# Biosafety Manual

Environmental Health, Safety, and Risk Management Department

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# I. INTRODUCTION

The Stephen F. Austin State University (SFA) Biosafety Manual was established to: promote safety in research studies, prevent disease, ensure safe handling of biological agents, ensure safe disposal of biohazardous waste, and comply with applicable institutional policies and regulatory requirements. This manual will describe the roles and responsibilities, biosafety procedures, work practices and engineering controls, spill response, emergency procedures, and proper waste disposal that should be implemented to minimize exposure to infectious agents.

This Biosafety Manual provides university-wide safety guidelines for all SFA faculty, staff, students, and visitors working with biohazards (biological agents, biological toxins, and recombinant DNA). Faculty members and research personnel that work with biohazardous agents should be familiar with the policies, procedures, and applicable regulations referenced in this manual.

SFA's Recombinant DNA and/or Infectious Biohazards in Teaching and Research policy 8.9, further defines the policy aspect and responsibilities addressed in this manual, and is available in the SFA Policy Manual online at: http://www.sfasu.edu/policies/8.9-recombinant-dna-and-or-infectious-biohazards-in-teaching-and-research.pdf.

For further assistance on how to categorize, handle, store, treat, or discard of any biologically derived material, call the Environmental Health, Safety, & Risk Management (EHSRM) Department at 468-6034. This Biosafety Manual is also available on the EHSRM web site at: http://www.sfasu.edu/safety/.

# II. RESPONSIBILITIES

Biosafety in teaching and research is a collaborative effort involving the SFA Institutional Biosafety Committee (IBC), Principal Investigators, laboratory personnel, and EHSRM Safety Officers. The provost and vice president for academic affairs is ultimately responsible for compliance with the rules and procedures described in this manual. It is the role of the IBC to review, approve/reject, and provide oversight and guidance to individuals at the university, or that use property owned by the university, who seek to use or possess rDNA and/or biohazardous materials for teaching or research. Specific responsibilities are described in detail in the following sections.

## A. Laboratory Supervisors, Faculty, and Staff Responsibilities:

1. Obtain IBC approval prior to initiating or modifying any teaching or research involving rDNA and/or biohazardous materials. The IBC Research Application Form is attached as *Appendix A* to this manual and located on the Office of Research and Sponsored Program (ORSP) website: http://www.sfasu.edu/orsp/forms.html. ORSP approval is also required for externally funded research projects.

2. Immediately report any significant problems or accidents and illnesses to the EHSRM, the IBC chair, and any other university committee or official that has reviewed and approved the research activity (e.g. the Institutional Animal Care and Use Committee or the Radiation Safety Officer);
3. Comply with all applicable local, state and federal requirements;
4. Develop Standard Operating Procedures (SOPs) incorporating biosafety procedures or a biosafety manual prepared specifically for the teaching or research classroom or laboratory;
5. Ensure that all personnel and students are properly trained on the potential hazards associated with the teaching or research activities and the necessary precautions to prevent exposures;
6. Provide personal protective equipment required for work with the respective rDNA and/or biohazardous material;
7. Adopt and implement emergency plans and guidelines for accidental spills and personnel contamination;
8. Supervise the safety performance of the teaching or research staff and personnel to ensure that the required safety practices and techniques are employed;
9. Correct work errors and conditions that may result in the release of rDNA and/or biohazardous materials;
10. Inspect biological and physical containment for damage affecting its integrity; and
11. Ensure the security of rDNA and/or biohazardous materials at all times.

## B. Environmental Health, Safety, & Risk Management Responsibilities:

1. Periodically inspect (minimum of one per fiscal year) all laboratories and classrooms conducting rDNA and/or biohazardous research to ensure that proper standards are strictly followed;
2. Ensure that each laboratory has up-to-date standard operating procedure manuals;
3. Report to the IBC Chair any significant problems, violations of NIH Guidelines, and any significant accidents or illnesses;
4. Assist laboratory personnel with the development of emergency plans for handling accidental spills and personnel contamination;
5. Investigate accidents involving rDNA and/or biohazardous materials;
6. Provide information on spills and incidents to public health officials as required;
7. Offer advice on laboratory safety to all personnel who have access to the laboratory (IBC, faculty, staff, and students); and
8. Assist laboratory personnel in meeting safety training requirements.

## C. IBC Responsibilities:

1. Review and consider for approval teaching or research activities involving rDNA and other potentially biohazardous agents that are sponsored by, or conducted at the university, for compliance with NIH Guidelines. This relates to initial and annual review of approval and modifications to all proposals and activities.
2. Assess facilities, procedures, practices, training, and expertise of personnel taking part in such teaching or research;
3. Assess, modify, and finalize containment levels for teaching or research;
4. Notify the EHSRM and the Office of Research and Sponsored Programs (for externally funded research) of the IBC's review results; and

5. Review and report any significant problems with or violations of the NIH Guidelines, accidents, or illnesses to the provost and vice president for academic affairs and to the NIH OBA as required by section IV-B-1-j of the NIH Guidelines.

# III. GENERAL INFORMATION AND PROCEDURES

The following sections describe general procedures that should be followed in any laboratory using biohazardous organisms and/or toxins.

## A. Regulatory Guidance

Stephen F. Austin State University is committed to providing a safe working environment for faculty, staff, students, and visitors. To achieve this goal, this manual is intended to be fully consistent with applicable federal, state, and local regulations. The primary rules and regulations related to biological safety in teaching and research at SFA are listed in detail in *Appendix B* of this manual.

## B. Laboratory Security

Certain biohazardous microorganisms and toxins may be of interest to persons or groups involved in terrorism or other illegal activities. Therefore infectious agents that could pose a serious threat to humans, agriculture, or the livestock industry should be kept under secure conditions within the laboratory.

If a request is received from another institution or corporate entity for a dangerous organism for academic purposes, Principal Investigators (PI) are responsible for ensuring that the receiving entity is a valid research organization and that the transfer has administrative approval from both institutions. When a request is received, the Principal Investigator must notify EHSRM for approval to send the biological agent.

## C. Signage and Postings

The universally accepted biological hazard warning symbol shall be used in laboratories and work areas to notify workers about the presence of infectious agents. It is the responsibility of the PI to ensure that all necessary postings are installed and properly maintained. The warning symbol must be removed when the hazardous agent is no longer in use or present.

The biohazard symbol on the postings should be orange or red in color with a contrasting background. As a general rule, the location of the posting is determined by how access is gained to the area where biological hazards are used. In most cases, the door to any laboratory containing a designated infectious agent should be posted. In addition, postings should also be displayed in other areas such as biological safety cabinets, refrigerators, freezers, waste containers, bags/containers of contaminated laundry or other specially designated work and storage areas or equipment where biological hazards are used. All individual containers of biological hazards should also be labeled to identify the content and any special precautionary measures that should be taken.

Acceptable color-coded (red or orange) and pre-labeled bags or containers may be substituted for the labeling requirement.

## D. Basic Biological Safety Practices

The following biosafety practices shall be followed in all SFA laboratories and work activities involving biohazardous agents. Strict adherence to these basic principles will greatly reduce the likelihood of laboratory exposures, accidents, or injuries.

1. Food and Drinks

    - No food or drinks are permitted in laboratories at any time.

2. Personal Protective Equipment (PPE)

***When hazards are present or used in the laboratory:***

- The instructor and students must utilize the appropriate PPE for the experiments.
- PPE is always selected and used based on the hazards presented and risk of exposure (i.e. when biological materials are present and nearby on bench tops, biological safety cabinets, etc. or laboratory procedures are being performed).
- Safety eyewear (goggles, safety glasses) must be used to prevent injury or exposure of the eyes.
- Protective clothing (lab coats) must be worn to prevent contamination of the body and street clothes. Protective clothing must be left in the lab or locker at the end of the period.
- Appropriate chemically resistant gloves must be used for handling chemicals to prevent contamination of the hands.

***When biological demonstrations are being performed for observational purposes:***

- The instructor and audience must be equipped with the appropriate PPE to protect them from the hazards associated with the demonstration.
- Instructors must not deviate from the established procedures or adjust quantities of materials during the demonstration without prior approval.

***When hazards are not present in the laboratory*** *(i.e. when all chemicals, biological, or radiological materials are secured in closed containers (chemical cabinets, biological freezers or incubators) and bench surfaces are clean and/or decontaminated:*

- PPE is unnecessary and does not need to be utilized

3. Street Clothes
    - Street clothing and footwear appropriate for laboratory work must be worn by the instructor and students for all activities (including lectures, lab sessions, and demonstrations) because some lectures are followed by lab sessions in the same course.

- Street clothing should be chosen so as to minimize exposed skin below the neck. Long pants are required. Avoid rolled up sleeves. Shorts (including cargo shorts), capris, miniskirts, tank tops, sleeveless shirts and midriff-length shirts are inappropriate clothing in laboratories.
- Shoes must cover the entire foot. Open-toed shoes and sandals are inappropriate footwear in laboratories. Fabric and athletic shoes offer little or no protection from chemical spills. Leather shoes with slip-resistant soles are recommended.
- When PPE is utilized for laboratory sessions and demonstrations (not lectures) long hair must be restrained and jewelry/watches removed.

4. Sharps, Spills, and Equipment
    - Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives; dispose of sharps in approved sharps disposal containers as it can cause transmission through sticks and inhalation.
    - Handle infectious fluids carefully to avoid spills and the production of aerosols as it can cause transmission through inhalation, ingestion, skin and mucous membrane contact.
    - Do not use mouth pipette as it can cause transmission through inhalation, ingestion, skin and mucous membrane contact.

## E. Decontamination

Sterilization, disinfection, and antisepsis are all forms of decontamination. Sterilization implies the killing of all living organisms. Disinfection refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. Antisepsis is the application of a liquid antimicrobial chemical to living tissue.

1. Chemical Disinfectants

*Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:*

- Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
- Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.
- Hospital Disinfectant - agent shown to be effective against S. aureus, S. choleresis (Staph Infection) and P. aeruginosa (Germs and Bacteria). It may be effective against M. tuberculosis (tuberculosis), pathogenic fungi or specifically named viruses.
- Antiseptic - agent formulated to be used on skin or tissue - not a disinfectant.

2. Disinfectants Commonly Used in the Laboratory

***Iodophors***

- Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- Effective against vegetative bacteria, fungi, and viruses.
- Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- Stable in storage if kept cool and tightly covered.
- Built-in color indicator; if solution is brown or yellow, it is still active.
- Relatively harmless to humans.

***Hypochlorites (bleach)***

- Working dilution is 1:10 to 1:100 household bleach in water.
- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions should be made daily.

***Alcohols (ethanol, isopropanol)***

- The effective dilution is 70-85%.
- Effective against a broad spectrum of bacteria and many viruses.
- Fast acting.
- Leaves no residue.
- Non-corrosive.
- Not effective against bacterial spores.

3. Important Characteristics of Disinfectants

| | Hypochorites (Bleach) | Iodoform (Wescodyne) | Ethyl Alcohol |
|---|---|---|---|
| Shelf-life > 1 week | | X | X |
| Corrosive | X | X | |
| Residue | X | X | |
| Inactivation by Organic Matter | X | X | |
| Skin Irritant | X | X | |
| Respiratory Irritant | X | | |
| Eye Irritant | X | X | X |
| Toxic | x | X | X |

4. Dilution of Disinfectants

***Chlorine Compounds (Household Bleach)***

| Dilution in Water | % Available Chlorine | Available Chlorine (mg/l or ppm) |
|---|---|---|
| Not diluted | 5.25 | 50,000 |
| 1/10 | 0.5 | 5,000 |
| 1/100 | 0.05 | 500 |

- Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 1%.

***Iodophor***

- Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces into 5 gallons water, or approximately 9 ml/liter.

***Alcohols***

- Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant.

## 6. Biosafety Levels

### 1. Laboratory Biosafety Levels

The CDC-NIH has established four biosafety levels that describe practices and techniques, safety equipment, and facility design features recommended for work with specific infectious organisms. Descriptions of the biosafety levels, as well as assigned biosafety levels for specific organisms, are contained in the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (BMBL), Section IV.

***Notice: There are currently no SFA facilities licensed to contain any biohazardous agents or organisms above Biosafety Level 2 (BSL 2).***

The following table describes the four different biosafety levels and the appropriate agents, practices, safety equipment, and facilities for each.

**As outlined in the BMBL, the four biosafety levels are summarized below:**

| Biosafety Level | Agents | Practices | Safety Equip. | Facilities |
|---|---|---|---|---|
| 1 | Not known to cause disease in healthy adults. | Standard Microbiological Practices | None required | Open bench top, sink required |
| 2 | Associated with human disease, hazard: autoinoculation, ingestion, mucous membrane exposure | BSL-1 practice plus: - Limited access - Biohazard warning signs - Sharps precautions - Biosafety manual | Primary barriers: Class I or II BSCs or other containment used for manipulations of agents that cause splashes or aerosols of infectious materials; PPE: lab coats; gloves; eye/face protection as needed | BSL-1 plus: Autoclave available |
| 3 *(Currently Prohibited at SFA)* | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences | BSL-2 practice plus: - Controlled access - Decontamination of all waste - Decontamination of lab clothing before laundering - Baseline serum | Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing; gloves; respiratory protection as needed | BSL-2 plus: - Physical separation from access corridors - Self-closing, double door access - Exhausted air not recirculated - Negative airflow into laboratory |
| 4 *(Currently Prohibited at SFA)* | Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agent with unknown risk of transmission | BSL-3 practices plus: - Clothing change before entering - Shower on exit - All material decontaminated on exit from facility | Primary barriers: All procedures conducted in Class III BSCs or Class I or Class II BSCs in combination with full-body, airsupplied, positive pressure personnel suit | BSL-3 plus: - Separate building or isolated zone - Dedicated supply/exhaust, vacuum, and decon systems - Other requirements outlined in BMBL |

### 2. Animal Biosafety Levels

A similar set of four biosafety levels are provided for work with vertebrate animals infected with agents which may infect humans. The Animal Biosafety Levels (ABSL), 1 thru 4,

include practices, equipment, and facilities that are comparable to the laboratory biosafety levels described below. However, there are unique hazards associated with infected animals that must be understood by those personnel with animal contact and addressed in the animal facility. Animal activity can create aerosols and bites and scratches can occur. A good summary of the Animal Biosafety Levels can be found in the CDC-NIH publication, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, 2009, http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf.

## 7. Training

All IBC members, PI's, and laboratory staff members conducting activities involving microorganisms or biotoxins, are required to receive training in biosafety and be familiar with the contents of this manual, regardless of the level of activity they propose to use (BSL1 or BSL2). The laboratory PI is responsible for the development and administering of site-specific biological safety training to their laboratory staff members and students. All training records should be maintained by the PI and a copy forwarded to the EHSRM Biological Safety Officer.

This training shall address biosafety and laboratory safety relative to the activities on-going in the laboratory. Training should include but not be limited to: procedures and techniques, laboratory safety rules, emergency response, spill containment and cleanup, and instructions on the operating procedures for use of laboratory equipment (chemical fume hood, biosafety cabinets, autoclaves, centrifuge, etc.). PIs must provide training to lab personnel regarding lab specific SOP's.

Training must be given to all new laboratory employees and/or students upon initial assignment with refresher training annually. Records are to be kept for a minimum of five (5) years. The EHSRM Biological Safety Officer can assist the PI in meeting training requirements upon request. PI's will also ensure that everyone involved in their laboratory operations also participate in other training requirements relative to health and safety as administered by EHSRM. Examples of this training would include Bloodborne Pathogens, Hazards Communication, and Chemical Safety.

## 8. Importing and Shipping Biological Materials

There are numerous regulations that apply to the shipping and transport of biohazardous 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.

Contact the EHSRM Biological Safety Officer for assistance with proper packaging, labeling, and shipping of biological materials.

# IV. ENGINEERING AND WORK PRACTICE CONTROLS

## A. Containment

The primary principle of biological safety 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 hazardous agents. There are two levels of biological containment: primary and secondary.

In addition, the three key elements of biological containment include laboratory practices and techniques, 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 techniques described in this manual appropriately.

### 1. Laboratory Practices and Techniques

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and trained to be proficient in the practices and techniques required for handling such materials safely.

### 2. Primary Containment (Safety Equipment)

Primary barriers protect 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:

- Biosafety cabinets,
- Enclosed containers,
- Safety centrifuge cups, and
- Personal protective equipment (PPE) such as safety glasses or goggles, lab coats, and gloves.

### 3. Secondary Containment (Facility Design)

Secondary barriers protect the environment outside of the laboratory from exposure to infectious agents.

Examples of secondary barriers include:

- Work areas that are separate from public areas,
- Decontamination facilities,
- Handwashing facilities, and
- Special ventilation systems.

## B. Biosafety Work Practice Controls

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

### 1. 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 materials; and
- Never eat, drink, or apply cosmetics in the work area.

**2. 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:
  - Remove shoes from the back.
  - Remove head covering from the top.
  - 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.

**3. 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.

**4. 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.
- Be careful not to stick yourself with a used needle.
- Place used syringes into a pan of disinfectant or approved sharps container.
- Do not place used syringes in pans containing pipets or other glassware that require sorting.
- Do not recap used needles.
- Dispose of needles in an approved sharps container.

**5. 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.
- Decontaminate all work surfaces daily and after each spill.

- Keep miscellaneous material (i.e., books, journals, backpacks, etc.) away from contaminated areas.
- Completely decontaminate equipment before having maintenance or repair work done.

## C. 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.

**1. General Guidelines for disinfection and sterilization:**

The proper method of disinfection and sterilization depends on the following:

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

Materials and equipment small enough to fit in an autoclave may be sterilized, while larger equipment such as laboratory benchtops must be disinfected. 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 autoclave compatible 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 time.
- Use indicators with autoclave loads to ensure sterilization.
- Clearly mark all containers for biological materials (e.g., BIOHAZARDOUS - TO BE AUTOCLAVED.).
- Stock solutions of disinfectants should be maintained at each bench top and biological safety cabinet work area such as:
  - Bleach, 10% sodium hypochlorite;
  - Ethanol, 70% solution; or
  - Other appropriate disinfectant.
- Vacuum lines should be protected by a disinfectant trap (aspirator suction flask containing bleach) and a HEPA filter between the vacuum port and the aspiration flask to prevent pathogens from entering the vacuum system.
- All infectious materials, contaminated plasticware/glassware, and contaminated waste should be disinfected prior to washing or disposal.
- Contaminated materials are to be placed in sealed biohazard bags with red or orange lettering indicating biohazard for disposal or appropriately labeled autoclave bags.
- Work surfaces should be decontaminated after each use.

2. **Disinfection Methods:**
   Sterilization is not practical for tables, cabinets, and some equipment, so disinfection must be utilized. The term disinfection implies the use of antimicrobial chemicals on inanimate objects with the purpose of destroying all non-spore forming organisms of pathogenic nature or which would compromise the integrity of the experiment. Note that disinfection does not mean the destruction of all organisms. Disinfectants destroy microorganisms by coagulating or denaturing proteins, injuring the cell membrane, and stopping normal enzymatic reactions.

   The range of susceptibility of microorganisms to disinfectants is relatively broad. The vegetative bacteria, fungi, and lipid containing viruses are highly susceptible to inactivating agents. Non-lipid containing viruses are moderately resistant to these agents. Spore forms are the most resistant. Once the waste has been treated, it should be double bagged in 2 mL thick opaque liners and placed in designated containers located in each autoclave room. Treated waste can then be disposed of into a municipal solid waste landfill.

   Refer to the table in *Appendix C* for a list of chemical disinfectants including associated target organisms, important characteristics, and potential applications.

3. **Sterilization Methods:**
   a. **Autoclave (Steam Sterilization):**
   - When used properly, the damp steam heat from an autoclave effectively sterilizes biohazardous waste. Steam Sterilization is defined as **121C for at least 15 minutes** peak temperature. A conventional autoclave used in laboratories may take as long as 15 minutes to reach peak temperature, and may take 15 minutes or more to be safe to open. Therefore, a standard autoclave "cycle" would be **at least 45 minutes total**, under ideal conditions. Any interfering factors would necessarily increase this treatment time.
   - An autoclave must be available for all BSL2 laboratories and must only be operated by personnel who have been properly trained in its use. Improper sterilization could result in laboratory personnel or other personnel involved in disposal of laboratory waste being exposed to potentially infectious agents.
   - Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.
   - Wrap packages to allow for steam penetration; aluminum foil does not allow steam penetration, and should not be used for wrapping.
   - Do not overload the chamber.
   - Avoid overpacking of autoclave bags.
   - Do not seal bags or close bottles and other containers tightly.
   - Do not stack containers.

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

***Safe work practices when using an autoclave include the following:***

- Read the operating manual and post proper work procedures near the autoclave.
- Never autoclave hazardous chemicals.
- Dry hypochlorites, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth or oil:

**OXIDIZER + ORGANIC MATERIAL + HEAT = MAY PRODUCE AN EXPLOSION!**

- Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or simultaneous opening of both doors on a double door autoclave.
- Open the door slightly to allow escape of steam before unloading.
- Wear insulated gloves or mitts when unloading.

**Biological Monitoring for Sterilization:**

Treated waste should be monitored with the appropriate biological Bacillus species indicator to test the effectiveness of the treatment. Biological indicators can be in the form of either an ampoule or strip containing the spore *Bacillus stearothermophilus*. 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. Autoclaves should be tested at least weekly. In addition to weekly monitoring, every load should be monitored with a sterilization indicator strip that turns color when the proper temperature is achieved.

For those autoclaves in which a continuous readout of operating procedures is available, routine parameter monitoring can be substituted for biological monitoring.

**Potential problems with autoclaves include the following:**

1. Heavy or dense loads require higher temperature for sterilization.
2. Poor heat conductors (e.g., plastic) take longer to sterilize.
3. Containers may prevent steam from reaching the materials to be sterilized.
4. Deep trays can interfere with air removal.
5. Tightly stacked loads can impede steam circulation and air removal.
6. Double-bagging will impede steam penetration.
7. Carcasses do not allow steam penetration.
8. Some bags and containers rated as autoclavable have thermal stability but they do not allow steam penetration.
9. Prior to autoclaving, materials should be placed in appropriate trays in case the autoclave bag leaks.

**Records/Logs:**
Records are an essential part of a biosafety management program. Every SFA department that autoclaves biohazardous waste should have written documentation to ensure the waste is sterile. Parameters for sterilization and standard operating procedures should include requirements for verifying sterilization. All departments that treat waste are required by State of Texas regulations to keep records that include the following:

1. Date of treatment
2. Method/Conditions of treatment
3. Quantity of waste treated (pounds)
4. Verification of operating parameters or biological monitoring
5. Written procedures for the operation and testing of equipment used
6. Printed name and initials of person treating the waste

These records can be kept on the generic form included in this manual or on a similar form, as long as all the information required is referenced.

**b. 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 60–170°C) to achieve sterilization. A *Bacillus* species biological indicator can verify dry heat sterilization.

## D. Biological Safety Cabinets

A biological safety cabinet (also referred to as: bio hood, tissue culture hood, or biological fume hood) 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.

1. 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).
2. The capability of a biological safety cabinet to protect laboratory personnel and the environment from exposure to potentially hazardous aerosols is primarily dependent on proper functioning of the cabinet. No biological safety cabinet should be used to contain hazardous materials unless it has been demonstrated by appropriate test procedures to meet the minimum safety specifications given in NSF Standard 49.
3. Certification of the biosafety cabinet by a qualified certification company is required when the following occur:
   - Before a newly installed cabinet is used;
   - After a cabinet is moved, relocated or partially dismantled for cleaning or repair ;
   - After filter or motor replacement; and/or
   - Must be performed at least annually

4. Primary researchers or their departments are responsible for scheduling their annual certification prior to the one-year date on the cabinet sticker. The personnel are expected to ensure that working surfaces of cabinets are effectively decontaminated and all hazardous work is safely contained prior to any scheduled inspection or certification.
5. A certification company label indicating the date of service and other pertinent information will be affixed to each safety cabinet that conforms to minimum performance standards. If a cabinet fails to meet performance standards, person(s) using the cabinet will be promptly informed as to the nature of the problem and how it can be corrected. The cabinet must not be used until repaired.
6. High Efficiency Particulate Air (HEPA) filters should only be replaced by a bio-safety cabinet certification company or other qualified personnel.

The table on the following page lists the different types of biosafety cabinets, the types of ventilation, and possible limitations.

**Description of Biosafety Cabinets**

| 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. |

**Guidelines for Using Biological Safety Cabinets**

1. **Preparation:**
   - Leave safety cabinets on at all times. If the cabinet is off, 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. Including a container for waste. (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.

**2. 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.
- Never use flammable solvents in a biological safety cabinet unless it is a total-exhaust cabinet (e.g., Class II B2).

**3. Open Flames inside the Cabinet:**

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. Additionally, heat buildup may damage the HEPA filters. Open flames are extremely dangerous around flammable materials. Avoid using open flames when flammable materials are present in the cabinet.

**Follow these tips for avoiding fires in your biological safety cabinet:**

- Use disposable pre-sterilized loops and spreaders.
- Replace Bunsen burners with alternative technology such as electric loop sterilizers.
- Use only Bunsen burners without pilot flame, excess-temperature protection, flame monitor, and regulated timer.
- Ensure that the gas supply is clearly labeled inside the cabinet. Inspect your gas lines inside the cabinet before use for kinks, tears, holes, and loose connections and replace worn/damaged lines.
- Stabilize the alcohol container so that it cannot be tipped over.
- Reduce the amount of flammable chemicals, equipment and supplies in the cabinet. Use only enough alcohol for one day’s work.
- Have a “snuffing” lid available in case the alcohol in the container catches fire. ***Avoid using water for putting out fires in biological safety cabinets.***
- If you smell gas, turn off the exterior gas valve and wait until the gas has fully dissipated before lighting any flames. Remember that a biological safety cabinet recirculates air and vapors may build up inside the cabinet.

**4. Experiment Completion:**

- Enclose or decontaminate all equipment that has been in direct contact with the infectious agent and 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 and 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 you from contamination.*

## E. Miscellaneous Equipment

Various other types of laboratory equipment commonly found in biological laboratories are described in the following sections. It is important to maintain laboratory equipment in good working order and follow the safety guidelines described below to help prevent accidents and injuries.

**1. Centrifuges – Proper use:**

Improperly used or maintained centrifuges can present significant hazards to users. Failed mechanical parts can result in release of flying objects, hazardous chemicals and biohazardous aerosols. The high-speed spins generated by centrifuges can create large amounts of aerosol if a spill, leak, or tube breakage occurs.

Cover all potentially contaminated material spun in a tabletop centrifuge with parafilm so that leakage from an improper seal will not spread into the centrifuge container. Ultracentrifuge rotors cannot be sealed in this manner, but should be constantly monitored for leaks. All opening of centrifuges must be performed slowly.

***To avoid contaminating your centrifuge:***

- Check glass and plastic centrifuge tubes for stress lines; hairline cracks and chipped rims before use. Use unbreakable tubes whenever possible.
- Avoid filling tubes to the rim.
- Follow manufactures recommendations for tube and rotor maximum safe operation speeds.
- Aerosol-free (sealed) centrifuge buckets or rotors are required for all centrifuging of infectious specimens and bacteria. Only the correct size tubes should be used in any centrifuge bucket.
- Buckets should be kept clean and free of broken glass and plastic.
- Use caps or stoppers on centrifuge tubes. Avoid using lightweight materials such as aluminum foil as caps.
- Once samples to be centrifuged are prepared, load tubes into buckets inside the biological safety cabinet and seal carefully before moving to centrifuge.
- After centrifugation, buckets should be opened in a biological safety cabinet to prevent exposure from aerosolized particles. Always visually inspect rotor for signs of tube leakage prior to opening buckets.
- Decontaminate the outside of the cups or buckets before and after centrifugation.
- Inspect O-rings regularly and replace if cracked or dry.

- Ensure that the centrifuge is properly balanced.

*When using **high-speed or ultra-centrifuges**, additional practices should include:*

- Connect the vacuum pump exhaust to a disinfectant trap.
- Record each run in a log book: keep a record of speed and run time for each rotor.
- Install a HEPA filter between the centrifuge and the vacuum pump.
- Never exceed the specified speed limitations of the rotor.
- Regularly inspect the rotor for contamination, corrosion, or cracks.

**2. Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers:**

The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should only be used in a biological safety cabinet when working with biohazardous materials.

**3. Microscopes:**

Tighten caps on flasks of infectious culture before transporting to the microscope. Infectious cultures in plates or other containers without tight fitting lids must be carried to the microscope in a tray. When using the hemocytometer to count cells, enclose the hemocytometer in a 70% ethanol-disinfected petri dish for transport to the microscope. Disinfect the viewing platform of the microscope after each use.

**4. Microtomes:**

Microtome blades are extremely sharp and must be handled with great care and stored safely when not in use. If the knife projects beyond the sectioning area, a suitable guard must be fitted. Handling and changing of microtome blades causes many (often serious) injuries, and great care must be exercised when performing these operations. Always carry the knife, in its case, to the microtome. Never leave the knife on a microtome. After use, always return the knife to its case. Disinfect the microtome by wiping with bleach or sodium hydroxide solution. Slide the "back" on to the knife before removing it.

**5. Water Baths:**

Pathogenic or nonpathogenic agents may contaminate water baths. It is recommended that either 1 oz. of bleach or 1 oz. of phenolic detergent be added to each gallon of water used in a bath. Phenolic disinfectants are preferred over bleach, but phenolic must be replenished regularly. Propylene glycol has been used effectively as an alternative in cold-water baths. Raise the temperature to 90ºC or higher for 30 minutes once a week for decontamination purposes. Avoid using sodium azide to prevent growth of microorganisms (sodium azide forms explosive compounds with some metals). Thimerosal should also be avoided as a bacteriostat or fungistat as it contains mercury. All forms of mercury are poisonous if absorbed. Treated water must be disposed of as hazardous waste. To prevent electrical shocks, unplug the unit before filling or emptying and have the continuity-to-ground checked on a regular basis.

### 6. Loop Sterilizers and Bunsen Burners:

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols, which may contain viable microorganisms. To eliminate the spattering and aerosolization associated with flaming of loops, char the material before fully inserting the loop into the flame: i.e., before flaming, hold the loop close to (but not into) the flame. In addition, the use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators are not available.

## F. Other Physical and Chemical Hazards

### 1. Liquid Nitrogen

Liquid nitrogen is frequently used in chemical research laboratories for the purpose of cooling. There are two major risks associated with the use and handling of liquid nitrogen which present potential hazards:

- Liquid nitrogen is extremely cold. The eyes can be damaged by exposure to this gas even when the contact is too brief to affect the skin.
- Liquid nitrogen produces a large amount of nitrogen gas.

**Do not allow objects cooled by liquid nitrogen to touch your bare skin.**
Contact with the skin may cause serious frostbite. Because it is extremely cold, it can freeze human flesh almost instantaneously. Use forceps or tongs to remove straws or canes from the storage container.

**Protective clothing can reduce the hazards of handling liquid nitrogen.**
Insulated or heavy leather gloves should always be worn when handling any object that has been in contact with liquid nitrogen. Loose fitting gloves are recommended so that they may be discarded quickly in the event that any liquid nitrogen splashes into them.

**Special containers are required.**
Cryobiological storage containers are specifically designed and constructed to withstand the extreme temperature variances involved in handling liquid nitrogen. These special containers should be filled slowly to avoid the expansion stress that occurs as a result of the rapid cooling. Too much stress can damage the container.

**Do not seal the containers.**
Cryobiological storage containers are designed to function with little or no internal pressure. The use of any tight-fitting stopper or plug that prevents the adequate venting of gas builds up pressure that could severely damage or even burst the container. Even icing or accumulated frost can interfere with proper venting and containers should be checked for such obstructions.

**Transfer liquid nitrogen with care.**
The primary hazards of transferring liquid nitrogen from one container to another are spilling and splashing. Special funnels (with the top partially covered) will reduce splashing. For cryobiological storage containers a self-pressurizing discharge device is available that allows controlled liquid nitrogen withdrawal up to two liters per minute. Always follow the instructions on containers or accessories carefully when transferring liquid nitrogen. NEVER overfill the containers. Filling above the specified level is likely to produce spillage when the necktube core is replaced.

**Use solid metal or wooden dipsticks.**
Because of the extremely low temperature of liquid nitrogen, plastic measuring devices tend to become very brittle or even shatter. NEVER use hollow rods or tubes; the gasification and expansion of the rapidly cooling liquid inside the tube will force liquid to spurt from the top of the tube. Always wear insulated or heavy gloves when measuring.

**Nitrogen gas is colorless, odorless, tasteless ... and deadly!**
Nitrogen gas reduces the concentration of oxygen and can cause suffocation. Since it cannot be detected by sight, taste or smell, it may be inhaled as if it were air. That is why liquid nitrogen must always be stored and used ONLY in areas that are fully ventilated. As liquid nitrogen evaporates, the resulting nitrogen gas displaces the normal air-and breathing air that is less than 18% oxygen may cause dizziness, unconsciousness and even death.

**Handle containers with care.**
Containers should always be stored in an upright position. Tipping the container or letting it lie on its side can result in spillage and may damage the container or the materials stored in it. Walking or dragging containers could result in a partial or complete vacuum loss. For containers that cannot be easily and safely carried, a roller base can provide safe and easy movement of containers.

**2. Ultraviolet light**
Biosafety cabinet UV lights have limitations that all researchers should know about before relying on them for protection against contamination. You should not rely on a biosafety cabinet UV light as the sole decontaminating agent. Surface disinfection should be performed before and after every cabinet use. UV light can only destroy microorganisms that it can reach, and whether a microorganism is exposed to UV light depends on several factors. For example, some biosafety cabinets are made with welds or seams where microorganisms can hide from UV light. Even the flat surfaces inside a biosafety cabinet are not perfectly flat at the microscopic level. This unevenness creates “microshadows” where microorganisms can stay safely tucked away. Additionally, microorganisms can hide behind dust or other particulates. And finally, any dust or dirt that has accumulated on the bulb itself will block UV light, and the bulb should be cleaned regularly.

The UV bulb of a biosafety cabinet light loses effectiveness over time and needs to be replaced regularly. When the intensity drops below a certain level, the percentage of microorganisms it's able to destroy drops, leaving research products and personnel vulnerable to contamination. It's not always easy to tell when it's time to change the bulb. The light stays on long after its germicidal effectiveness has ceased. Manufacturers have developed lamp life ratings for their products, which vary depending on the type of UV light. Following these ratings will help you determine when it's time to change the bulb.
UV light not only kills microorganisms in the biosafety cabinet, but it also destroys living cells in your body. In particular, it can cause painful damage to both your skin and your eyes, even after its intensity drops below the effective level. Additionally, the surface inside a biosafety cabinet can reflect UV light into the lab, putting personnel at risk.

Visualization of DNA often involves the use of UV light. Ethidium bromide fluoresces under UV light. The lamp used to generate the UV light is usually a mercury arc lamp. The primary emission of the light is 354 nm. There are other emissions from the visible range (400 nm+) down to and below 254 nm. The most hazardous region for human skin is 270-310 nm. Some mercury arc lamps put out a significant portion (20-30%) of their power in this range. To reduce exposure employees should not use the lamp facing up. While using the lamps, wear a protective face shield and cover exposed skin. The effects of UV are erythema (red skin), photokeratitis (small lacerations of the cornea, or "welder's flash) and skin cancer. When using a UV microscope personnel must wear protective goggles or glasses. In addition, anyone else in the room during UV use should also wear similar protective equipment.

**3. Electrical Hazards**

Electrical hazards can be present in electrophoresis because electricity is fundamental to the process. New electrophoresis machines come with UL and CE designations. These pieces of equipment have passed stringent tests for electric shock protection. If the electrophoresis machines do not have these approvals, the operator must ensure that no exposed live wires or contact are exposed. A ground fault interrupter (GFI) can be added to automatically shut off the electricity in the event of an electrical fault. The voltages used for electrophoresis are sufficient to cause electrocution. Cover the buffer reservoirs during electrophoresis. Always turn off the power supply and unplug the leads before removing a gel.

**4. Ethidium Bromide**

Ethidium bromide is a moderately toxic chemical. It has been shown to be mutagenic. It is suggested that ethidium bromide be treated as a carcinogen. No skin contact is permitted. When working with ethidium bromide, try to minimize the potential for spills. Purchase ready-made stock solutions in lieu of mixing your own when possible. If you prefer to mix your own solutions of ethidium bromide, protect yourself by doing this process in a fume hood. Perform all processes that generate ethidium bromide dusts or mists inside the fume hood to minimize inhalation exposures. Prevent accidents by transporting small quantities of ethidium bromide in a secondary container instead of carrying large quantities.

**5. Acrylamide**

Acrylamide is a common research laboratory chemical. Widely used as a cross linking agent for electrophoresis separation procedures, acrylamide is a basic requirement for various biochemical techniques. This familiarity may cause some lab personnel to overlook the hazardous nature of this toxic substance. Acrylamide is a powerful central and peripheral nervous system toxicant. Acute (short-term) exposures to low levels of the monomer can damage nerves and cause effects such as drowsiness, lack of coordination, hallucinations, and confusion. Chronic (long-term) exposures can cause severe nerve damage and result in sensory and motor impairment marked by numbness and weakness in the hands and legs, and difficulty walking and speaking. The U.S. EPA has classified acrylamide as a probable human carcinogen.

All measuring, mixing, and handling of the acrylamide monomer should take place in a chemical fume hood while wearing latex gloves, which extend over the cuffs of the lab coat. Once the monomer has polymerized it is no longer hazardous, however, since there is never 100% polymerization, there will always be toxic monomer contamination. For this reason polymerized gels should be treated with the same caution as the monomer.

Elimination of the hazardous powder is one of the best methods to decrease the risk of acrylamide exposure in the lab. Where practical, purchase pre-mixed acrylamide solutions. These solutions have the added advantage of being specifically designed for each application and can provide a high level of purity and reproducibility.

**6. Phenol**

Phenol is a very caustic organic solvent that is used to extract protein from DNA preps. Phenol can be readily absorbed through the skin, whereupon it can affect the central nervous system and cause damage to the liver and kidneys. It is also a mutagen, and there is some evidence that phenol may be a reproductive hazard. When heated, phenol will produce flammable vapors that are highly toxic and explosive. Whenever possible, work with phenol in a chemical fume hood, especially when heating it. Never heat or melt phenol in an incubator, microwave, drying oven, or similar appliance. Prevent phenol from contacting skin by wearing neoprene gloves and a laboratory coat. Change gloves frequently. Wear chemical goggles to protect the eyes. Always wash hands thoroughly after handling phenol, even if gloves are used.

**7. Chloroform**

Chloroform is widely used in molecular biology as a solvent in organic extraction. It has been shown that generation of phosgene from chloroform has occurred with or without the exposure to flames, electrical arcs, intense sunlight and hot surfaces. Over time chloroform can break down and form phosgene in older, particularly un-stabilized, chloroform containers.

Purchase stabilized chloroform whenever possible.

If un-stabilized chloroform is necessary for your work, you must treat it like peroxide forming compounds; date the container when received, use it quickly, and discard as hazardous waste after one year. If you have opened un-stabilized chloroform that has been in the laboratory for more than one year, contact the EHSRM Department for proper disposal. Storing chloroform in a dark place (cabinet) in an amber bottle can reduce the rate of chloroform decomposition. Open chloroform containers in a hood and let the headspace vent for a few minutes before bringing the container back into the laboratory. If possible dispense chloroform in the chemical fume hood.

## G. Select Agents, Toxins, and Bloodborne Pathogens

### 1. Select Agents and Toxins

A Select Agent is one of approximately 40 viruses, bacteria, rickettsiae, fungi, and toxins that are defined by CDC and USDA as biological agents or toxins deemed as a threat to the public, animal or plant health, or to animal or plant products. The list of select agents in 42 CFR 73 is available in *Appendix D* and online at: http://www.cdc.gov/od/sap/docs/salist.pdf. Certain strains of organisms and quantities of toxins are exempt from the regulations.

In accordance with SFA policy 11.31 (USA Patriot Act), the Public Health Security and Bioterrorism Preparedness and Response Act, the Department of Health and Human Services (HHS), and the U.S. Department of Agriculture (USDA) rules for access to Select Agents and Toxins have been established. This rule requires registration of possession, use, and transfer of Select Agents and Toxins capable of causing death or disease in a human, animal, plant, or any other living organism, or is detrimental to food, water, equipment, supplies, or the environment. The existing Select Agent Rule requires facilities to register if they possess select agents.

An important component of the rule includes the security risk assessment of individuals who have access to the select agents and toxins. Any person who meets the criteria of a "restricted person" as defined in the USA PATRIOT Act of 2001 and SFA policy 11.31, is not eligible to work in a laboratory that is doing work with Select Agents and Toxins.

**A “restricted person” is a person who:**

1. is under indictment for a crime punishable by imprisonment for a term exceeding 1 year;
2. has been convicted in any court of a crime punishable by imprisonment for a term exceeding 1 year;
3. is a fugitive from justice;
4. is an unlawful user of any controlled substance;
5. is an alien illegally or unlawfully in the United States;
6. has been adjudicated as a mental defective or has been committed to any mental institution;
7. is an alien (other than an alien lawfully admitted for permanent residence) who is a national of a country as to which the Secretary of State has made a determination (that remains in effect) that such country has repeatedly provided support for acts of international terrorism. As of April 30, 2001, these countries were or Iran, Iraq, Syria, Libya, Cuba, North Korea, and Sudan; or
8. has been discharged from the Armed Services of the United States under dishonorable conditions.

See the SFA USA Patriot Act policy 11.31 located online at: http://www.sfasu.edu/policies/usa-patriot-act.pdf for more information.

**2. Bloodborne Pathogens**

Bloodborne pathogens are biological agents that cause human disease such as Hepatitis B, Hepatitis C, and HIV/AIDS. Individuals who come in contact with infected blood or bodily fluids containing blood may be at risk of exposure. It is important that you treat all human blood and body fluids as if they are infected. See the SFA Bloodborne Pathogens Exposure Control Plan located on the EHSRM website at: http://www.sfasu.edu/safety for detailed information and procedures related to bloodborne pathogens.

# V. SPILL RESPONSE AND EMERGENCY PROCEDURES

## A. Biological Spill Response

The following procedures are provided as a guide to bio-hazardous spill cleanup. In each of the following cases, depending on the size of the spill, notify everyone in the lab, and call the EHSRM Department at 468-6034. If a spill is considered too large or too dangerous for laboratory personnel to safely clean up, secure the area (including the entire lab) and call the EHSRM Department immediately for assistance. Call 911 after normal business hours.

**1. Spills inside a Bio-safety Cabinet (BSC):**

- Wait at least five minutes to allow the BSC to contain aerosols.
- Wear lab coat, safety glasses and gloves during cleanup.
- Allow cabinet to run during cleanup.
- Apply disinfectant and allow a minimum of 20 minutes contact time.
- Wipe up spillage with disposable disinfectant-soaked paper towel.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant-soaked paper towel.
- Discard contaminated materials using appropriate bio-hazardous waste disposal procedures (e.g., autoclave or biohazard bag for disposal). Refer to the Waste Disposal section on page 27 for additional information.
- Remove protective clothing used during cleanup and place in an autoclave bag for autoclaving or biohazard bag for proper disposal.
- Run cabinet 10 minutes after cleanup before resuming work or turning cabinet off.
- Disinfect or sterilize reusable items.

2. **Spills in the Lab (outside a BSC):**
   - Clear the area of all personnel.
   - Wait at least 15 minutes for aerosols to settle before entering the spill area.
   - Remove any contaminated clothing and place in a biohazard bag to be disposed of properly, or an autoclave bag to be autoclaved.
   - Put on a disposable gown, safety glasses, and gloves.
   - Initiate cleanup with disinfectant as follows:
     - Place dry paper towels on the spill (to absorb liquids); then place a second layer of disinfectant soaked paper towels over the spill.
     - Encircle the spill with additional disinfectant being careful to minimize aerosols while assuring adequate contact.
     - Decontaminate all items within the spill area.
     - Allow 20 minutes contact time to ensure germicidal action of disinfectant.
     - Wipe equipment with appropriate disinfectant.
     - Discard contaminated disposable materials using appropriate bio-hazardous waste disposal procedures (e.g., autoclave or biohazard bag for disposal). Refer to the waste disposal section on page 27 for additional information.
     - Disinfect or sterilize reusable items.

3. **Spill inside a Centrifuge:**
   - Clear the area of all personnel.
   - Wait at least 15 minutes for aerosols to settle before attempting to clean up the spill.
   - Wear a lab coat, safety glasses, and gloves during cleanup.
   - Remove rotors and buckets to nearest biological safety cabinet for cleanup.
   - Thoroughly disinfect the inside of the centrifuge.
   - Discard contaminated disposable materials using appropriate bio-hazardous waste disposal procedures (e.g., autoclave or biohazard bag for disposal). Refer to the waste disposal section on page 27 for additional information.
   - Disinfect or sterilize reusable items.

4. **Spills outside the Lab, During Transport:**
   - Transport labeled bio-hazardous materials in an unbreakable, well-sealed primary container placed inside of a second unbreakable, lidded container (cooler or plastic bucket) labeled with the biohazard symbol.
   - Should a spill occur in a public area, do not attempt to clean it up without appropriate personal protective equipment.
   - Secure the area, keep people out of the room or hallway until cleanup is complete.
   - Call the EHSRM Department at 468-6034 to assist in cleanup.
   - Standby during spill response and cleanup activity and provide assistance only as requested or as necessary.

## B. Emergency Contact Information and Procedures

Biohazardous spills may result in an emergency situation when there is an injury to personnel, serious exposure to biohazardous agents, or a large spill that laboratory personnel are not equipped to handle. In the event of such emergency situations, refer to the emergency contacts and procedures described on the following page.

**Emergency Contact Information:**

| | |
|---|---|
| **Serious or Life Threatening Emergencies** | **911**<br>**Routed through UPD if dialed from a campus phone.* |
| **University Police** (Non-Emergency) | **468-2608** |
| **Env. Health, Safety, & Risk Management** | **468-4514** |
| **Safety Officer – Biohazards & Lab Safety** | **468-6034** |
| **SFA Health Clinic** (Students) | **468-4008** |
| **Poison Control Center** | **800-222-1222** |
| **Power Outage** | **468-3206 or 888-313-4747** (after 5 pm) |

### *IN A LIFE THREATENING EMERGENCY:*

- Do not move the individual! (unless the scene of the accident is unsafe).
- Check the victim for consciousness, breathing, pulse, and bleeding.
- Call 911.
- Comfort and provide care for the individual as best as possible.

*Always use your best judgment and remain calm. Inform responders of any biohazard(s) that may constitute a threat to their safety.*

The exact procedure for responding to a biological spill depends on the material, amount, and location of the spill. For large spills, contact EHSRM at 468-6034 as soon as possible or 911 after normal business hours and when a life threatening emergency exists.

The following information will help expedite the response:

- Location of incident (Room number and building/Lab number)
- Hazardous material (Biohazardous agent/Name/Biosafety Level)
- If medical assistance is needed (type of injury or exposure & number of individuals needing assistance).

## C. Reporting Accidents and Exposure to Infectious Agents

Accidents (i.e., incidents resulting in injury or exposure to infectious agents) should be reported and documented to the principal investigator, instructor, or supervisor then to EHSRM at 936-468-6034. A Biological Incident Form located in *Appendix E* should be filled out and attached to the container of spilled material and a copy mailed to the EHSRM Department (Box 6113).

Employees who are injured on the job shall receive medical attention through a Worker's Compensation health care provider such as Urgent Doc, Nacogdoches Medical Center, or Nacogdoches Memorial Hospital. Contact the EHSRM Worker's Comp. Coordinator at 468-4514 to report an injury and file a claim. Additionally, students needing medical attention may go to the SFA Health Clinic between the hours of 8-5, Monday thru Friday.

Accidents and injuries may also be reported to EHSRM by using the "Accident/Injury 24 Hour Report Quick Link" provided on the EHSRM website at: http://www.sfasu.edu/safety. Employees must report workplace injuries to EHSRM within 24 hours to be eligible for Worker's Compensation benefits and treatment.

# VI. WASTE DISPOSAL

The Texas Department of State Health Services (TDSHS) has identified biological waste as waste that requires special handling to protect human health or the environment. Materials that may be effectively sterilized in an autoclave may be placed in an autoclave bag, sterilized, and placed in the regular trash. If an autoclave is not available, or the materials are not appropriate for autoclaving, place them in an approved red/orange biohazard waste bag and contact EHSRM for proper disposal through a SFA contracted biological waste contractor. Biological waste is comprised of the following:

## A. Microbiological Waste

- Discarded cultures and stocks of infectious agents and associated biologicals.
- Discarded cultures of specimens from medical, pathological, pharmaceutical, research, clinical, commercial, and industrial laboratories.
- Discarded live and attenuated vaccines, but excluding the empty containers thereof.
- Discarded, used disposable culture dishes.
- Discarded, used disposable devices used to transfer, inoculate, or mix cultures. Note: In vitro tissue cultures that have not been intentionally exposed to pathogens are exempt from these regulations.

## B. Animal Waste

Animal waste items known to be pathogenic must be autoclaved prior to disposal in the regular trash or placed in an approved red/orange biohazard waste bag for proper disposal. Animal waste that does not contain pathogens may be placed in the regular trash. Use sealable containers such as buckets for carcasses, body parts, or blood and take them directly to the dumpster or landfill to minimize odors and additional handling by custodial staff. Animal waste includes the following:

- Carcasses of animals.
- Body parts of animals.
- Whole blood, serum, plasma, and/or other blood components from animals.
- Bedding of animals intentionally exposed to pathogens.

## C. Human Blood and Blood Products

- Human blood, serum, plasma, other blood components, and body fluids.
- Disposable items contaminated with human blood or body fluids.

## D. Pathological Waste

- Laboratory specimens of blood and tissue after completion of laboratory examination.
- Anatomical remains, human materials, and tissues.

## E. Sharps

All sharps described below must be disposed of in an approved sharps container displaying the universal biohazard symbol.

Sharps include but are not limited to the following, regardless of contamination:

- Hypodermic needles.
- Hypodermic syringes with attached needles.
- Scalpel blades.
- Razor blades, disposable razors, and disposable scissors used in dissection, surgery or other laboratory procedures.
- Glass pipettes.
- Broken glassware.
- Specimen tubes.
- Blood culture bottles.
- Microscope slides.

# Appendix A

## Stephen F. Austin State University
## Application for review of proposed research by the Institutional Biosafety Committee (IBC)

Refer to SFA policy 8.9 - Recombinant DNA and/or Infectious Biohazards in Teaching and Research http://www.sfasu.edu/policies/8.9-recombinant-dna-and-or-infectious-biohazards-in-teaching-and-research.pdf, and the SFA Biosafety Manual http://www.sfasu.edu/safety/442.asp for specific policies and procedures related to biological safety.

| **SECTION A** *Principal Investigator and personnel information (please type or print)* | | |
|---|---|---|
| ***P.I. Name*:** | ***Title*:** | ***Dept*:** |
| ***Phone No*:** | ***Alternate Phone No.*:** | ***Fax*:** |
| ***Building and Lab Room No(s):*** | ***E-mail:*** | |

**SECTION B Brief description of the research understandable to scientist working in different fields.**

**☐ New Protocol ☐ Renewal Protocol ☐ Amendment Protocol**

**Title of the protocol:**

_____

**This project will use: ☐Biohazardous Material ☐Biological Toxins ☐Recombinant DNA**

*****Notice: Biohazardous agents above Biosafety Level 2 are currently prohibited at SFA facilities.***

**B.1. Provide the date when you propose to begin research and the date when you anticipate research will be completed:**

**B.1.a. Proposed Start date:**

**B.1.b. Anticipated completion date:**

**B.2. General description of research:**

**B.3. Hypothesis:**

**B.4. Types of biological agents and toxins, their quantity, duration of experiment, and/or the rDNA technology to be applied:**

**B.5. Significance of the project**

**B.6. Please include any additional information that may assist the IBC in the review of this protocol (e.g. description of experimental design, procedures, etc)**

**SECTION C** *To determine if your project is **Exempt** or **Non Exempt,** complete the six questions below:*

1. Does the construct contain viral DNA that represents more than ½ of any eukaryotic viral genome or is the viral construct from DNA or Risk Group 2,3,4 virus or restricted agents? ☐ Yes ☐ No

***Notice: Biohazardous agents above Biosafety Level 2 are currently prohibited at SFA facilities.***

Use the links below to determine relevant Risk Group and/or select agent of your research materials:
https://my.absa.org/tiki-index.php?page=Riskgroups
http://www.selectagents.gov/SelectAgentsandToxinsList.html

2. Does this study involve the deliberate transfer of rDNA; or DNA or RNA that is derived from rDNA into humans, other vertebrates, invertebrates, or plants; or consist of DNA transferred from a prokaryotic or eukaryotic host that is not a closely related strain or species?
NIH Guidelines Section III-D-2.
☐ Yes ☐ No

3. Does the study involve the use of a microorganism from a Risk Group 2,3, 4 or select agent as a Host-Vector System, or cloned DNA from a Risk Group 2,3,4 into nonpathogenic prokaryotic or lower eukaryotic Host-Vector System or if using RG-2 organisms, does it involve the movement of DNA between organisms from different Appendix sublists?
☐ Yes ☐ No

Use the links below to determine relevant Risk Group, select agent, or natural exchanger sublist of your research materials:
https://my.absa.org/tiki-index.php?page=Riskgroups
http://www.selectagents.gov/SelectAgentsandToxinsList.html
http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html ***(See Appendix A in NIH Guidelines)***

4. Does the research involve the generation of Toxin Molecules lethal for vertebrates at an $LD_{50}$ of less than 100 nanograms per kilogram body weight (or 100 µg/kg of body weight) (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin)? NIH Guidelines Section III-B-1.
☐ Yes ☐ No

Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight.

5. Does the research involve the generation of more than 10 liters of culture at one time?
NIH Guidelines Section III -D-6.
☐ Yes ☐ No

6. Does the research involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally and if so, could this acquisition compromise the use of the drug to control the disease agents in humans, animals and/or plants?

☐ Yes ☐ No

7. Project Title

| **Nature/Source of inserted DNA:** Examples: include genus/Species, name of protein pathway or function | **Host(s)** Examples: E.coli K-12 | **Methods of gene transfer/vector(s):** Examples:Virus; Plasmid; naked DNA; conjugation; chemical; mechanical; other – specify type & name | **Intended Use of rDNA:** Examples: cloning; transgenic generation; modification of natural gene expression; new protein expression |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

**Principal Investigator Assurance:**

- Agree to use at least Biosafety Level (BSL -1) containment practices with all exempt rDNA work.
- Acknowledge that I will notify the IBC of any changes to this research study by promptly amending this form

NIH Guidelines - Section III-F. Exempt Experiments:
*The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required:*
**Section III-F-1.** Those that are not in organisms or viruses.
**Section III-F-2.** Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
**Section III-F-3.** Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
**Section III-F-4.** Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
**Section III-F-5.** Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), *Major Actions*). See Appendices A-I through A-VI, *Exemptions Under Section III-F-5--Sublists of Natural Exchangers*, for a list of natural exchangers that are exempt from the *NIH Guidelines*.
**Section III-F-6.** Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment.
See Appendix C in the NIH Guidelines, *Exemptions under Section III-F-6* for other classes of experiments which are exempt from the *NIH Guidelines*.

Signature of Principal Investigator _____ Date _____

Submit to:

## Institutional Biosafety Committee (IBC)

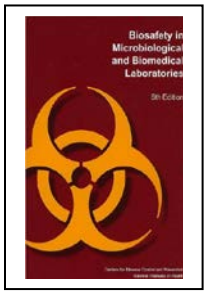
Campus Mail: Attn: Dr. Odutayo Odunuga, PO Box 13006, SFA Station • Nacogdoches, Texas 75962-3046 Phone (936) 468-6468 • Fax (936) 468-7634 • E-mail odunugaoo@sfasu.edu (Please email fully completed application)
Ph. 936-468-3601
Fax: 936-468-2056

| For IBC Use Only | For ORSP Use Only |
|---|---|
| Protocol #: | PCF #: |
| IBC Approval Date: | Submission Date: |
| NIH Guidelines exemption category: | Completion of Biosafety Training: |

# Appendix B

## Regulatory Guidance for Biosafety in Teaching and Research

**The agencies listed below provide guidelines covering the use of biological agents:**




US Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* (5th Ed)(CDC-NIH Standards).
(December 2009)
http:/ /www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf.

Centers for Disease Controls and Prevention (CDC) and the National Institutes of Health (NIH): Biosafety in Microbiological and Biomedical Laboratories (BMBL). This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety.

NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) http://www.nih.gov

National Institutes of Health (NIH): *Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines). This document provides guidelines for constructing and handling recombinant DNA molecules (rDNA), and organisms containing rDNA. Institutions that receive any NIH funding for rDNA research are required to comply with these guidelines as a condition of funding. This document requires that each institution establish an Institutional Biosafety Committee with the authority to approve proposed rDNA research using the NIH Guidelines as a minimum standard.

OSHA Bloodborne Pathogens Standard (29 CFR Part 1910.1030)
http://www.osha.gov

Occupational Safety and Health Administration (OSHA): Bloodborne Pathogens. This regulation covers occupational exposure to human blood and other potentially infectious material, including human tissue and cells. OSHA specifies a combination of engineering controls, work practices, and training to reduce the risk of infection. Personnel exposed to human blood and other potentially infectious material must be offered immunization against Hepatitis B and receive annual training.

CDC Select Agent Program (42 CFR Part 73)
http://www.cdc.gov/od/sap

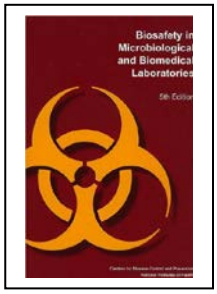
Centers for Disease Control and Prevention (CDC): *Possession, Use, and Transfer of Select Agents and Toxins* and USDA Animal and Plant Health Inspection Service (APHIS): *Agricultural Bioterrorism Protection Act of 2002: Possession, Use, and Transfer of Biological Agents and Toxins.* These regulations require institutions that possess, use, or transfer certain biological agents and toxins ("Select Agents") to be registered and approved by the CDC and/or APHIS.

Texas Commission of Environmental Quality (TCEQ)
http://www.tceq.state.tx.us

The TCEQ is the local regulatory authority concerning hazardous waste.


Laboratory Biosafety Manual - Third Edition
http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf

Published by the World Health Organization in 1983, the Laboratory Biosafety Manual has provided practical guidance on biosafety techniques for use in laboratories at all levels. The third edition of the manual has been extensively revised and expanded. The manual now covers risk assessment and safe use of recombinant DNA technology, and provides guidelines for the commissioning and certification of laboratories.




CDC/NIH: Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets – 5th Ed (December 2009)
http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixa.pdf

U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health.
This document is currently printed as Appendix A of the Biosafety in Microbiological and Medical Laboratories.

Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens. World Health Organization – 1997
http://www.who.int/csr/resources/publications/biosafety/whoemc973.pdf

## Other regulations and guidelines that may apply:

1. Environmental Protection Agency
   a. Resource Conservation and Recovery Act (RCRA) – Hazardous Waste Disposal
   b. Toxic Substances Control Act (TSCA) – new products
   c. Biotechnology TSCA Requirements – (rDNA)
   d. Emergency Planning, Continuity Right-To-Know Act (EPCRA) – reportable quantities

2. Occupational Safety and Health Administration
   a. Chemical Hygiene
   b. Bloodborne Pathogens
   c. Personal Protective Equipment
   d. Formaldehyde

3. Public Health
   a. Texas Department of Health Regulations
   b. Etiologic Agent Regulations of United States Public Health Service (USPHS) Division of Quarantine
   c. Health and Hygiene in Research Laboratories
   d. Laboratory Animal Handling Guidelines
   e. Good Laboratory Practices Act

# Appendix C

## Summary of Chemical Disinfectants

| Disinfectant | Use Parameters | Effective Against | | | | | Important Characteristics | Potential Application |
|---|---|---|---|---|---|---|---|---|
| | | Vegetative Cells | Lipophilic Viruses | Tubercle bacilli | Hydrophilic Viruses | Bacterial Spores | | |
| **Alcohol** (ethyl, isopropyl) | conc.: 70-85% contact time: 10-30 min. | + | + | + | ± | | eye irritant, toxic, flammable, inactivated by organic matter | surfaces - work & equipment |
| **Chlorine Compounds** | conc.: 0.05-0.5% (commercial bleach ≈ 5%) contact time: 10-30 min. | + | + | + | + | ± | may leave residue; corrosive; skin, eye & respiratory irritant; inactivated by organic matter; makeup at least weekly | spills, equipment surfaces, instruments, glassware, water baths |
| **Quaternary Ammonium Compounds** | conc.: 0.1-2% contact time: 10-30 min. | + | + | | | | toxic, inactivated by organic matter; Not effective against P. aeruginosa | surfaces (work & equip.), BSCs, floor maintenance, glassware, instruments |
| **Phenolic Compounds** | conc.: 0.2-3% contact time: 10-30 min. | + | + | + | ± | | leaves residue; corrosive, skin, eye & respiratory irritant; toxic; inactivated by organic matter | surfaces (work & equip.), BSCs, floors, spills, glassware, instruments, water baths |
| **Iodophor Compounds** | conc.: 0.47% contact time: 10-30 min. | + | + | + | ± | | leaves residue; corrosive, skin & eye irritant; toxic; inactivated by organic matter | surfaces (work & equip.), BSCs, glassware, water baths |
| **Formaldehyde[a] (Formalin)** | conc.: 4-8% contact time: 10-30 min. | + | + | + | + | ± | leaves residue; skin, eye & respiratory irritant; toxic (carcinogen) | less effective than other disinfectants but can be used for equipment surfaces, glassware, instruments |
| **Glutaraldehyde[b]** | conc.: 2% contact time: 10-600 min. | + | + | + | + | + | leaves residue; skin, eye & respiratory irritant; toxic | equipment surfaces, glassware, instruments |

From: Laboratory Safety: Principles and Practices, second edition, Diane O. Fleming, John H. Richardson, Jerry J. Tulis, and Donald Vesley, eds., American Society for Microbiology, Washington, D. C.

1. + = very positive response, ± = less positive response. A blank denotes a negative response or not applicable.

a. Due to its irritating characteristics and status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation.

b. Glutaraldehyde also requires a well ventilated exhaust system due to its chemical toxicity.

# Appendix D

## List of Select Agents and Toxins

**HHS & USDA SELECT AGENTS & TOXINS:**
**7 CFR Part 331, 9 CFR Part 121, & 42 CFR Part 73**

**Health and Human Services (HHS) SELECT AGENTS AND TOXINS**

Abrin
Cercopithecine herpesvirus 1 (Herpes B virus) *Coccidioides posadasii*
Conotoxins
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Ebola viruses
Lassa fever virus
Marburg virus
Monkeypox virus
Ricin
*Rickettsia prowazekii*
*Rickettsia rickettsii*
Saxitoxin
Shiga-like ribosome inactivating proteins
South American Haemorrhagic Fever viruses
- Flexal
- Guanarito
- Junin
- Machupo
- Sabia

Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses
- Central European Tick-borne encephalitis
- Far Eastern Tick-borne encephalitis Swine
- Kyasanur Forest Disease
- Omsk Hemorrhagic Fever
- Russian Spring and Summer encephalitis

Variola major virus (Smallpox virus)
Variola minor virus (Alastrim)
*Yersinia pestis*

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**USDA PLANT PROTECTION & QUARANTINE (PPQ) SELECT AGENTS AND TOXINS**

*Candidatus* Liberobacter africanus
*Candidatus* Liberobacter asiaticus
*Peronosclerospora philippinensis*
*Ralstonia solanacearum* race 3, biovar 2
*Schlerophthora rayssiae* var *zeae*
*Synchytrium endobioticum*
*Xanthomonas oryzae* pv. *oryzicola*
*Xylella fastidiosa* (citrus variegated chlorosis strain)

**OVERLAP SELECT AGENTS AND TOXINS**

*Bacillus anthracis*
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
*Brucella abortus*
Brucella melitensis
Brucella suis
*Burkholderia mallei* (formerly *Pseudomonas mallei*)
*Burkholderia pseudomallei*
(formerly Pseudomonas pseudomallei)
*Clostridium perfringens* epsilon toxin
*Coccidioides immitis*
*Coxiella burnetii*
Eastern Equine Encephalitis virus
*Francisella tularensis*
Hendra virus
Nipah virus
Rift Valley fever virus
Shigatoxin
Staphylococcal enterotoxins
T-2 toxin
Venezuelan Equine Encephalitis virus

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**USDA SELECT AGENTS AND TOXINS**

African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (Exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
*Cowdria ruminantium* (Heartwater)
Foot-and-mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
(Alcelaphine herpesvirus type 1)
Menangle virus
*Mycoplasma capricolum*/ M.F38/*M. mycoides capri*
(contagious caprine pleuropneumonia)
*Mycoplasma mycoides mycoides*
(contagious bovine pleuropneumonia)
Newcastle disease virus (velogenic)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (Exotic)

# S AND USDA SELECT AGENTS AND TOXINS

## 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

**HHS SELECT AGENTS AND TOXINS USDA SELECT**

**AGENTS AND TOXINS**

Abrin African horse sickness virus
Cercopithecine herpesvirus 1 (Herpes B virus) African swine fever virus
*Coccidioides posadasii* Akabane virus
Conotoxins Avian influenza virus (highly pathogenic)
Crimean-Congo haemorrhagic fever virus Bluetongue virus (Exotic)
Diacetoxyscirpenol Bovine spongiform encephalopathy agent
Ebola virus Camel pox virus
Lassa fever virus Classical swine fever virus
Marburg virus *Cowdria ruminantium* (Heartwater)
Monkeypox virus Foot-and-mouth disease virus
Reconstructed replication competent forms of the 1918 pandemic Goat pox virus
influenza virus containing any portion of the coding regions of Japanese encephalitis virus
all eight gene segments (Reconstructed 1918 Influenza virus)
Lumpy skin disease virus
Ricin Malignant catarrhal fever virus
*Rickettsia prowazekii* (Alcelaphine herpesvirus type 1)
*Rickettsia rickettsii* Menangle virus
Saxitoxin *Mycoplasma capricolum*/ M.F38/*M. mycoides Capri*
Shiga-like ribosome inactivating proteins (contagious caprine pleuropneumonia)
South American Haemorrhagic Fever viruses *Mycoplasma mycoides mycoides*
Flexal (contagious bovine pleuropneumonia)
Guanarito Newcastle disease virus (velogenic)
Junin Peste des petits ruminants virus
Machupo Rinderpest virus
Sabia Sheep pox virus
Tetrodotoxin Swine vesicular disease virus
Tick-borne encephalitis complex (flavi) viruses Vesicular stomatitis virus (Exotic)
Central European Tick-borne encephalitis
Far Eastern Tick-borne encephalitis **USDA PLANT PROTECTION AND QUARANTINE (PPQ)**
Kyasanur Forest disease **SELECT AGENTS AND TOXINS**
Omsk Hemorrhagic Fever *Candidatus* Liberobacter africanus
Russian Spring and Summer encephalitis *Candidatus* Liberobacter asiaticus
Variola major virus (Smallpox virus) and *Peronosclerospora philippinensis*
Variola minor virus (Alastrim) *Ralstonia solanacearum* race 3, biovar 2
*Yersinia pestis Schlerophthora rayssiae* var *zeae*
*Synchytrium endobioticum*
**OVERLAP SELECT AGENTS AND TOXINS** *Xanthomonas oryzae* pv. *oryzicola*
*Bacillus anthracis Xylella fastidiosa* (citrus variegated chlorosis strain)
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
*Brucella abortus*
*Brucella melitensis*
*Brucella suis*
*Burkholderia mallei* (formerly *Pseudomonas mallei*)
*Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)
*Clostridium perfringens* epsilon toxin
*Coccidioides immitis*
*Coxiella burnetii*
Eastern Equine Encephalitis virus
*Francisella tularensis*
Hendra virus
Nipah virus
Rift Valley fever virus
Shigatoxin
Staphylococcal enterotoxins
T-2 toxin
Venezuelan Equine Encephalitis virus

2/23/06

# Appendix E

**BIOLOGICAL SPILL INCIDENT REPORT**
Stephen F. Austin State University

DATE: _____ TIME: _____

NOTIFIED BY_____

PHONE: _____ DEPARTMENT: _____

EXACT LOCATION OF INCIDENT: _____
(be specific)

TYPE OF BIOHAZARD _____

AMOUNT _____

LIST SUPPLIES USED FOR CLEAN-UP: _____
_____

SPECIAL PROBLEMS ENCOUNTERED _____
_____

SIGNATURE_____