitis B virus** are to be autoclaved for a minimum of 30 minutes. Once treated, allow the bags to cool to room temperature, place in a second bag secured according to the above technique and then discard into a medical waste contractor-provided receptacle.

b. Any material under federal permit (e.g. USDA APHIS) requiring onsite inactivation in an approved and validated autoclave. These must be autoclaved according to validated parameters. Once treated, allow the bags to cool to room temperature and then discard into a medical waste contractor-provided receptacle or dispose in the dumpster.

4. Properly packaged and permanently closed sharps containers may also be discarded into the medical waste contractor receptacles. **Sharps containers must be secondarily enclosed in a securely tied (as described above) biohazard bag prior to disposal.** Permanently closed sharps containers must be wiped down with a disinfectant prior to removal from the lab for disposal. If there are any liquids present in the biohazardous sharps container, it must be placed in a leak-proof secondary container with a secure lid (and a biohazard label) for transport to the waste collection site or prior to bagging for depositing in the medical waste contractor bins. Disposal of biohazardous sharps containers will be accomplished through a medical waste disposal contractor coordinated through EH&S. Alternatively, sharps containers can be taken to the nearest hazardous chemical waste room (recommended if the sharps containers are very large). Collection of sharps containers in the waste collection rooms will be on the same schedule as chemical waste. Contact EHS for the current chemical waste collection schedule at 974-5084. **Do not dispose of biohazardous sharps containers in the regular trash, regardless of treatment status.**

5. Be sure that all materials being placed into the medical waste contractor receptacles are double bagged prior to depositing packaged waste from the labs (including sharps containers). Lab wastes are not to be placed in the white 'Autoclave-treated Wastes' Brute® bins after the medical waste contractor receptacles are placed. Contact Facilities Services for pick up if white bins pictured below remain in your area after red bins have been delivered.

#### Autoclave validation program:

Once the Medical waste contractor process is in place, there is no longer a need to do quarterly autoclave validations to satisfy TDEC rules for landfilling treated biohazardous wastes. A subset of volunteers will be asked to continue quarterly validations with biological indicators per USDA permit requirements (as applicable). Autoclaves used to treat Category A Infectious Substances will also be tested at least annually. Otherwise, autoclave testing will be at the discretion of the respective users.

**Do not discard wastes not mentioned here into medical waste contractor containers. If a waste is not described here, contact RSA for further guidance.**

#### On-site Autoclave Treatment (if required above)

Autoclavable waste bags must be used in biohazardous waste collection containers. Bags must be placed in a secondary container (i.e., tray with raised sides), which is placed on a cart for movement to the autoclave facilities.

Practice notes on biohazard bags:

- Biohazard bags are a one-way means of disposal. Do not “dump” the contents from one biohazard bag into another as this action spreads contamination and increases your exposure to this waste.
- Biohazard bags need to be contained at all times during the collection, treatment, and disposal process. Some lab items may puncture bags and this can lead to leaks and spills. Bags awaiting autoclave treatment should be stored in trays, tubs, buckets, etc. (The only exception to this practice is when small quantities of biohazardous wastes that do not contain liquids are collected temporarily in benchtop containers.)
- Biohazard bags must not be used for collection of other hazardous wastes (i.e., ethidium bromide gels).

Biohazardous waste bags awaiting treatment should always be stored in pans or secondary containment to prevent spills! Ethidium bromide waste is to be collected in a leak-proof container with a lid lined with a sturdy, non-descript bag. The UT Hazardous Waste label should be placed on the storage container as well. No biohazard labels!

Autoclave treatment of this waste must be performed in accordance with the biohazardous waste treatment parameters established for the autoclave. Note: Only personnel who have received training regarding the operation of the autoclave should use this device.

#### 3.12.4 Biohazardous Waste Training:

Any lab worker handling untreated biohazard waste must:

1. Complete training offered by the RSA.
2. Complete and sign the lab-specific training form after being trained in lab biosafety practices by the PI or lab supervisor.
3. Complete the Bloodborne Pathogens (BBP) training annually, if applicable.
4. Complete Lab Safety Training on Blackboard if packaging waste for UT Health Science Centers regulated medical waste contractor. Instructions for packaging regulated medical waste are included in the biosafety unit.

#### 3.12.5 Autoclave Use and Safety:

Autoclaves pose physical (e.g., heat, steam and pressure) and biological hazards. Each autoclave has unique characteristics and resulting hazards. Review and understand the owner’s manual before using any autoclave for the first time and as needed thereafter. Employ the following autoclave safety practices:

1. Before using, check inside the autoclave for any items left by the previous user that could pose a hazard (e.g., sharps).
2. Load properly (per manufacturer’s directions).
3. Loosen container caps.
4. Place containers with liquid in a tray with solid bottom and walls to catch spills.
5. Add water to the autoclave bags to facilitate steam generation. Add ¼ to ½ inch water to tray to ensure even heating.
6. Check plastic materials to ensure they are compatible with the autoclave.
7. Do not autoclave hazardous chemicals or radiological materials!!
8. Never place glassware directly on the autoclave bottom or floor.
9. Make sure the door of the autoclave is fully closed and latched and the correct cycle has been chosen before starting the cycle.
10. When removing items from the autoclave, wear the following PPE (at a minimum): heat resistant gloves, safety glasses with side shields, and a lab coat.
11. For non-liquid loads, let stand for a full hour before touching with ungloved hands. Be sure others in the area know a heat hazard is present.

#### 3.12.5.1 Autoclave Use and Safety: Validations:

In accordance with local and state regulations, all biohazardous waste must be biologically-inactivated before it is disposed of as trash. This can only be achieved if the waste is exposed to the right temperature for the right amount of time. Optimally, the waste should be exposed to: 121°C, at a pressure of 15 PSIG for at least 20 minutes.

If you are an autoclave validation volunteer, employ the following autoclave validation practices:

1. Validate quarterly using 3M Comply Thermalog TM Steam chemical integrator strips.
2. Place a full, medium-sized biohazard bag (e.g. 25” x 35”) into an autoclavable secondary container.
3. Add 1 cup (~250 mL) warm water to bag.
4. Place a 3M Comply Thermalog TM Steam chemical integrator strip inside the bag near the center of the bagged contents.
5. Place bag into the autoclave, leaving the top of the bag open for facilitate adequate steam penetration into the bag.
6. Autoclave the biohazard bag for a minimum of 30 minutes at 250F/121C. 30 minutes is the recommended minimum, but sterilizations of >1 hour

are not abnormal depending on the autoclave and load volume/contents.

7. After cycle completion, note the status of the chemical integrator. A successful test is achieved only if the blue indicator line reaches any portion of the “safe” window.
8. Document the validation test. Documentation should include the following:
  - Date and time of test.
  - Load contents.
  - Parameter setting for autoclave.
  - Type of test and results of test.
  - Name of person performing test.
  - Failed attempts and remedial actions as necessary.
9. If the validation was successful, the biohazard bag may be tied up and discarded into the designated white bins for final disposal.

Note: biological indicators (Geobacillus stearothermophilus spore) testing may be required in some circumstances. Contact the Institutional Biosafety Officer at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu) for instructions.

#### 3.12.5.2 Autoclave Use and Safety: Bagged Waste:

Employ the following autoclave treatment protocol for biohazardous waste:

1. Use secondary containment (i.e., pan and/or cart with containment walls) for transporting waste bags to the autoclave for treatment to reduce the possibility of a spill during transport.
2. Add one cup of water to each bag to facilitate air displacement and enhance steam generation.
3. Leave bags open or loosely closed at the top to facilitate steam penetration.
4. Place bags in autoclavable secondary containment pan for autoclave treatment to reduce the possibility of a spill during treatment.
5. Follow waste cycle parameters established for the autoclave to assure effective decontamination of waste.
6. Unload waste after cycle is complete and chamber pressure has returned to 0 PSIG. Do NOT override safety features to open the autoclave.
7. Use autoclave gloves, appropriate eye protection, and lab coat to avoid injury from contact with hot surfaces or liquids when removing waste from the autoclave.
8. Allow to cool, then tie or band the treated bags closed to reduce the possibility of a spill.
9. Discard in “Autoclave-treated wastes” bins located in or near the autoclave rooms.

CAUTION: Do not autoclave wastes that are contaminated with hazardous chemicals or radiological materials!!

#### 3.13 Spill Prevention and Response

Spills are a part of life in the laboratory and will occur. This means that when working with infectious or recombinant agents, precautions must be taken to

reduce the number of spills and that a spill response plan must be in place.

Spill prevention strategies:

- Use shatter resistant containers with tight fitting lids or stoppers.
- If handling multiple tubes, employ a tube rack or other device to prevent drops.
- Use a leak-proof secondary container such as a cooler or tray to contain leaks or spills (critical for movement outside the lab).
- Always use a cart when transporting large volumes of materials or when moving from floor to floor.

For spills involving potentially infectious materials:

#### **Cleaning up a biohazardous spill in a biosafety cabinet**

1. Let the BSC run. Do not turn off.
2. Remove broken glass with forceps, tweezers or other tools and place glass in a sharps container. Do not wipe up broken glass.
3. Cover spill with paper towels.
4. Pour (don't spray) disinfectant to contaminated surface by pouring it around the periphery of the covered spill moving inward. Allow the appropriate contact time for the disinfectant and agent.
5. After the contact time, wipe up the spilled material.
6. Reapply disinfectant to the affected area and after the appropriate contact time, wipe up the area. Repeat if necessary.
7. Perform disinfection before removing items.
8. Segregate contaminated cleanup materials into the appropriate biohazardous waste containers.

#### **Cleaning up a biohazardous spill outside of a biosafety cabinet:**

1. Close off the area and allow aerosols to settle.
2. Notify others including supervisor.
3. Assemble all spill cleanup materials and review procedure.
4. Don appropriate PPE: laboratory coat, safety glasses or chemical splash goggles (depending on risk of splashes), Nitrile gloves.
5. Cover spill with paper towels.
6. Pour (don't spray) disinfectant to contaminated surface that is covered with paper towels by pouring it around the periphery of the spill area moving inward. Allow the appropriate contact time for the disinfectant and agent.
7. Wipe up spill. Dispose of the waste in the biohazardous waste containers.
8. Re-apply disinfectant to contaminated surface and allow it to stand for proper contact time.
9. Wipe up disinfectant, repeat if necessary.
10. Segregate contaminated cleanup materials into the appropriate biohazardous waste containers.

#### **Cleaning up a biohazardous spill inside a centrifuge:**

1. Close off the area and allow aerosols to settle.

2. Notify others including supervisor.
3. Wait 30 minutes to allow the aerosols to settle.
4. Don appropriate PPE: laboratory coat, safety glasses and Nitrile gloves.
5. Remove rotors and bucket and place in Biosafety Cabinet.
6. Thoroughly disinfect the inside and outside of the centrifuge rotor, cups and accessories and allow proper contact time. After disinfection, move to sink for a thorough rinse, dry thoroughly.
7. For the centrifuge interior, follow the steps above for cleaning up a spill outside a Biosafety Cabinet.
8. Segregate contaminated cleanup materials into the appropriate biohazardous waste containers.

*Note: The response outlined above refers to spills that are <500 mL. For larger spills, please contact the RSA.*

#### **3.14 Emergency Response and Notification**

Proper response to personal exposure is to flush the exposed area for a minimum of 15 minutes.

After initial first aid, report the incident to your supervisor and seek medical attention as soon as possible. The need for these steps can arise from an incident where (not an all-inclusive list):

1. The skin is cut or punctured with a contaminated sharp.
2. Broken skin comes into contact with a hazardous or infectious material.
3. You experience a splash, splatter, or spray to the eyes, nose or mouth.
4. You suspect you have inhaled a material that is infectious to humans.

It is important to have pathogen/strain information for the materials you are working with available to carry with you when you seek medical attention.

To ensure proper agency reporting and other remedial actions, notify your supervisor and RSA in the event of:

1. Accidental exposures to Risk Group 2 (or higher) organisms.
2. Accidental exposures to organisms containing recombinant or synthetic nucleic acids.
3. Accidental releases, discharges, or spills in public areas involving Risk Group 2 (or higher) organisms.
4. Accidental releases, discharges, or spills in public areas involving organisms containing recombinant or synthetic nucleic acids.
5. Discovery or diagnostic confirmation of a select agent or toxin.
6. Biohazardous materials appear to be tampered with or missing.

Emergency procedures, reporting, and medical evaluation are summarized below:

- For all infectious biological material and human derived material exposures, paid staff must report exposure to CorVel at 1-866-245-8588 to obtain a

claim number per UT Risk Management procedures (this step can be concurrent with emergency reporting). Follow CorVel instructions for medical follow-up with health care provider. Paid employees must complete the Worker's Compensation forms as soon as possible. Forms are to be remitted to the Risk Management Office. For additional information, see [riskmanagement.tennessee.edu](http://riskmanagement.tennessee.edu) or contact 865.974.5409.

- Unpaid students and visitor must report exposures or injuries using the online incident report form accessible through the UT Office of Risk Management website (select General Liability). Student may also seek follow-up care from UT Health Science Center University Health Services (901-448-5630) or their primary care physician. Unpaid volunteers may report to the health care provider of their choice. Individuals not listed on the UT payroll may be personally responsible for medical costs. Unpaid employees must complete the Incident Report Form found at [riskmanagement.tennessee.edu](http://riskmanagement.tennessee.edu) or contact 865.974.5409.

#### **3.14.1 Medical Emergencies:**

Medical emergencies can only be responded to successfully if they're reported. In many BSL-2 environments, the work may be done by one individual in a culture room or an isolated area of the lab where a person may not be readily visible. Therefore, anyone who will be working at BSL-2 should assure that at least one other person in the lab group knows that they will be working, and the person notified should check on them frequently to assure that they are alright.

If a medical emergency arises for someone in your lab who is working with materials requiring BSL-2 containment, call UT Health Science Center police at 448.4444 and ask for them to send EMS (by way of 911) immediately. Have location of incident and any other pertinent information ready. Campus police will meet and direct EMS to your location. While life safety is first and foremost, some basic actions should be taken to minimize the spread of contamination.

- Restrict foot traffic to the area.
- Provide emergency responders with information about the materials that the person was working with so that they know what contamination is present in the area.

#### **3.14.2 Minor Injury/Exposure:**

For injuries not related to an exposure event (i.e. back strain, paper cut, etc.) follow the regular occupational injury reporting process.

In the event of biological exposure incident, follow the procedures outlined in Section 3.14 of this biosafety manual. Remember to flush the exposed skin or mucous membranes for 15 minutes, report it to your supervisor and to seek medical attention.

#### **3.14.3 Incident Reporting and Investigation:**

All personnel experiencing an exposure to potentially infectious materials must contact CorVel (employees) by calling 1-866-245-8588 or complete the online incident

report form (students or visitors). RSA be notified by either CorVel or UT Office of Risk Management and will follow up to investigate.

RSA encourages all lab personnel to report near miss events and unsafe conditions as well as exposures. Follow the exposure guidelines above for near misses and unsafe conditions or call RSA at 901-448-6114 or email [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu).

## **4 CONTROLLING BIOLOGICAL HAZARDS**

### **4.1 Maintaining a Secure Lab Environment**

The NIH Guidelines, the BMBL, and institutional policy require recombinant and potentially infectious agents to be secured when not in use. Beyond this requirement, maintaining a secure lab protects the environment, and protects both work related materials and private property. Entry to any laboratory is granted at the sole discretion of the laboratory principal investigator, but the following standards should be followed:

- Ensure entry doors are locked at the end of the shift. Note that labs under the purview of the Radiation Safety program require the doors be locked anytime a lab is unoccupied.
- Individuals who do not routinely work in the lab are encouraged to wear identification or make their identity known upon entry.
- Lab staff should question any unrecognized individual who enters the lab. Don't provide access codes or keys to individuals who are not authorized to be in the lab.
- Contractors, service personnel, and visitors should be made aware of risks and entry requirements prior to beginning work in the lab.
- Report any security breaches to the next higher level of authority (supervisor, department head) and UT Police as soon as possible.
- Children under the age of 18 are prohibited from entering BSL-2 (or higher) laboratories at the University unless the risk(s) have been evaluated by RSA and/or IBC.
- Be aware of your surroundings and do not allow strangers to wander through the lab. If you notice someone who seems out of place, make other personnel aware and offer to direct them to their desired person or location. This does not mean for you to put yourself at risk. If you are uncomfortable with the situation or are alone, please do not hesitate to seek help from others in the lab or surrounding labs, or the UT Police Department.
- Lock the laboratory when not in use. Unlocked and empty facilities are easy targets for theft. Secure the lab if you will be out for more than 2-3 minutes.

#### **4.1.1 Security measures for Biohazards, including Select Agents:**

The RBL High Containment Biosafety Officer is the primary contact for required security measures under

this section and can be contacted at 901-448-6115. Security measures associated with biohazards include:

1. Infectious agents categorized as Risk Group 2 (or higher) must be secured. Security measures include lockable storage devices, locked laboratory doors (when personnel not present), card/code-restricted areas/zones, or combination thereof. Stringency may vary based on the agents, regulatory requirements, or other special considerations identified by safety and security risk assessments.
2. Storage devices located in unlocked/unrestricted common areas shall be locked and appropriately labeled with biohazard signage and contact information.
3. Biological materials under regulatory permit (e.g., USDA, CDC, etc.) must be secured according to the specified permit provisions.
4. Department of Health and Human Services (DHHS)/ United States Department of Agriculture (USDA) select toxins under the de minimis threshold quantity must be secured in a locked container (refrigerator, freezer, cabinet, etc.), which is maintained in a secured laboratory of storage area. An inventory must be maintained by laboratory personnel. SOPs for toxin amounts to, use, and storage/security must be approved by the Institutional Biosafety Committee. (IBC).
5. DHHS/USDA select agents and toxins (exceeding de minimis threshold quantity) are subject to a comprehensive plan inclusive of equipment, laboratory, building, and campus security measures. This plan will be developed by RSA in collaboration with campus security authorities. The plan must be approved by the IBC and federal authorities as applicable.
6. Wastes containing biological hazards from any of the above must remain within the control of the laboratory or approved personnel until it has been inactivated and/or disposed in accordance with biological waste disposal requirements.

**Properly house materials by:**

- Making sure biological hazards are kept in primary tubes and secondary storage boxes that will prevent leaks in the event of containment failure.
- Sealing necropsy and pathological specimens in primary containers and housing in leak-proof secondary containment vessels.
- Strategies for the short- and long-term storage of research materials should consider environmental requirements to preserve the viability of samples, the security of material, and provide a means of clear and simple identification of agents by others.

**Prevent surprise discoveries of old research materials by:**

- Keeping track of laboratory hazards, including biohazards.
- Clearly labeling all prokaryotic and eukaryotic cell stocks, necropsy specimens, and other biological hazards.
- Neutralizing and disposing of old or unwanted research materials and/or equipment at the end of a project or during laboratory check-out procedures.

**4.1.2 Report Mechanical Vectors: Insect and Rodent Control**

To control the inadvertent spread of infectious agents and other biological contaminants beyond the lab, it is important to control arthropod vectors and other vermin by doing the following:

- Laboratory windows (if present) should not be open to the exterior. If a lab does have windows that open to the exterior, they must be fitted with fly screens.
- The UT Health Science Center campus has an integrated pest management program. If pests are making their way into the laboratory, contact the department or submit a work order to have the area evaluated more closely.
- Do not store food in or near the laboratory. All food should be contained and inaccessible to pests.
- Animals and plants not associated with the work being performed are not permitted in the laboratory.

**5 USE OF VIRAL VECTORS**

Viruses are inherently capable of binding to mammalian cells and transferring genetic information into those cells. Each virus has evolved to utilize the host cell's machinery in order to replicate itself. The amount of genetic material that can be packaged in each virus is determined, in part, by the structure and volume of its capsid (shell).

Using viruses for vectors takes advantage of this cell targeting and gene expression system. In designing gene vectors, the virus is generally engineered so it cannot reproduce (replication-deficient). This is accomplished by removing a gene from the virus genome that is critical for replication. Removing this gene also creates space to allow the insertion of the gene desired for expression (gene of interest). This vector can now be reproduced by incubating it with cells that can compensate for the gene that was deleted, allowing the virus to replicate within the cell (packaging cell line). In some cases, another virus can supply the missing replication machinery (helper virus). The goal is to end up with a large number of viral particles with the gene of interest, but to not allow the virus to exert any pathogenic properties associated with the whole or wild-type virus.

There are two basic biosafety concerns regarding research using viral vectors. First, it is impossible to completely control cellular processes to ensure that a replication-deficient virus will not naturally gain back genes that it requires for replication (become replication-competent). If a virus becomes replication-competent, it may re-acquire any pathogenic characteristics associated with the wild-type virus and could cause illness.

In addition, the gene of interest may be acquired by viruses or cells not expected to be associated with that gene. Because of these concerns, biosafety containment recommendations are made according to the properties of the wild type virus, and must also take into consideration the nature and necessary containment of the gene of interest.

**6 ACUTE BIOLOGICAL TOXINS**

**6.1 Toxins of Biological Origin**

**Biological toxins** are produced by certain bacteria, fungi, protozoa, plants, reptiles, amphibians, fish, echinoderma

(spiny urchins and starfish), mollusks, and insects.

The following table lists the **LD<sup>50</sup>** values for commonly used biological toxins:

TOXIN	LD50 (MG/KG)*	TOXIN	LD50 (MG/KG)*
Abrin	0.7	Diphtheria toxin	0.1
Aerolysin	7	Listeriolysin	3-12
Botulinin toxin A	0.0012	Leucocidin	50
Botulinin toxin B	0.0012	Modeccin	1-10
Botulinin toxin C1	0.0011	Nematocyst toxins	33-70
Botulinin toxin C2	0.0012	Notexin	25
Botulinin toxin D	0.0004	Pertussis toxin	15
Botulinin toxin E	0.0011	Pneumolysin	1.5
Botulinin toxin F	0.0025	Pseudomonas aeruginosa toxin A	3
b-bungarotoxin	14	Ricin	2.7
Caeruleotoxin	53	Saxitoxin	8
Cereolysin	40-80	Shiga toxin	0.25
Cholera toxin	250	Shigella dysenteriae neurotoxin	1.3
Clostridium difficile enterotoxin A	0.5	Streptolysin O	8
Clostridium difficile cytotoxin B	220	Staphylococcus enterotoxin B	25
Clostridium perfringens lecithinase	3	Staphylococcus enterotoxin F	2-10
Clostridium perfringens kappa toxin	1500	Streptolysin S	25
Clostridium perfringens perfringolysin O	13-16	Taipoxin	2
Clostridium perfringens enterotoxin	81	Tetanus toxin	0.001
Clostridium perfringens beta toxin	400	Tetrodotoxin	8
Clostridium perfringens delta toxin	5	Viscumin	2.4-80
Clostridium perfringens epsilon toxin	0.1	Volkensin	1.4
Conotoxin	12-30	<i>Yersinia pestis</i> murine toxin	10
Crotoxin	82		

\*Note that the LD50 values are from a number of sources (see below). For specifics on route of application (i.v., i.p., s.c.), animal used, and variations on the listed toxins, please go to the references listed below. (Table courtesy University of Florida EHSO)

**References:**

1. Gill, Michael; 1982; Bacterial toxins: a table of lethal amounts; Microbiological Reviews; 46: 86-94

2. Stirpe, ; Luigi Barbieri; Maria Giulia Battelli, Marco Soria and Douglas A. Lappi; 1992; Ribosome-inactivating proteins from plants: present status and future prospects; Biotechnology; 10: 405-412.
3. Registry of toxic effects of chemical substances (RTECS): comprehensive guide to the 1997. Doris V. Sweet, ed., U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; Cincinnati, Ohio.

## 6.2 Additional Biological Toxins

Aflatoxins	Fusarium sp toxins	Streptonigrin
DTX-1 (Dinophysistoxin-1)	Ricin toxins – all	Cobra venoms and all derived toxins
Palytoxin	Brevetoxins	Microcystins
Amanitin	Gliotoxin	Taipoxin
Echnioderm venoms – all	Sapintoxin	Cobratoxin
Palytoxin	Bungarotoxins	Mojave toxin
Amphibian venoms	Joco Spider Toxin JSTX – 3	Tetanus toxins – all
Endotoxins – all	Sarafotoxin	Conotoxins – all
Pertussis toxins – all	Cardiotoxin	Mycotoxins – all
Anatoxin A	Lappaconitines	Tetrodotoxins – all
Enterobacteriaceae toxins – all	Saxitoxin	Crotamine
Phalloidin	Charybdotoxin	Myotoxins
Anthrax toxin	Leiurotoxins	Textilotoxin
Enterotoxins – all	Short Neurotoxins	Dendrodotoxins
Psilocybine	Cholera toxins – all	Neurotoxins – all
Aspergillus sp toxins	Lipid A – all types	Thymeleatoxin
Escherichia coli toxins – all	Snake Venoms – all	Dinoflagellate neurotoxins
Pseudomonas sp toxins	Ciguatera toxin	Notexin
Bacillus sp toxins – all	Lipopolysaccharides from all species	Tinyatoxin
Exotoxin A	Stable toxins	Diphtheria toxins
Reptile venoms – all	Clostridia species toxins – all	Nodularin
Bordetella sp toxins	Maitotoxin	Toxin II – all types
Fish venoms – all	Staphylococous sp toxins	Domoic acid
Resiniferatoxin	Cluepeotoxins	Ochratoxin
Botulinum toxins – all	Medamine	

## 6.3 Toxins Classified as Select Agents

Some biological toxins are classified by the CDC and USDA as Select Agents ([selectagents.gov/index.html](https://selectagents.gov/index.html) or [aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/sa\\_ag\\_select\\_agent](https://aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/sa_ag_select_agent)) due to their potential to pose a severe threat to public health and safety. Possession, use, and transfer of these toxins are highly regulated. A complete list can be found at [selectagents.gov/SelectAgentsandToxinsList.html](https://selectagents.gov/SelectAgentsandToxinsList.html).

In small quantities, some of these toxins are exempt from select agent registration. See the table on the next page. Exempt Amounts of Select Agent Toxins Permissible Per Principal Investigator.

HHS (CDC-LISTED) TOXINS	EXEMPTED QUANTITY
Abrin	1,000 mg
Botulinum neurotoxin	1 mg
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X1CCX2PACGX3X4X5X6CX7)	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg
Saxitoxin	500 mg
Staphylococcal enterotoxins A,B,C,D,E subtypes	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

## 6.4 Working with Biological Toxins

Because they can be extremely hazardous, even in minute quantities, biological toxins require strict safeguards against their inhalation, absorption through skin or mucous membranes (typically due to a splash), ingestion, or percutaneous injury. A chemical hygiene plan and Safety Data Sheet specific for the toxin must be on hand in the laboratory for inspection.

Key points of the guidelines are:

1. Written safety protocols to cover the use of the specific toxin(s) in use;
2. Security measures in place to protect against unauthorized access to toxin(s);
3. Inventory control system in place; all entries in a hardbound book, in ink;
4. Written plan for toxin-related emergencies (spill, exposure, ) posted; and
5. BSL-2 or BSL-3 containment and practices in

## 6.5 Disposal of Biological Toxins

Specific inactivation and disposal requirements should be in place for acute biological toxins. Some toxins are quite resistant to conventional methods of inactivation. These agents cannot be simply placed in an autoclave.

Toxins may be destroyed by several methods as shown in the table below. Some toxins are inactivated by autoclaving for one hour at 121°C. Others are inactivated by exposure to sodium hypochlorite and/or sodium hydroxide.

### 1. Chemical Destruction of Toxins:

When using sodium hypochlorite and / or sodium hydroxide to destroy toxins, the procedure(s) must be performed in a laboratory fume hood or a biological safety cabinet. At a minimum, personal protective equipment for all procedures should include:

- Long sleeved protective clothing (lab coat, gown)
- Gloves
- Eye protection

1. If the toxin is classified as a select agent, even in exempt amounts, notify the RSA prior to the destruction of the agent.
2. Work in a fume hood or biosafety cabinet with the sash at the lowest reasonable sash height for safe and effective work.
3. Place plastic-backed absorbent paper (bench diaper) on the work surface of the fume hood or biosafety cabinet.
4. CAREFULLY put the toxin into solution in the primary container. **DO NOT USE A GLASS CONTAINER.**

5. Place the primary container in a secondary container, such as a beaker or rack.
6. Slowly dispense an equal volume of the concentrations of sodium hypochlorite and/or sodium hydroxide designated in table 1 below into the primary container of toxin solution to be destroyed.
7. Do not replace the cap on primary container.
8. Place a “**WARNING / DO NOT USE**” sign on the hood/ cabinet.
9. Allow a **minimum 60 minutes** exposure (See table 1 below for additional exposure time recommendations.).
10. Document the destruction of the toxin in the laboratory inventory log book.
11. Secure the cap on the primary container. **DOUBLE BAG** the material in zip- lock plastic bags and label it “**Inactivated/denatured (TOXIN NAME)**”.
12. Contact the RSA for disposal as hazardous waste.

### 4. Steam Sterilization (Autoclaving) of Toxins:

If acceptable as a method in table 1 below, destroy toxins by autoclaving them using the procedure outlined below:

1. If the toxin is classified as a select agent, even in exempt amounts, notify the RSA prior to the destruction of the agent.
2. In a fume hood or biological safety cabinet, loosen the cap of the primary toxin container to allow steam penetration.
3. Place the primary container into a secondary biohazard container.
4. Place the container in a loosely closed biohazard bag.
5. Place the bag in an autoclavable pan.
6. Autoclave at 121° C for 1 hour on liquid cycle (slow exhaust).
7. Document the destruction of the toxin the laboratory inventory logbook.
8. After autoclaving, allow time for materials to cool before handling.
9. Discard the autoclave bag and its containers as treated biological waste.

DO NOT use steam sterilization for destruction of any of the low molecular weight toxins (i.e. mycotoxins, marine and reptile venoms).

### 5. Inactivation Procedures for Selected Toxins

Allow at least a 60-minute chemical contact time for complete inactivation of toxin. Any procedure labeled “yes” is an approved procedure for inactivation of the toxin specified.

TOXIN	AUTOCLAVE (1 HOUR @ 121° C, LIQUID EXHAUST)	2.5% NAOCL +0.25 N NAOH	1.0%NAOCL	2.5%NAOCL
Abrin (1)(8)	Yes	N/A	N/A	N/A
Botulinum Neurotoxin (1) (7)	Yes	Yes	Yes	Yes
Clostridium perfringens epsilon toxin (2)	Yes	N/A	N/A	N/A
Conotoxin(3)	CONTACT the Research Safety Affairs			
Diacetoxyscirpenol(5)	No	Yes	No	Yes (3-5%)
Ricin (1)(7)	Yes	Yes	Yes	Yes
Saxitoxin(1)(7)	No	Yes	Yes	Yes
Shigatoxin & Shiga-like ribosome inactivating proteins(4)	Yes	Yes	Yes	Yes
Staphylococcal Enterotoxins (1)(7)	Yes	Yes	Yes	Yes
Tetrodotoxin(1)(7)	No	Yes	Yes	Yes
T-2 Toxin (1)(6)(5)	No	Yes	No	No

References:

1. Wannemacher W. 1989. Procedures for Inactivation and Safety Containment of Toxins. Proc. Symposium on Agents of Biological Origin, U.S. Army Research, Dev. And Engineering Center, Aberdeen proving Ground, MD. Pp. 115-122
2. Factsheets on Chemical and Biological Warfare [PDF], [dhs.gov/publication/biological-attack-fact-sheet](https://www.dhs.gov/publication/biological-attack-fact-sheet)
3. For complete inactivation of T-2 mycotoxin extend exposure time for liquid samples, spills, and non-burnable waste in 5% sodium hypochlorite and 0.25 N sodium hydroxide to 4 hr. Expose cages/bedding from animals exposed to T-2 mycotoxin to 0.25% sodium hypochlorite and class=GramE>0.025 N sodium hydroxide for 4 hrs.
4. For inactivation of saxitoxin, tetrodotoxin, ricin, botulinum toxin, or staphylococcal enterotoxins, expose work surfaces, solutions, equipment, animal cages, spills to 10% sodium hypochlorite for 60 minutes.
5. [inchem.org/documents/pims/plant/abruspre.htm](https://www.inchem.org/documents/pims/plant/abruspre.htm)

## 7 TRAINING

Based on federal and institutional guidelines all personnel working in UT Health Science Center laboratories are required to complete UT Health Science Center Laboratory Safety Training. This course covers the requirements of the UT Health Science Center Chemical Hygiene Plan, standard practices for working at BSL-1, hazardous waste management, and emergency response procedures. Lab Safety Refresher training must be completed annually. These courses or accessible through Blackboard. Lab personnel must complete the course Principles of Biosafety (BSL2) if their research involves the following:

- Agents infectious to humans.
- Recombinant or synthetic nucleic acids.
- Acute biological toxins.
- Human or nonhuman primate-derived materials.
- Animal specimens or environmental samples that likely harbor infectious agents affecting humans (per risk assessment).

In addition to these courses provided by RSA, lab-specific training is required. This training is directed by the PI or senior laboratory personnel, must be completed

prior to beginning work in the lab and must address the specific hazardous materials and procedures performed in the lab in which personnel are working.

### Other Biosafety Training

Other specialized training modules are provided depending on the type of work being carried out. Examples of specialized training are below:

- **Bloodborne Pathogens (BBP) training:** Required if manipulating unfixed and untested human tissues and cells in a research setting or if handling wastes contaminated with these materials (note: BBP is included in this initial training, but can be offered separately depending on the activity/department).
- **IATA Dangerous Goods Shipping for infectious substances (Exempt biological materials, category A and B infectious substances) and dry ice shipping:** Required if packaging and shipping microbiological cultures, diagnostic specimens, or biological materials (with or without dry ice) by air or ground.
- **Animal Biosafety:** Personnel working in Lab Animal Care Unit (LACU) vivaria must the AALAS Learning Library courses prescribed by the LACU and IACUC.
- **Animal Hazard Safety Evaluation Form:** Required if performing procedures involving microbiological agents, human-derived materials, or acute biological toxins in an animal model that requires BSL-2 containment facilities.
- **IBC committee (IBC) and regulatory compliance training:** Required if serving as a member of the IBC and for those initiating or supervising research projects involving recombinant or synthetic nucleic acids (covered in this training), select agents, or other biological materials covered by the IBC or various U.S. federal, state, or local authorities (e.g., USDA APHIS permit review/training).
- **Agent specific training:** Required if manipulating infectious agents, acute biological toxins, or other biological hazards covered by the Biosafety Program. This training may be provided by the PI (or designate) or in conjunction with RSA (note: this a required item on the site-specific training checklist).

## 8 OCCUPATIONAL HEALTH

### 8.1

As a condition of authorization, personnel who work with animals, are required to be enrolled in an Occupational Health and Medical Surveillance Program related to their potential risks. This program is administered by UT Health Science Center University Health Services (UHS). The goal of the Occupational Health and Medical Surveillance Program is to prevent occupational injury and illness, and protect the vulnerable populations of students, employees, and research animals. The program includes a risk assessment intended to identify individual risk associated the work they expect to perform. In part, this risk assessment evaluated personnel atopic for lab animal allergies.

### 8.2

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

### 8.3

All employees required to wear a respirator must complete an OSHA Respirator Medical Evaluation Questionnaire before using a respirator. The respiratory protection programs are overseen by the Research Safety Affairs and Campus Safety and Emergency Management.

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

These potentially “immunocompromising” conditions include the following:

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

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

- Heart conditions (e.g., valve disease, heart failure)
- Iron-overload conditions (e.g., hemochromatosis)
- Chronic liver disease (e.g., cirrhosis, hepatitis, fatty liver disease)

- Hemoglobinopathies (e.g., sickle cell disease, beta thalassemia)
- Gastrointestinal conditions (or medications used to treat these)
- Eczema or other skin diseases which may present with open lesions

#### Post-Exposure Prophylaxis and Medical Monitoring

Plans to address how a biological exposure incident will be addressed should be prepared by the laboratory and provided to the RSA for review prior to working with these agents.

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

## 9 BLOODBORNE PATHOGENS

What is a bloodborne pathogen (BBP)?

A BBP is a disease-causing organism that can be found in human blood and certain body fluids. Common BBP include: Human immunodeficiency virus (HIV); hepatitis B Virus (HBV); hepatitis C virus (HCV). However, there are several other microbes that may be considered BBP, including:

- Viruses: HTLV-1; Epstein-Barr virus (infectious mononucleosis), Ebola virus, various arthropod-borne viruses (e.g. Zika)
- Parasites: Plasmodium falciparum (malaria)
- Bacteria: Treponema pallidum (syphilis)

### 9.1 The OSHA BBP Standard

The Occupational Safety and Health Administration's (OSHA) Occupational Exposure to Bloodborne Pathogens (BBP) Standard [29CFR1910.1030] applies to anyone that will handle or may come into contact with human blood or other potentially infectious materials (OPIM) due to the risk of Bloodborne pathogens. OSHA mandates initial and annual recurrent training for all affected personnel.

### 9.2 Exposure Control Plan (ECP)

All employers with workers who have a reasonably anticipated risk for BBP exposure must provide an ECP. This plan is a workplace-specific document that outlines jobs/tasks with BBP risk, methods of exposure control, and employer and employee administrative responsibilities. It is imperative that all lab personnel working with human derived materials know the location of and be able to access the current year's ECP. The UT Health Science Center Exposure Control Plan is accessible online in the UT Health Science Center Safety Manual.

### 9.3 BBP – Agent Summary

The table below summarizes key features of the most commonly occurring BBP (in the U.S.):

### 9.4 BBP – Infectious Materials

Researchers and lab personnel are among the occupations that are at risk of being exposed to BBPs and will fall under the specific OSHA regulations designed to reduce occupational risk of infection.

BBPs may be present in infectious concentrations in human blood; semen/vaginal secretions; fluid from the spine, joints, lungs and other vascularized body cavities; any body fluid contaminated with blood; any body fluid that you can't identify; and other potentially infectious materials (OPIM).

OPIMs in clinical or lab settings include: human blood products (serum, plasma, albumin, various factors, etc.); unfixed tissues/organs (other than intact skin of human origin); cell or tissue cultures that may contain BBPs; organ cultures, culture medium or other solutions that may contain BBPs; experimental animals infected with BBPs.

Note: Tissue cultures of concern include primary and immortalized or established cell lines regardless of origin/vendor unless documented to be free of BBPs. Otherwise, risk assessment indicates coverage under the BBP Standard.

Some human body fluids are not considered to be a BBP hazard unless they are visibly contaminated with blood. These include: urine, feces, vomit, sweat, tears, saliva, or nasal secretions. Even so, use universal precautions and wear appropriate personal protective equipment (PPE) when handling these fluids as they may contain other types of pathogenic microbes.

### 9.5 BBP – Routes of Exposure

Needle sticks and percutaneous injuries are the primary ways BBP infections occur. Other ways infections can occur are: 1) through contact with human derived materials through breaks in the skin that may occur if there is a fresh flesh wound; your skin is chapped; or you have acne, eczema, split cuticles, etc.; 2) splashes of blood or OPIM come into contact with mucous membranes around the eyes, nose or mouth; or 3) transmission occurs through sexual intercourse or from the mother to the unborn child.

An important thing to keep in mind when considering the risk of BBPs is that they are not all equally infectious. The risk of infection through a needlestick exposure for HBV is much higher than for HIV or HCV.

This is in part due to the fact that HIV breaks down quickly once it is outside the human body, while HBV is more environmentally stable. Also, HBV-infected blood/body fluids contain significantly more virus particles relative to HIV-infected blood. This is why the Hepatitis B vaccine is so important.

### 9.6 BBP – Hepatitis B Vaccine

OSHA requires employers to offer the Hepatitis B vaccine to all at risk employees as follows: 1) those with risk are

offered the vaccine at no cost, 2) the vaccine is offered at a convenient time and location, 3) the offer of vaccine must be documented through a waiver/request to be completed by the employee, and 4) if an employee declines the offer, they can request vaccination at a later date if still at-risk.

The vaccine is typically administered in 3 separate doses spanning ~6 months. Once vaccinated and neutralizing antibodies have been confirmed, boosters are generally not required.

While there are minor risks associated with the vaccine (localized pain, swelling, redness; fatigue; and malaise), it is widely considered one of the safest.

Contact the University Health Services (UHS) at 901.448.5630 for more information about the Hepatitis B vaccine if interested.

### 9.7 BBP – Universal Precautions

The best way to protect yourself from BBP is to practice universal precautions. Universal precautions approximate BSL-2 practices and include the following:

- Treat all blood and OPIM as if they were known to be infectious.
- If you are aware of a hazard, communicate it to fellow lab personnel.
- Good laboratory and personal hygiene practices. Routine handwashing and disinfection of potentially contaminated surfaces are critical for mitigating risk!
- Clean the surfaces of visible debris before disinfection with a 1:10 bleach solution or with a disinfectant that is EPA-registered for the destruction of HIV and HBV. Refer to the manufacturer's instructions for proper dilution, contact time, and use of the disinfectant.
- Eliminate or reduce the use of sharps devices if possible.
- Employ engineering controls when available.
- Wear PPE that is appropriate for the task.
- Segregate and treat wastes (disinfection, autoclaving, etc.).

### 9.8 BBP – Needlestick Prevention

All employers are required to minimize the risk of needlesticks under the authority of the Needlestick Safety and Prevent Act which is part of the Bloodborne Pathogens standard. The Act specified that employers must provide safer sharps devices if available; employees who work with sharps and blood must be involved in device evaluation and selection process; initial and annual sharps evaluations must be completed by anyone using sharps on LIVE HUMANS (e.g. phlebotomy, finger sticks, etc.); and a sharps injury log must be maintained.

Safer sharps resources and evaluation forms can be found in the UT Health Science Center Exposure Control Plan or on the by contacting RSA at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu).

### 9.9 BBP – Exposure Response

In the event you are exposed or potentially exposed to a Bloodborne pathogen, it is very important to flush the exposed area for 15 minutes, report the exposure to your supervisor, and seek prompt medical care. It is important because it:

- Allows accurate evaluation of exposure risk by a medical professional;
- Increases the chance of identifying and testing the source of blood/OPIM; and
- Provides lead time to administer treatments that can reduce the chance of infection (if high risk event) – antiretroviral therapies for HIV are most effective if started within 2-4 hours of exposure, and it is important to administer HBV immunoglobulin or vaccine booster.

It is important to take any information that you may have regarding the materials to which you were exposed. This helps to inform the medical risk assessment and course of treatment. Without it, physicians are likely to take the most conservative approach and start antiviral therapies (some of which can have serious side effects).

### 9.10 BBP – Exposure Reporting

For all infectious biological material and human derived material exposures, paid staff must report exposure to CorVel Corp. at 1-866-245-8588 to obtain a claim number per UT Risk Management procedures (this step can be concurrent with emergency reporting). Follow CorVel instructions for medical follow-up with health care provider. Paid employees must complete the Worker's Compensation forms as soon as possible. Forms are to be remitted to the Risk Management Office. For additional information, see [riskmanagement.tennessee.edu/incident-reporting/](http://riskmanagement.tennessee.edu/incident-reporting/) or contact 865.974.5409.

Unpaid students may report to UT Health Science Center University Health Services 901.448.5630 or their primary care physician. Unpaid volunteers may report to the health care provider of their choice. Individuals not listed on the UT payroll may be personally responsible for medical costs. Unpaid employees must complete the online Incident Report Form found at [riskmanagement.tennessee.edu](http://riskmanagement.tennessee.edu) or contact 865.974.5409.

## 10 LAB CLOSEOUT PROCEDURES

### 10.1 Lab Moves and Closeouts

The lab commissioning process is detailed in UT Health Science Center procedure RS105: Procedure for New Laboratories. The procedure for departing Principal Investigators or decommissioning labs is detailed in RS104: Lab Closures. These and other research safety procedures are accessible online in the campus Safety Manual.

## 11 EQUIPMENT DECONTAMINATION

### 11.1 Surplus Equipment Decontamination

Equipment that is destined for surplus must be properly decontaminated from chemical, biological, or radiological hazards.

Surplus equipment is managed by Procurement Services. Surplus inventory that is to be discarded or redistributed must be decontaminated by the owner or another individual familiar with its use history and operation. Proper decontamination must be documented by completion of the S-3 Surplus Equipment Decontamination Form. A copy of this form must be signed by the individual that performed the decontamination and affixed to the surplus equipment before it will be removed from by Procurement Services or the Facilities Logistics Team. A copy of the form should also be emailed to RSA at [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu).

### 11.2 Considerations for Surplus Equipment Management

All personnel are asked to bear in mind that the surplus warehouse workers have no special knowledge about laboratory practices and potential contaminants. When they receive something from a lab that looks dirty, they must assume a hazardous contaminant.

These items going to surplus, if not purchased by another department on campus, are auctioned off to the general public, so items must be cleaned out and decontaminated by lab personnel so that no one is unintentionally harmed.

#### 11.2.1 Steps to Take Before Decontamination

1. Find out the history of the equipment
  - a. What was it used for?
  - b. What might the equipment contaminated with?
2. What PPE is appropriate during decontamination and spill clean-up?
3. What will be the disposition plan for the containers inside the equipment (if applicable)?
4. Clean up all spills in or on the equipment prior to decontaminating (spill clean-up materials may need to be disposed of as hazardous waste or biohazardous waste, or radiological waste as appropriate. Contact the appropriate safety office for assistance with this.
5. Determine if the design, materials, or construction of the equipment will have an effect on the decontamination process. Cavities, joints and pores constitute barriers to the penetration of liquid disinfectants and prolonged contact time may be required to accomplish decontamination, depending on the intricacy of the design and the amount of soil present.
6. Select the appropriate disinfectant or decontamination solution depending on the contaminant.

#### 11.2.2 Biological Contamination

When incubators, refrigerators, freezers or other equipment are disinfected, they should also be wiped out and dried out completely. Also, the doors should be left open, if possible, to prevent mold growth.

For specific guidance on how to disinfect an item with biological contamination, please contact the RSA at 901.448.6114 or email [labsafety@uthsc.edu](mailto:labsafety@uthsc.edu).



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