Biological Safety Program

Texas A&M University-Central Texas
Office of Safety & Risk Management

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Concurrence and Approval

This Biological Safety Program document was developed for use by Texas A&M University-Central Texas and has been reviewed and approved by the following approvers.

Document Custodian:

Shawn Kelley	Original Signed	07/26/16
Safety & Risk Management Officer	Signature	Date

Approval:

Gaylene Nunn	Original Signed	07/26/16
Vice President for Finance & Admin	Signature	Date
Marc Nigliazzo	Original Signed	07/26/16
University President	Signature	Date

Change History

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Section 1. Introduction & Scope

The following information is provided to assist Texas A&M University-Central Texas (A&M-Central Texas) in establishing procedures to meet biological safety requirements to protect faculty, staff, students, and the environment.

Objectives

This program sets forth the minimum biological safety requirements to ensure the protection of faculty, staff, students, and the environment from biological hazards.

Scope

The guidelines and procedures outlined in the Biological Safety Program are applicable to all A&M-Central Texas employees and students who work with biological materials.

Principles of Biological Safety

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 a biological safety cabinets, personal protective equipment (PPE), 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 principles appropriately.

IMPORTANT: Employees who will be working with infectious agents or potentially infectious materials must complete the TrainTraq Bloodborne Pathogen Online Training, course number 2111525, and be aware of hazards associated with their work ***before*** beginning that work. These workers must be trained and proficient in biosafety procedures and techniques.

Section 2. General Biological Safety 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 and PPE Guidelines

As a general rule, take prudent steps to avoid exposure when working with biological agents, in keeping with the principle of universal precautions when dealing with potentially infectious agents. Engineering, work practice, and administrative controls are the BEST first lines of defense to avoid harmful physical and health effects from working with biological agents and any chemicals associated with the work. If these are not feasible or do not provide sufficient protection, employees are provided personal protective equipment (PPE) necessary, as appropriate, to protect:

- Hands
- Body
- Feet and legs
- Eyes and face
- Respiratory tract
- Hearing

To provide a basic level of biological and, as necessary, chemical safety in laboratory work, certain minimum PPE is recommended. Consult additional resources for higher-hazard work (e.g., respiratory protection when working with infectious materials that may aerosolize). PPE selection guides cover the categories of protection:

Standard Attire for a Biological Laboratory

Always wear appropriate clothing and PPE as standard attire in a biological laboratory.

- Long pants, sleeved shirts, closed-toed shoes of non-mesh materials, and no loose hair or jewelry
- Lab coat
    - A standard long-sleeved laboratory coat is required when working with microorganisms.
    - A wrap-around gown or scrub suit, gloves and a surgical mask must be used when working with infected animals.

- Eye protection – Always wear safety glasses and never wear contact lenses around infectious agents.
- Gloves (disposable latex or nitrile when handling low hazard materials).

Clothing and PPE Work Practices

Do not wear potentially contaminated clothing outside of the laboratory area. To remove contaminated clothing, follow these steps:

- Remove booties from the back.
- Remove head covering from the peak.
- Untie gown while wearing gloves.
- Remove gloves by peeling them from the inside out.
- Remove the gown by slipping your finger under the sleeve cuff of the gown.

Handling Guidelines for Biological Work

- 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 and needles – Avoid using syringes and needles whenever possible. If a syringe is necessary, minimize your chances of exposure by following these guidelines.
    - Use a safety-engineered device (e.g., needle-locking or disposable needle unit).
    - Take care not to stick yourself with a used needle.
    - Place used syringes into a pan of disinfectant without removing the needles.
    - Do not recap used needles.
    - Handle and dispose all needles as if they are contaminated sharps. Dispose needles in an approved sharp container.

Work Area Management

- 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, handbags, etc.) away from contaminated areas; utilize the student lockers in the hallway.
- Completely decontaminate equipment before having maintenance or repair work done.

Section 3. 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.

General Guidelines for 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.

Disinfection

The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are:

- The nature and number of contaminating microorganisms (especially the presence of bacterial spores).
- The amount of organic matter present (e.g., soil, feces, and blood).
- The type and condition of instruments, devices, and materials to be disinfected.
- The temperature.

Use Table 1 to aid in the selection of disinfectants.

Table 1. Disinfectant selection guide

Disinfectant	Uses
EPA-registered Disinfectants	Sterilants and disinfectants are essentially pesticides and are governed by the U.S. EPA. The agency maintains lists of registered antimicrobial products that are effective against common pathogens. EPA's lists are found at: https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants For broad-spectrum disinfection, consult List B: EPA's Registered Tuberculocide Products.
Alcohols	Ethyl or isopropyl alcohol at 70-80% concentration is a good general purpose surface disinfectant; not effective against bacterial spores.
Phenols	Effective against vegetative bacteria, fungi, and viruses containing lipids, unpleasant odor.
Formaldehyde	Concentration of 5-8% formalin is a good disinfectant against vegetative bacteria, spores, and viruses; known carcinogen; irritating odor.
Quaternary Ammonium Compounds	Cationic detergents are strongly surface active; extremely effective against lipoviruses; ineffective against bacterial spores; may be neutralized by anionic detergents (i.e., soaps). Works best in pH >7.
Chlorine	Low concentrations (50-500 ppm) are active against vegetative bacteria and most viruses, higher concentrations (2,500 ppm) are required for bacterial spores, corrosive to metal surfaces, must be prepared fresh, laundry bleach (5.25% chlorine) may be diluted and used as a disinfectant.

Iodine	Recommended for general use, effective against vegetative bacteria and viruses, less effective against bacterial spores, Wescodyne diluted 1 to 10 is a popular disinfectant for washing hands.

Sterilization

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

Wet Heat

When used properly, the steam heat from an autoclave effectively sterilizes contaminated materials and biohazardous waste. Sterilization occurs when contaminated materials reach 15 psi pressure at 121°C (250°F) for at least 30 minutes. Additional sterilization should be added for large loads (over 10 lbs.).

For the autoclave process to be effective, sufficient temperature, time, and direct steam contact are essential. A&M-Central Texas has two autoclaves that are located in the Biology Preparation room number 409 in Warrior Hall. All biohazardous waste should have written documentation in the autoclave log to ensure the waste has been sterilized. Parameters for sterilization and standard operation procedures should be included in the log for verifying sterilization. See APPENDIX C for an example Autoclave Usage Log.

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.

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 means to monitor and ensure sterility. Commercially available strips or vials of *Bacillus* species endospores, for example, are suitable biological indicators. Autoclaves should be tested once a month using biological indicators to ensure that they are functioning properly. A log of the monthly test results must be kept. See APPENDIX D for an example of an Autoclave Testing Log.

Dry Heat

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 is lethal to all microorganisms however, 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.

General Disinfection Practices

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

- Frequently disinfect all floors, counter 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 used 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 time.
- Use indicators with autoclave loads to ensure sterilization.
- Clearly mark all containers for biological materials (e.g., BIOHAZARDOUS - TO BE AUTOCLAVED).

Section 4. Biological Safety Cabinets

A biological safety cabinet (BSC) — also called a biosafety cabinet, microbiological safety cabinet, biohood, tissue culture hood, or biological fume hood — is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens requiring a defined biosafety level.

Several different types of BSC exist, differentiated by the degree of biocontainment required (Class I, Class II A & B, or Class III). A BSC 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.

All BSCs above Class I contain at least one High Efficiency Particulate Air (HEPA) filter. Class II and above cabinets operate with a laminar air flow (i.e., the air flows with uniform velocity, in one direction, along parallel flow lines.).

BSC Certification

Biological safety cabinets must be inspected and certified:

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

Before working with a BSC, ensure that it has been inspected and maintained within the last year, that its components are working properly, and that the planned work is appropriate for the given BSC class.

Biological Safety Cabinet Operations

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).
- 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.

- Keep the laboratory door shut. 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.

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.

Section 5. Importing and 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.

If you plan on transporting or shipping biological materials, consult Safety & Risk Management for guidance on applicable methods, restrictions and required training/certification (if any).

Section 6. 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:

- Warn others.
- Leave the room, close the door.
- Remove contaminated garments.
- Wash your hands.
- Notify your supervisor.

Follow these steps to clean up a biological spill:

- Wait for any aerosols to settle.
- Put on appropriate PPE.
- Apply disinfectant to the contaminated area and allow sufficient time for treatment (see disinfectant instructions for required contact time).
- Cover the area with paper towels to absorb the biological material and disinfectant.
- Wipe up the towels and mop the floor.
- Treat (autoclave) contaminated, potentially infectious wastes and or properly dispose of untreated wastes as biohazardous, as required.

Spill cleanup must be appropriate for the hazards involved. Call the Office of Safety & Risk Management for assistance.

If a spill occurs inside a biological safety cabinet, follow these steps.

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

Section 7. Biological Waste Treatment and Disposal

Biohazardous Waste

The Texas Department of State Health Services (DSHS) and the Texas Commission on Environmental Quality (TCEQ) regulate the treatment and disposal of biohazardous waste.

- DSHS uses the term "Special waste from health care-related facilities" (SWFHCRF) and defines it as "a solid waste" which if improperly treated or handled may serve to transmit an infectious disease(s) and which is comprised of the following: (A) animal waste; (B) bulk blood, bulk human blood products, and bulk human body fluids; (C) microbiological waste; (D) pathological waste; and (E) sharps" (25 TAC §1.132(44)). The DSHS definition of a "health care-related facility" is found at 25 TAC §1.134 Application and is very broad, including (*but not limited to*) locales not necessarily connotative of health care, for example educational institution research laboratories, pharmacies, tattoo studios, veterinary clinical, and research laboratories.

  DSHS maintains the regulations governing approved methods for treatment and disposition for these wastes at 25 TAC Chapter 1 Subchapter K. On-site treatment in an autoclave is termed "moist heat disinfection" in 25 TAC §1.136 Approved Methods of Treatment and Disposition.

- TCEQ relies on DSHS regulations, and then expands coverage to include newly revised (May 26, 2016) rules to complete the cycle of handling, shipping and disposal. TCEQ, however, uses the term *medical waste* for such wastes (30 TAC §326.3(23)). TCEQ's rules are found at 30 TAC Chapter 326 Medical Waste Management.

  - A&M-Central Texas is by definition a small quantity generator (SQG) because it produces 50 pounds or less per month of medical waste (30 TAC §326.3(14)(A)).
  - Medical waste may be stored on-site without a permit, registration, notification, or other authorization provided that it is stored in a secure manner and location that does not create a nuisance and that affords protection from theft, vandalism, inadvertent human or animal exposure, rain, water, and wind (30 TAC §326.31(a)).
  - Biohazardous waste (i.e., SWFHCRF or Medical Waste) must be identified and segregated from ordinary trash. It must be conspicuously marked with the international symbol for biohazardous material and the words "CAUTION, contains medical waste which may be biohazardous" and "PRECAUCIÓN, contiene desechos medicos que pueden ser peligro biológico" (30 TAC §326 Subchapter B).

    

  - Prior to shipping, it must also be labeled with the name and address of the generator and the date of shipment (30 TAC §326.21(b)).
  - Prior to shipping, the generator must place the container which contains medical waste in an outer container that is rigid, leak resistant, impervious to moisture, of sufficient strength to prevent tearing and bursting under normal conditions of use and handling, and sealed to prevent leakage. Place sharps in a rigid, marked, and puncture-resistant container designed for sharps (30 TAC §326.19).

- Since A&M-Central Texas will not be transporting their own untreated waste, medical waste may only be released to TCEQ-registered transporters and treatment / disposal facilities (30 TAC §326.23(a)).
- The generator, A&M-Central Texas, shall obtain from the transporter a signed manifest for each shipment of untreated medical waste. The generator shall maintain accurate and complete manifests regarding all shipments of untreated medical waste for a period of three years following the date of each shipment (30 TAC §326.23(c) and (d)).

On-Site Treatment of Biohazardous Waste (SWFHCRF/Medical Waste)

If a dedicated autoclave is used solely (***only***) for the treatment of SWFHCRF/Medical Waste, the waste generator is required to provide one-time, written notification to TCEQ (30 TAC §326.39(a)). A mixed-use autoclave does not trigger this notification.

SWFHCRF or Medical Waste that has been ***treated*** (e.g., autoclaved) in accordance with DSHS approved methods may be managed as routine municipal solid waste provided that it is accompanied by a written statement to the solid waste landfill that the shipment has been treated by an approved method in accordance with 25 TAC §1.136 (relating to Approved Methods of Treatment and Disposition) (30 TAC §326.23(e)). Use the procedure in the following inset box.

Required Records

Maintain on-site a written record that contains the following information:

- Contact information for the generator.
- The method/conditions of treatment.
- The name (printed) and initials of the person(s) performing treatment.
- The dates of treatment.
- The amounts of waste treated.

Handling & Disposal of Biohazardous Waste by Autoclaving

- Collect solid biohazardous waste in a red or orange biohazard autoclave bag for autoclaving.
- Label the waste bag or container with the name of the principal investigator (PI)/generator, building number, and lab room number and date.
- After autoclaving, affix a preprinted sticker (shown below) over the biohazard symbol on the bag and place the biohazard bag inside a second black trash bag.
- Dispose of autoclaved waste in the appropriate dumpster.
- Do not leave it on the curb or street or, place it in container labeled for composting or other waste.
- NEVER dispose of anything sharp inside of the biohazard trash bag. This would include serological pipets and pipet tips, as they can poke through the bag and cause injury to personnel.

If you have any questions about proper biohazardous waste disposal, please contact _____.

Treated in Accordance with

Texas Administrative Code

25 TAC §1.136

(Texas Dept. of State Health Services-Approved Methods of Treatment for Special Waste from Health Care-Related Facilities)

Biosafety Program

Texas A&M University-Central Texas

254-519-5771 or

safetyandriskmanagement@tamuct.edu

Printed on Avery® 3 1/3" x 4"shipping label

Section 8. Bloodborne Pathogens

Bloodborne pathogens are biological agents in human or primate blood that cause human disease. Examples of blood borne diseases include: Hepatitis, Syphilis, Malaria and Human Immunodeficiency Virus (HIV). Two significant and deadly blood borne diseases are hepatitis B virus (HBV) and HIV. These pathogens may be present in the following materials:

- 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 include 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.

For more information, refer to the **Bloodborne Pathogen Program** or contact the Office of Safety and Risk Management.

Appendix A: CDC and NIH Biosafety Levels

Biosafety Levels

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 risk criteria: 1) Nature of work being conducted, 2) Transmissibility, 3) Infectivity, and 4) Severity of disease. Refer to Supplemental Table 1.

Biosafety Level 1 (BSL-1): Facilities that work with defined and characterized strains of viable organisms 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 (BSL-2): 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.

Biosafety Level 3 (BSL-3): 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 three agents are autoinoculation, ingestion, and inhalation. Level 3 precautions emphasize primary and secondary barriers. For primary protection, 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 (BSL-4): 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 A&M-Central Texas.

Supplemental Table 1: Summary of Laboratory Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Primary Barriers	Secondary Barriers
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices.	None Required	Laboratory bench and sink required
2	Agents associated with human disease. • Routes of transmission include: Percutaneous injury, ingestion, mucous membrane exposure.	BSL-1 practices plus: Limited access. Biohazard warning signs. "Sharps" precautions. Biosafety manual defining any needed waste decontamination or medical surveillance policies.	Primary Barriers: Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials. PPEs:	BSL-1 plus: Autoclave available.

			• Laboratory coats, gloves, and face protection as needed.	
3	Indigenous or exotic agents with the potential for aerosol transmission. • Disease may have serious or lethal consequences.	BSL-2 practices plus: Controlled access. Decontamination of all waste. Decontamination of laboratory clothing before laundering. • Baseline serum collected and stored.	Primary Barriers: Class I or II BSCs or other physical containment devices used for all open manipulation of agents. PPEs: Protective laboratory clothing; gloves, and respiratory protection as needed	BSL-2 plus: Physical separation from access corridors. Self-closing, double door access. Exhausted air not re-Circulated. Negative airflow into Laboratory.
4	Dangerous/exotic agents which pose high risk of life-threatening disease. • Aerosol transmitted infections have occurred, or related agents with unknown risk of transmission.	BSL-3 practices plus: Clothing change before entering. Shower upon exit. • All material decontaminated upon exit from facility.	Primary barriers: • All procedures conducted in Class III BSCs or in Class I or II BSCs in combination with full-body, air supplied, positive pressure personnel suit.	BSL-3 plus: Separate building or isolated zone. Dedicated supply/exhaust, vacuum, and decontamination systems. • Other requirements outlined in the text.

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 (ABSL) 1, 2, 3, and 4. Refer to Supplemental Table 2.

Supplemental Table 2: Summary of Laboratory Animal Biosafety Levels for Animal Experiments

ABSL	Agents	Practices	Primary Barriers	Secondary Barriers
1	Not known to cause disease in healthy adults.	Standard animal care and management practices, including appropriate medical surveillance programs.	As required for normal care of each species.	Standard animal facility: No recirculation of exhaust air Directional air flow recommended • Hand washing sink available
2	Agents associated with human disease. • Hazard: percutaneous injury, ingestion, mucous membrane exposure.	ABSL-1 practices plus: Limited access. Biohazard warning signs "Sharps" precautions. Biosafety manual. • Decontamination of all infectious wastes and all animal cages prior to washing.	ABSL-1 equipment plus primary barriers: Containment equipment appropriate for animal species. PPEs: • Laboratory coats, gloves, face and respiratory protection as needed.	ABSL-1 plus: Autoclave available Hand washing sink available Mechanical cage washer recommended
3	Indigenous or exotic agents with the potential for aerosol transmission. • Disease may have serious health effects.	ABSL-2 practices plus: Controlled access. Cages decontaminated before bedding removed. Decontamination of laboratory clothing before laundering. • Disinfectant footbath as needed.	ABSL-2 equipment plus: Containment equipment for housing animals and cage dumping activities. Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs:	ABSL-2 plus: Physical separation from access corridors. Self-closing, double door access. Sealed penetrations. Sealed windows. • Autoclave available in facility.

			Appropriate respiratory Protection.	
4	Dangerous/exotic agents which pose high risk of life-threatening disease. • Aerosol transmission, or related agents with unknown risk of transmission.	ABSL-3 practices plus: Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower upon exit. • All wastes are decontaminated before removal from facility.	ABSL-3 equipment plus: • Maximum containment equipment (i.e. Class III BSC or partial containment equipment in combination with full-body, air supplied, positive pressure personnel suit) used for all procedures and activities.	ABSL-3 facility plus: Separate building or isolated zone. Dedicated supply/exhaust, vacuum, and decontamination systems. • Other requirements outlined in the text.

Refer to Laboratory Safety for more information regarding the use of hazardous materials with laboratory research animals. A copy of the CDC/NIH criteria for laboratory and animal biosafety levels is available from the Office of Safety and Risk Management.

Appendix B: Types of Biological Safety Cabinets

Supplemental Table 3: Types of Biological Safety Cabinets (BSC)

Type of Cabinet	Operation and Use
Class I	Only exhaust air is filtered. The user and environment are protected but the experiment is not. Operator's hands and arms may be exposed to hazardous materials inside the cabinet. This cabinet may be used with low to moderate-risk biological agents.
Class II	Vertical laminar air flow with filtered supply and exhaust air. The user, product, and environment are protected.
Type A	Recirculates 70% of the air inside the cabinet. Do not use with flammable, radioactive, carcinogenic, or high-risk biological agents.
Type B1	Recirculates 30% of the air inside the cabinet and exhausts the rest to the outside. May be used with low to moderate-risk agents and small amounts of chemical carcinogens or volatiles.
Type B2	Offers total exhaust with no recirculation.
Type B3	Same as Class II Type A, but vented to the outside of the building.
Class III or Glovebox	Gas-tight and maintained under negative air pressure. Used to work with highly infectious, carcinogenic, or hazardous materials. All operations are conducted through rubber gloves attached to entry portals.

Appendix C: Example Autoclave Usage Log

AUTOCLAVE LOG SHEET

Department: ____ Room Number: ___ Machine ID: _____

Date	User	Item	Run Time (min)	Run Temp. (°C)	Comments

Appendix D: Example Autoclave Testing Log

AUTOCLAVE TESTING LOG

Department: _____ Room Number: ____ Machine ID: _____

Date	User	Bioindicator Info. (Item #, Lot #, and Expiration	Run Time (min)	Run Temp. (°C)	Indicator Results/ Comments