The following sections provide general guidelines and requirements for biological safety. This chapter covers the following topics:

<table>
<thead>
<tr>
<th>TOPIC</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosafety Principle</td>
<td>12-2</td>
</tr>
<tr>
<td>General Biosafety Guidelines</td>
<td>12-3</td>
</tr>
<tr>
<td>CDC and NIH Biosafety Levels</td>
<td>12-4</td>
</tr>
<tr>
<td>Recombinant DNA Research</td>
<td>12-6</td>
</tr>
<tr>
<td>Disinfection and Sterilization</td>
<td>12-7</td>
</tr>
<tr>
<td>Biological Safety Cabinets</td>
<td>12-10</td>
</tr>
<tr>
<td>Clean Benches</td>
<td>12-12</td>
</tr>
<tr>
<td>Importing and Shipping Biological Materials</td>
<td>12-12</td>
</tr>
<tr>
<td>Biological Spill Response</td>
<td>12-12</td>
</tr>
<tr>
<td>Biological Waste Disposal</td>
<td>12-13</td>
</tr>
<tr>
<td>Bloodborne Pathogens</td>
<td>12-17</td>
</tr>
</tbody>
</table>
Biosafety Principle

The primary principle of biological safety (i.e., biosafety) is containment. The term *containment* refers to a series of safe methods for managing infectious agents in the laboratory. The purpose of containment is to reduce or eliminate human and environmental exposure to potentially harmful agents.

Primary and Secondary Containment

There are two levels of biological containment---primary and secondary. Primary containment protects people and the immediate laboratory environment from exposure to infectious agents. Good microbial techniques and safety equipment provide sufficient primary containment. Examples of primary barriers include safety equipment such as biological safety cabinets, enclosed containers, and safety centrifuge cups. Occasionally, when it is impractical to work in biological safety cabinets, personal protective equipment, such as lab coats and gloves may act as the primary barrier between personnel and infectious materials.

Secondary containment protects the environment external to the laboratory from exposure to infectious materials. Good facility design and operational practices provide secondary containment. Examples of secondary barriers include work areas that are separate from public areas, decontamination facilities, handwashing facilities, special ventilation systems, and airlocks.

Elements of Containment

Ultimately, the three key elements of biological containment are laboratory practices, safety equipment, and facility design. To ensure minimal exposure, employees must assess the hazards associated with their work and determine how to apply the biosafety principle appropriately.

**IMPORTANT:**

*Employees working with infectious agents or potentially infectious materials must be aware of the hazards associated with their work. These workers must be trained and proficient in biosafety procedures and techniques.*
General Biosafety Guidelines

Biohazardous materials require special safety precautions and procedures. Follow these guidelines when working with infectious agents:

Personal Hygiene Guidelines:
- Wash your hands thoroughly, as indicated below:
  - After working with any biohazard
  - After removing gloves, laboratory coat, and other contaminated protective clothing
  - Before eating, drinking, smoking, or applying cosmetics
  - Before leaving the laboratory area
- Do not touch your face when handling biological material.
- Never eat, drink, smoke, or apply cosmetics in the work area.

Clothing Guidelines:
- Always wear a wrap-around gown or scrub suit, gloves, and a surgical mask when working with infectious agents or infected animals.
- Wear gloves over gown cuffs.
- Never wear contact lenses around infectious agents.
- Do not wear potentially contaminated clothing outside the laboratory area.
- To remove contaminated clothing, follow these steps:
  1. Remove booties from the back.
  2. Remove head covering from the peak.
  3. Untie gown while wearing gloves.
  4. Remove gloves by peeling them from the inside out.
  5. Remove the gown by slipping your finger under the sleeve cuff of the gown.

Handling Procedures:
- Use mechanical pipetting devices.
- Minimize aerosol production.
- Add disinfectant to water baths for infectious substances.
- Use trunnion cups with screw caps for centrifuging procedures. Inspect the tubes before use.
- Use secondary leak-proof containers when transporting samples, cultures, inoculated petri dishes, and other containers of biohazardous materials.

Syringes:
Avoid using syringes and needles whenever possible. If a syringe is
necessary, minimize your chances of exposure by following these guidelines:

- Use a needle-locking or disposable needle unit.
- Take care to stick yourself with a used needle.
- Place used syringes into a pan of disinfectant without removing the needles.
- Do not place used syringes in pans containing pipers or other glassware that requires sorting.
- Do not recap used needles.
- Dispose of needles in an approved sharps container.

Work Area:

- Keep laboratory doors shut when experiments are in progress.
- Limit access to laboratory areas when experiments involve biohazardous agents.
- Ensure that warning signs are posted on laboratory doors. These signs should include the universal biohazard symbol and the approved biosafety level for the laboratory.
- Ensure that vacuum lines have a suitable filter trap.
- Decontaminate work surfaces daily and after each spill.
- Decontaminate all potentially contaminated equipment.
- Transport contaminated materials in leak-proof containers.
- Keep miscellaneous material (i.e., books, journals, etc.) away from contaminated areas.
- Completely decontaminate equipment before having maintenance or repair work done.

CDC & NIH Biosafety Levels

**Universal Precautions:**
Clinical and diagnostic laboratories often handle specimens without full knowledge of the material's diagnosis; these specimens may contain infectious agents. To minimize exposure, observe universal precautions when handling any biological specimen. Consider all specimens to be infectious and treat these materials as potentially hazardous.

**Biosafety Level 1**
The Centers for Disease Control (CDC) and the National Institutes of Health (NIH) have established four biosafety levels consisting of recommended laboratory practices, safety equipment, and facilities for various types of infectious agents. Each biosafety level accounts
for the following:

- Operations to be performed
- Known and suspected routes of transmission
- Laboratory function

Biosafety Level 1 precautions are appropriate for facilities that work with defined and characterized strains of viable organism that do not cause disease in healthy adult humans (e.g., *Bacillus subtilis* and *Naegleria gruberi*). Level 1 precautions rely on standard microbial practices without special primary or secondary barriers. Biosafety Level 1 criteria are suitable for undergraduate and secondary education laboratories.

---

**Biosafety Level 2**

Biosafety Level 2 precautions are appropriate for facilities that work with a broad range of indigenous moderate-risk agents known to cause human disease (e.g., Hepatitis B virus, salmonellae, and *Toxoplasma* spp.). Level 2 precautions are necessary when working with human blood, body fluids, or tissues where the presence of an infectious agent is unknown. The primary hazards associated with level 2 agents are injection and ingestion. Most TSU research laboratories should comply with Biosafety Level 2 criteria.

---

**Biosafety Level 3**

Biosafety Level 3 precautions apply to facilities that work with indigenous or exotic agents with the potential for aerosol transmission and lethal infection (e.g., *Mycobacterium tuberculosis*). The primary hazards associated with level 3 agents are autoinoculation, ingestion, and inhalation. Level 3 precautions emphasize primary and secondary barriers. For primary protection,

**Biosafety Level 3, cont’d**

all laboratory manipulations should be performed in a biological safety cabinet or other enclosed equipment. Secondary protection should include controlled access to the laboratory and a specialized ventilation system.
Biosafety Level 4

Biosafety Level 4 precautions are essential for facilities that work with dangerous and exotic agents with a high risk of causing life-threatening disease, the possibility of aerosol transmission, and no known vaccine or therapy (e.g., Marburg or Congo-Crimean viruses). Level 4 agents require complete isolation. Class III biological safety cabinets or full-body air-supplied positive-pressure safety suits are necessary when working with level 4 agents. In addition, isolated facilities, specialized ventilation, and waste management systems are required. There are no Biosafety Level 4 facilities at TSU.

Animal Biosafety

Four biosafety levels are also described for infectious disease work with laboratory animals. Safety practices, equipment, and facilities are designated by Animal Biosafety Levels 1, 2, 3, and 4.

Refer to the Laboratory Safety chapter for more information regarding the use of hazardous materials with laboratory research animals.

A copy of the CDC/MIH criteria for laboratory and animal biosafety levels is available from the Office of Risk Management & Safety (RMS).

Recombinant DNA Research

As an institute that receives NIH funding, TSU is obligated to ensure that all recombinant DNA (rDNA) work conducted by its faculty and staff conforms with Federal rDNA guidelines. This task falls jointly to the Institutional Biosafety Committee (IBC) and the Safety & Health Office. The IBC reviews all protocols involving rDNA, rules on the appropriateness of proposed containment procedures, and sets suitable biosafety levels. RMS inspects individual laboratories and verifies assigned by the IBC.

The Federal rDNA guidelines define rDNA as "...molecules which are constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell."

The Federal definition also includes the replicated progeny of these molecules as well as cells, plants, and animals that harbor such molecules. Transgenic plants and animals also come under the
guidelines, even if the transgenic DNA was not cloned prior to introduction.

Investigators who possess rDNA in any form must file an rDNA protocol with the IBC. A copy of the TSU Policies and Procedures for Research Involving Recombinant DNA is available from the Safety & Health Office.

---

**Disinfection and Sterilization:**

Biological safety depends on proper cleanup and removal of potentially harmful agents. Disinfection and sterilization are two ways to help ensure biological safety in the laboratory.

- **Disinfection:**
  Reduction of the number of pathogenic organisms by the direct application of physical or chemical agents.

- **Sterilization:**
  Total destruction of all living organisms.

The following sections discuss guidelines and procedures for biological disinfection and sterilization.

Choosing the best method for disinfection and sterilization is very important. The proper method depends on the following:

- Target organisms to be removed
- Characteristics of the area to be cleaned

Once you have chosen the proper method for disinfection or sterilization, follow these guidelines to ensure laboratory safety:

- Frequently disinfect all floors, cabinet tops, and equipment where biohazardous materials are used.
- Use autoclavable or disposable materials whenever possible. Keep reusable and disposable items separate.
- Minimize the amount of materials and equipment present when working with infectious agents.
- Sterilize or properly store all biohazardous materials at the end of each day.
- Remember that some materials may interfere with chemical disinfectants---use higher concentrations or longer contact
Sterilization Methods

There are three common methods for sterilizing laboratory materials: wet heat, dry heat, and ethylene oxide gas.

**WET HEAT**

When used properly, the damp steam heat from an autoclave effectively sterilizes biohazardous waste. Sterilization occurs when contaminated materials reach 15 psi pressure at 250°F or 121°C for at least 30 minutes.

*IMPORTANT:*

*For the autoclave process to be effective, sufficient temperature, time, and direct steam contact are essential.*

Every TSU department that autoclaves biohazardous waste should have written documentation to ensure the waste is sterile. Parameters for sterilization and standard operation procedures should include requirements for verifying sterilization.

Potential problems with wet heat sterilization and autoclaves include the following:

- Heavy or dense loads require higher temperature for sterilization.
- Poor heat conductors (e.g., plastic) take longer to sterilize.
- Containers may prevent steam from reaching the materials to be sterilized.
- Incomplete air removal from the chamber can prevent contact between the steam and the load.
- Deep trays can interfere with air removal.
- Tightly stacked loads can impede steam circulation and air removal.
- Double-bagging will impede steam penetration.
- Carcasses do not allow steam penetration.
- Some bags and containers rated as autoclavable have thermal stability but they do not allow steam penetration.
Sterilization Methods, cont.

To ensure that all materials are sterile, always test autoclave loads. Remember, however, that some sterilization indicators are incomplete. Autoclave tape, for example, verifies sufficient external temperature exposure, but it does not indicate internal equipment temperature, exposure time, or steam penetration. Thermocouples or other instrumentation can also indicate temperature, but they do not verify sterility. A biological indicator is the most effective monitor to ensure sterility. Commercially available strips or vials of *Bacillus* species endospores, for example, are suitable biological indicators.

**DRY HEAT**

Dry heat is less effective than wet heat for sterilizing biohazardous materials. Dry heat requires more time (two to four hours) and a higher temperature (320-338°F or 160-170°C) to achieve sterilization. A *Bacillus* species biological indicator can verify dry heat sterilization.

**ETHYLENE OXIDE GAS**

Ethylene oxide gas is lethal to all microorganisms. Because it is also a known carcinogen and potentially explosive (freon and carbon dioxide mixtures are stable), minimize your exposure and use extreme care when working with this gas. Ethylene oxide sterilizers and aerators must be properly vented. Ethylene oxide gas is most effective with heat-resistant organisms and heat sensitive equipment. The effectiveness of ethylene oxide gas may be affected by the following:

- **Temperature:**
  The antimicrobial activity of ethylene oxide increases with increased temperature. Normal sterilization temperature is 120-140°F or 49-60°C.
- **Ethylene Oxide Concentration:**
  Sterilization time decreases with increased gas concentration. Normal concentration is 500-1000 mg/L.
- **Humidity:**
  Relative humidity of 30-60% is necessary.
- **Exposure Time:**
  Follow the manufacturer's recommendations.
Biological Safety Cabinets:

A biological safety cabinet is a primary barrier against biohazardous or infectious agents. Although biological safety cabinets surround the immediate workspace involving an agent, they do not provide complete containment (i.e., aerosols can escape). Therefore, careful work practices are essential when working with agents that require a biological safety cabinet.

NOTE:
A biological safety cabinet is often referred to by other names such as: biohood, tissue culture hood, or biological fume hood.

All biological safety cabinets contain at least one High Efficiency Particulate Air (HEPA) filter. These cabinets operate with a laminar air flow (i.e., the air flows with uniform velocity, in one direction, along parallel flow lines.).

Biological safety cabinets must be inspected and certified:

- When newly installed
- After filter or motor replacement
- After being moved
- Annually

Contact RMS for more information about inspection.

The following sections discuss safety procedures and guidelines for working with various types of biological safety cabinets.

Using Biological Safety Cabinets

Follow these guidelines for using biological safety cabinets properly:

Preparation:

- Leave safety cabinets on at all times. Otherwise, turn the blower on and purge the air for at least five minutes before beginning work.
- Never turn off the blower of a biological safety cabinet that is vented to the outside.
- Turn off the UV light if it is on. Never work in a unit with the UV light illuminated. (UV light will damage your eyes.)
- Do not depend on the UV germicidal lamp to provide a sterile work surface; wipe down the surface with a disinfectant (70% alcohol is usually suitable).
Using Biological Safety Cabinets, cont’d

**NOTE:**
For more information on ultraviolet lights, refer to the Radiation Safety chapter.

- Place everything needed for your procedure inside the cabinet prior to beginning work. Arrange the equipment in logical order.
- Provide a container for wastes inside the cabinet. (Remember, nothing should pass through the air barrier until the entire procedure is complete.)
- Never place any items on the air-intake grilles.
- Place a disinfectant-soaked towel on the work surface to contain any splatters or spills that occur.
- Keep the laboratory door shut and post signs stating "CABINET IN USE" on all the doors. Restrict activities that will disturb the cabinet's airflow, such as entry, egress, and walking traffic.

**Cabinet Use:**
- Conduct work at least four inches from the glass view panel. The middle third area is ideal.
- Limit arm movement and avoid motions that could disturb airflow.
- If a burner is necessary, use the Touch-O-Matic type with a pilot light. Since flames cause air turbulence, place burners to the rear of the workspace.
- Never use flammable solvents in a biological safety cabinet unless it is a total-exhaust cabinet (e.g., Class II B2).

**Experiment Completion:**
- Enclose or decontaminate all equipment that has been in direct contact with the infectious agent.
- Cover all waste containers.
- To purge airborne contaminants from the work area, allow the cabinet to operate for five minutes with no activity inside the cabinet.
- Remove all equipment from the cabinet.
- Decontaminate interior work surfaces.

**IMPORTANT:**
Biological safety cabinets are not a substitute for good laboratory practices. Because aerosols can escape, take precautions to minimize aerosol production and to protect yourself from contamination.
Clean Benches

A clean bench has horizontal laminar air flow. The HEPA-filtered air flows across the work surface towards the operator, providing protection for the product, but no protection for the user. Because clean benches offer no protection, use a clean bench only to prepare sterile media. Do not use clean benches when working with pathogenic organisms, biological materials, chemicals, or radioactive materials.

Importing & Shipping Biological Materials

The Public Health Service provides Foreign Quarantine regulations for importing etiologic agents and human disease vectors. Other regulations for packaging, labeling, and shipping, are administered jointly by the Public Health Service and the Department of Transportation. The U.S. Department of Agriculture regulates the importation and shipment of animal pathogens. It prohibits the importation, possession, and use of certain animal disease agents that pose a serious threat to domestic livestock and poultry.

Biological Spill Response

The exact procedure for responding to a biological spill depends on the material, amount, and location of the spill.

In general, follow these steps immediately after a biological spill occurs:
1. Warn others.
2. Leave the room; close the door.
3. Remove contaminated garments.
4. Wash your hands.
5. Notify your supervisor.

Follow these steps to clean up a biological spill:

1. Wait for any aerosols to settle.
2. Put on protective clothing, as appropriate.
3. Apply disinfectant to the contaminated area.
4. Cover the area with paper towels to absorb the disinfectant.
5. Wipe up the towels and mop the floor.
6. Autoclave all contaminated wastes.

**NOTE:**
Spill cleanup must be appropriate for the hazards involved. Call
If a spill occurs inside a biological safety cabinet, follow these steps:

1. Decontaminate materials while the cabinet is operating to prevent contaminants from escaping.
2. Spray or wipe all affected equipment with an appropriate disinfectant. (Wear gloves while doing this.)
3. If the spill is large, flood the work surface with disinfectant and allow it to stand for 10 to 15 minutes before removing it.

**Biological Waste Disposal**

The Texas Department of State Health Services (TSHS) and the Texas Commission of Environmental Quality (TCEQ) regulate the disposal of biohazardous waste. Waste that contains infectious materials and waste that may be harmful to humans, animals, plants, or the environment is considered biohazardous. Examples of biohazardous waste include the following:

- Waste from infectious animals
- Bulk human blood or blood products
- Microbiological waste (including pathogen-contaminated disposable culture dishes, and disposable devices used to transfer, inoculate, and mix pathogenic cultures)
- Pathological waste
- Sharps
- Hazardous rDNA and genetic manipulation products

TSU's Biological Waste Disposal Program (available from RMS) stipulates that biohazardous waste meets strict safety requirements for the following:

- Segregation
- Treatment
- Labels
- Packaging
- Transportation
- Documentation

Biohazardous waste mixed with hazardous chemical or radioactive waste must be treated to eliminate the biohazard prior to disposal. After treatment, manage the hazardous waste through RMS.
IMPORTANT:
Disinfect all infectious material prior to disposal.

The following sections offer general safety guidelines and procedures for disposing of biological waste.

Segregation

Segregation is necessary when working with hazardous biological agents.

- Any waste that could cause a laceration or puncture must be disposed of as "Sharps." Sharps must be segregated from other waste.
- Do not mix waste that requires incineration with glass or plastics.
- Do not mix biological waste with chemical waste or other laboratory trash.
- Segregate hazardous biological waste from nonhazardous biological waste.

Handling & Transport

Follow these guidelines for handling and transporting biohazardous waste:

- Properly trained personnel (not the custodial staff) are responsible for transporting treated biological waste to the dumpster or incinerator. Only properly trained technical personnel may handle untreated biohazardous waste.
- Contain and label all treated waste before transporting it to the incinerator or dumpster.
- Avoid transporting untreated biohazardous materials and foul or visually offensive materials through nonlaboratory areas.
- Do not use trash/laundry chutes, compactors, or grinders to transfer or process untreated biohazardous waste.

Labeling Biohazardous Waste

Follow these guidelines for labeling biohazardous waste:

- Clearly label each container of untreated biohazardous waste and mark it with the Biohazard Symbol.
- Label containers intended for landfill disposal to indicate the method
Disposal Methods

Different materials require different disposal methods to ensure safety. Follow these guidelines for physically disposing of biological waste.

- **Animal Carcasses and Body Parts:**
  Incinerate the material or send them to a commercial rendering plant for disposal.

- **Solid Animal Waste:**
  All animal waste and bedding that is infectious or harmful to human, animals, or the environment should be treated by incineration, thermal disinfection, or chemical disinfection.

- **Liquid Waste:**
  Liquid waste, including bulk blood and blood production, cultures and stocks of etiological agents and viruses, cell culture material, and rDNA products should be disinfected by thermal or chemical treatment and then discharged into the sanitary sewer system.

- **Metal Sharps:**
  All materials that could cause cuts or punctures, must be contained, encapsulated, and disposed of in a manner that does not endanger other workers. Needles, blades, etc. are considered biohazardous even if they are sterile, capped, and in the original container.

- **Pasteur Pipers and Broken Glassware:**
  Place in a rigid, puncture resistant container. Disinfect by thermal or chemical treatment, if contaminated. Label the container as "Broken Glass" and place it in a dumpster.

**NOTE:**
*If broken glass in commingled with metal sharps, encapsulation is required for disposal.*

- **Plastic Waste:**
  Contaminated materials must be thermally or chemically treated and placed in a properly labeled, leak-proof container for disposition in
the dumpster. Materials that are not contaminated may be placed directly in the dumpster.

- **Microbiological Waste:**
  Solids must be thermally or chemically treated and placed in a properly labeled, leak-proof container for disposition in the dumpster. Liquids must be thermally or chemically treated and then discharged into the sanitary sewer system.

- **Human Pathological Waste:**
  Human cadavers and recognizable body parts must be cremated or buried. Other pathological waste from humans and primates must be incinerated.

- **Genetic Material:**
  Materials containing rDNA or genetically altered organisms must be disposed of in accordance with NIH Guidelines and the TSU Biological Waste Disposal Program.

### Nonhazardous Biological Waste

Most biological waste that is not infectious or otherwise hazardous to humans, animals, plants, or the environment may be discarded as regular waste or sewage. The only exceptions are animal carcasses and body parts. These wastes must be incinerated or sent to a commercial rendering plant for treatment. In addition, there are no record-keeping requirements for nonhazardous biological waste.

Follow these guidelines for nonhazardous biological waste:

- It is recommended to autoclave or disinfect all microbial products, even if they are not biohazardous.
- Avoid disposing of waste in a manner that could cause visual or odorous problems.
- Do not label nonhazardous biological waste as hazardous (e.g., do not use the Biohazard Symbol, red bags, etc.). Instead, it is recommended to label the container as "NONHAZARDOUS BIOLOGICAL WASTE."
- Use nonhazardous animal bedding and manure for compost or fertilizer when possible.

### Recordkeeping Requirements

Each TSU department that generates biohazardous waste must comply with the recordkeeping requirements of the TSU Biological waste Disposal Program and State regulations. Written records must
contain the following information:

- Date of treatment
- Amount of waste treated
- Method/conditions of treatment
- Name (printed) and initials of person performing the treatment

If a department generates more than 50 pounds per calendar month of biohazardous waste, the records must also include a written procedure for the operation and testing of any equipment used and a written procedure for the preparation of any chemicals used in treatment. The records must also include either the results of a biological indicator or a continuous readout (e.g., strip chart) to demonstrate proper parameters for effective treatment.

---

**Bloodborne Pathogens**

Bloodborne pathogens are biological agents that cause human disease. Examples of Bloodborne diseases include the following:

- Hepatitis
- Syphilis
- Malaria
- Human Immunodeficiency Virus (HIV)

Two significant and deadly Bloodborne diseases are hepatitis B virus (HBV) and HIV. These pathogens may be present in the following:

- Human blood
- Body fluids, such as saliva, semen, vaginal secretions, phlegm, and other body fluids visibly contaminated with blood
- Unfixed human tissues or organs other than intact skin
- HIV or HBV cultures
- Blood, organs, or other tissues from experimental animals infected with HIV or HBV.

Bloodborne pathogens may enter the body and infect you through a variety of means, including the following:

- Accidental injury with a sharp object contaminated with infectious material.
- Open cuts, nicks, and skin abrasions that come into contact
with infectious materials. Other potential sites of transmission includes acne sores and the mucous membranes of the mouth, nose, or eyes.

- Unprotected sexual activity with someone who is infected with the disease.
- Indirect transmission, such as touching a contaminated object and then transferring the pathogen to the mouth, eyes, nose, or open skin.

Currently, TSU is not covered by Federal or State regulations concerning Bloodborne pathogens. If you suspect you have been exposed to a Bloodborne pathogen, report the incident to your supervisor immediately.