# Biological Safety Level 3 Manual
# (BSL-3)

## Environmental Health, Safety and Risk Management
## Laboratory Safety Program
## July 2022

## EREBL BSL-3 FACILITY

### Site Specific Biosafety, Containment, Security and Incident Response Plan

The Research Education Building (EREBL) Biosafety Level 3 (BSL-3) laboratories are designed to work safely with microorganisms that can cause serious human disease. In addition to biological hazards, employees may be exposed to a range of chemical, radiological, and physical hazards. Therefore, it is incumbent upon each employee to understand the potential dangers inherent in biological research and to work in a manner that minimizes risk to them and to others. It is the goal of this plan manual to provide guidelines to prevent unnecessary exposure to hazardous situations and to prepare an adequate response in an emergency situation. This manual is to be used in conjunction with the University of Texas Rio Grande Valley *Laboratory Safety/Hazard Communication Manual, Biological Safety Level 2 (BSL-2) Manual, Bloodborne Pathogen Exposure Control Plan and Post Exposure Policy, Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6$^{th}$ Edition, Fire Safety Emergency Response and Evacuation Program Manual.*

### Manual Review

This manual and appendices will be reviewed annually or as necessary by the following individuals for compliance with current regulations and guidelines and for effectiveness in the safe handling of the agents.

1. Principal Investigator(s) or Laboratory Director
2. Director of Environmental Health & Safety and Risk Management (EHSRM)
3. The UTRGV Chief of Police and the Animal Care Supervisor (ACS) and Risk management review this manual when there are significant changes to security or ACS procedures, respectively.

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## Section 1: General Responsibilities

1. **The Laboratory Director / Principal Investigator (PI) is responsible…**
   - **a.** For communicating experimental design through written or verbal instructions
   - **b.** For ensuring the safety of all employees under his/her direction.
   - **c.** To ensure that employees are properly trained to safely handle and contain hazardous agents and animals used in the course of their duties.
   - **d.** To ensure his/her employees receive adequate medical surveillance and are provided with the equipment to safely perform their duties.
   - **e.** To make sure employees understand the proper post exposure procedures for any hazardous agents or chemicals where they are at risk of exposure.
     - **i.** To investigate, follow up and report any potential exposures immediately to the Biosafety Officer/ EHSRM as appropriate.
   - **f.** Report any significant changes in employee status affecting use and possession of biological agents or toxins as soon as he/she becomes aware of such changes.
   - **g.** Report all incidents involving exposure, theft, security breaches, to UTPD and EHSRM.
   - **h.** To maintain an accurate inventory of infectious agents, chemicals and radioactive materials in his/her laboratories.
   - **i.** Immediately notify EHSRM and UTPD when an authorized person will terminate employment, transfer to another laboratory, or is reassigned to other areas not requiring BSL-3 access.
   - **j.** Responding to and correcting any deficiencies as a result of inspections/evaluations performed by the Environmental Health and Safety department.

2. **The Environmental Health & Safety (EHSRM) Department is responsible …**
   - **a.** For monitoring compliance of activities in this facility with applicable federal, state, local, and institutional regulations and policies as they pertain to the safety of employees and the environment.
   - **b.** For performing routine evaluations (inspections) to ensure compliance with applicable regulations and UTRGV's policies and reporting these findings to the Institutional Biosafety Committee (IBC).
   - **c.** For assisting facility staff in implementing safe policies and procedures.
   - **d.** To assist in training personnel, Facilities Management workers, in safe work practices as they pertain to this facility.
   - **e.** To provide review of ongoing activities, including use of biohazardous materials, recombinant DNA, chemicals and radioactive materials through the Director of EHSRM, Biological Safety Officer, Radiation Safety Officer, and other appropriate personnel and to ensure proper review and approval of these activities by the appropriate Institutional Committee.
   - **f.** Providing, and coordinating scheduling for, routine certification of all Class I & II Biological Safety Cabinets (BSCs), and Annual Certification of the BSL-3 facility to requirements as set forth in the most current edition of *Biosafety in Microbiological and Biomedical Laboratories*.

3. **Department of Animal Care (ACS) is responsible for…**
   - **a.** Ensuring proper operation of the biohazard containment area in animal research facilities at UTRGV.

**b.** Ensuring that the Animal Attendants are trained in the proper care for animals involved in studies being carried out by the Principal Investigators at this facility.
**c.** Ensuring that the Research staff and animal care complies with proper animal husbandry / research procedures as required by ACS policy and the Institutional Animal Care and Use Committee (IACUC).
**d.** To ensure his/her employees receive adequate medical surveillance and are provided with the equipment to safely perform their duties.
**e.** Report all incidents involving exposure, theft, security breeches, or animal inventory discrepancies with infectious agents to the institutional Biosafety Officer.
**f.** Responding to and correcting any deficiencies as a result of inspections/evaluations performed by the Environmental Health and Safety department.

**4. The Chief of the UTRGV Police Department (UTPD) is responsible for…**

**a.** Assisting the Principal Investigator and EHSRM in restricting access into the BSL-3 facility to include:
  **i.** Granting and controlling access to the BSL-3 facility
    **1.** For new, transfer, or terminated employees having authorized access to infectious agents in the BSL-3 – this includes issuance, and final collection of keys, key cards, or hand key access codes.
    **2.** Maintaining an accurate database of all individuals with access and provided to the EHSRM on request.

**b.** Implementation and oversight of the Security Plan to include:
  **i.** Investigate the loss of keys, keycards, passwords, or combinations with access to the BSL-3 facility.
  **ii.** Investigate any suspicious persons reported and removal of any unauthorized persons from the premises.
  **iii.** Securing facilities containing infectious agents with personnel, or other means, when normal personnel are not present, or normal security systems are compromised in any way. (Note: Security of the main building is delegated to UTRGV police.)
  **iv.** Provide security monitoring of areas where infectious agents are used, stored, or transported within UTRGV facilities.
  **v.** Reporting any lapses or breaches in security to UTPD and EHSRM.

**5. Each Employee has the responsibility to …**

**a.** Comply with the safety, containment and security practices outlined in this manual.
**b.** Comply with specific departmental standard operating procedures, particularly as they relate to the BSL-3 facility and handling of infectious agents.
**c.** Complete the required annual training.
**d.** Report immediately to the Laboratory Director or PI any change in personal status that may affect his/her ability to work in the BSL-3 facility.
**e.** Report any hazardous agent / material exposure immediately to his / her supervisor.
**f.** Report any lost or stolen key, lock, keycard, keypad under his/her control immediately to his / her supervisor, UTPD, and EHSRM.
**g.** Report any compromised password, or signs that indicate that security has been compromised to his / her supervisor, Responsible Official, or UTPD.
**h.** Perform routine cleaning and maintenance of equipment.
**i.** Perform other duties as assigned.

## Section 2: Overview of Facilities and Operations

**Purpose:** This section provides an overview of the Research Education Building (EREBL) physical laboratories and provides guidelines for action to be taken in case of an emergency situation. Additional information is contained in the most current version of the UTRGV's *Fire Safety Emergency Response and Evacuation Program manual.*

1. **EREBL Facilities.** The 2nd floor laboratories consist of laboratories, lab support areas and offices for the routine daily experiments and workings of the laboratory up to Biosafety Level 2. Experiments involving biohazardous organisms infectious by aerosolization are performed in the 1st floor Biosafety Level 3 facility (BSL-3 facility) located within the vivarium of the Animal Care (ACS).
   a. **The BSL-3 Facility:** The BSL-3 facility consists of a suite of 3 main research laboratories (1.500.30, 1.500.26, 1.500.12), a pass-thru autoclave and two general purpose labs (1.500.4 and 1.500.6), and 4 animal rooms. Current studies involve the bacterial agent, *Mycobacterium tuberculosis,* Chikungunya, Dengue and Zika viruses which <u>are not</u> Select Agents, and live animal (mosquitos) are limited to the vivarium section of the BSL-3 facility. The BSL-3 facility is accessed through the anteroom/airlock 1.500.2 and the labs and animal rooms are accessed from an interior corridor 1.500, which contains an eyewash/safety shower, alarm stations and telephone and a large pass-thru autoclave between corridors 1.500.8 (BSL-3) and the outer BSL-2 corridor. The BSL-3 labs are under negative airflow with respect to the rest of the ACS ABSL/BSL-2 facilities and EREBL building, utilizing separate air handlers and high efficiency particulate air (HEPA) filtered exhaust units to control the BSL-3 facility. Airflow and temperature to the entire EREBL building and the BSL-3 facility are monitored by a Facilities Management Utilities 24 hours per day ECULP Control Room. (956-665-2796)

2. **ALARMS:** There are 3 basic types of alarms for the EREBL BSL-3 facility.
   a. **Airflow Indicators.** "Ping-pong ball" type pressure airflow indicators are present at doorways throughout the BSL-3/ABSL-3 facility, and at the entrance to both airlocks and each lab in the BSL-3 facility suite. These indicators should be visually checked that the air pressure is negative before entering the BSL-3 facility or individual labs and animal rooms. Should the negative exhaust fail in the BSL-3 facility, an audible (loud) alarm sounds both inside and outside the BSL-3 facility suite in the corridors. A visual flashing amber light also activates in each BSL-3 lab, the inner and outer corridors and a warning signal in the EREBL utility operators' room. Note that the Class II BSCs are also fitted with separate audio-visual airflow indicator alarms. Should you notice positive air pressure prior to entering the individual lab or hear or see the alarm while you are working in the BSL-3 labs, evacuate immediately following exit procedures to quickly remove PPE and contact the Utilities control room operator at 956-665-2796. Also refer to 2(c), *Biological alarms* below.

   b. **Fire alarms.** The entire EREBL building, including the BSL-3 facility is equipped with fire alarms that sound and visually strobe. Should this alarm sound/strobe while you are working in the BSL-3 labs, follow the acronym (R.A.C.E. on pg. 50) as outlined in the section on the emergency plan below, evacuate immediately and contact physical plant control room

operator (956-665-2796) as soon as possible. The BSL-3 facility has an emergency exit located off the main BSL-3 corridor, which may be used if the main exit is blocked.

**c. Biological alarms.** There are 2 pull stations in the BSL-3 for activating visual (flashing amber lights) and an audible alarm to notify workers within the facility and the control room of a major biological spill. If the biological alarms sound, evacuate the BSL-3 labs following the emergency plan. A panel in the Utilities operator's room will also alarm, and panel lights indicate the basic cause of the alarm. The alarm may be silenced after all personnel are safely evacuated from the BSL-3 facility suite, but may not be reset until Research, ACS, Safety, and Utilities personnel are all in agreement that the problem has been addressed and it's safe to do so (exception: this does not apply to routine systems testing and maintenance).

## Section 3: Security Plan for the EREBL BSL-3 Facility

## Overview:

The purpose of the security plan is to promote a secure laboratory environment and to secure and safeguard infectious agents against unauthorized access, theft, loss, or release at the Edinburg EREBL BSL-3 facility. The EREBL BSL-3 facility is located on the grounds of the UT Rio Grande Valley campus, located at 1201 Schunior Drive, Edinburg, Texas.

This security plan was developed by the University of Texas Rio Grande Valley Environmental Health & Safety (EHS) in conjunction with the University Police (UTPD), the Principal Investigator (PI), ACS and Facilities Management.

**PHYSICAL SECURITY**

### 1. Control of Access to Infectious agents

**a.** There are several layers of security in order to access the BSL-3 laboratories housing infectious agents. A UTRGV Police Department security guard is located, in the adjacent EMEBL building, where an officer monitors the entrance to the central campus, 24 hours a day. Other access doors are locked with access granted by use of personal magnetic key card, key or PIN access. The BSL-3 facility is located in the Animal Care Services (ACS) area in which access is restricted through magnetic key card access and key lock. Access into the BSL-3 facility is monitored by UTPD 24/7 by a camera located at the entrance to the BSL-3 facility. There is additional restricted access into the EREBL BSL-3 facility by magnetic key card access, or key. Each individual laboratory is restricted in access and also has magnetic key card or key access. Facilities Management personnel, visitors, and contractors shall be escorted by authorized personnel at all times while in the BSL-3 facility.

**b.** Personnel who are granted access to this facility are not to share their magnetic key cards, keys, or personal access code(s) with another individual.

### 2. WORKING AFTER HOURS

**a.** Normal hours of operation are from 7:00 AM to 7:00 PM. If an individual employee is working after hours, the door should be locked and never left propped open for any reason.

**b.** Access after hours and on weekends to the EREBL Building and the BSL-3 Facility are controlled by the magnetic key cards or key. The UTRGV's Police Department (956-882-7777) and the UTRGV guard on duty are notified of after hour entrance and exit times.

**c.** Research, ACS, & Environmental Health and Safety and Risk Management employees are to note that they are in the BSL-3 Facility by marking the white Dry-Erase Occupancy Board. Facilities Management personnel, contractors, and visitors are to be escorted into the BSL-3 facility by authorized personnel and must sign in and out of the facility as described in Appendix A: *Access (Entry/Exit) Procedures for Facilities Maintenance / Contractors / Visitors.*

## 3. Control of Biological Agents Inventory

All stocks of *M. tuberculosis*, Chikungunya, Dengue and Zika viruses and other biological agents are inventoried by the Principal Investigator and are stored in designated boxes and racks in a freezer in controlled access laboratories inside the BSL-3 facility. An inventory record book is maintained and stored in each laboratory. A separate key to freezers storing infectious agents shall be kept in a secure location only known to authorized lab personnel.

## 4. Information Systems Control

UTRGV has a system of firewalls that safeguard against unauthorized use and is managed by Computing Resources. All accounts on UTRGV computers require that users log on with a username and password. These passwords are changed on a routine basis. In addition, a second password is required to access the database from within a user account. Five minutes of terminal inactivity result in the need for the user to re-log onto the machine before database access can continue.

## 5. Procedures for Routine Cleaning, Maintenance, and Repairs

Personnel that need to enter the BSL-3 facility for cleaning, maintenance, or other required activities shall be escorted at all times by an approved individual. An approved individual is the Principal Investigator, laboratory staff member, or the UTRGV Environmental Health and Safety and Risk Management department who has undergone the proper training. The escorted individual must be under visual contact at all times.

Visitors shall be escorted by an authorized individual and will be required to receive training including a brief overview of the hazards associated with the infectious agents, security requirements, and emergency response procedures. A Visitor Training manual is available for review by the visitor/contractor. The visitor training shall be documented and kept on file in the visitor training manual and by the Principal Investigator. All visitors must have a need to enter the area and show government issued picture identification prior to entering the ACS area. All escorted individuals (visitors/contractors/Facilities mgmt.) must wear the appropriate personal protective equipment and sign in and out upon entry and exit to the BSL-3 area. Visitor information must include printed name, affiliation, and reason for the visit, entry time, exit time, signature, and escort name. Escort must also sign on the log sheet.

All scheduled repairs/maintenance should be scheduled at least two days in advance with the research staff and EHSRM. For the safety of the non-authorized personnel and the security of the biological agents, during these scheduled repairs/maintenances, there will be no active work with these agents. All biological agents shall be stored and secured. At least two hours of air changes (preferably overnight) is required before any maintenance work is started. Facilities Management personnel / contractors (electricians, plumbers, etc.) may enter to effect repairs only after receiving proper training

and wearing appropriate personal protective equipment for the agent(s) in use, and after the area has been decontaminated as much as possible and cleared by Environmental Health and Safety personnel.

**a.** Security Procedures for Facilities Management or Contractor staff:

**i.** Must pass basic UTPD security clearance and be identified with a UTRGV ID badge.

**ii.** Must sign in and out of the facility as described in Appendix A: *Access (Entry/Exit) Procedures for Facilities Maintenance / Contractors / Visitors.*

## 6. Removal/reporting of Unauthorized or Suspicious persons

If an individual's appearance or actions arouse your suspicion, follow the procedure as outlined in the UTRGV Fire Safety Emergency Response and Evacuation Program Manual as follows:

- Immediately call 911 from any university phone, or 956-882-4911 and state your emergency.
- If the person is requesting to see a particular UTRGV employee, be polite. Ask the person to be seated…
- Then call 956-882-4911; identify yourself and the office in which you work and *say **"Code 33"** or use the phrase **"Code 33"** in a sentence.* If possible, give the dispatcher your name, location, describe the activity, and let them know it involves the security of a biological agent or toxin.
- This will alert UTRGV Police Department that a potential problem exists, and an officer will be dispatched to your area.
- If you notice someone who is not authorized to be there and they refuse to leave the area when asked, do not get confrontational with them – go to a safe location (do not proceed into the BSL-3 facility) and contact UTPD (as noted above) to dispatch an officer for assistance.

## 7. Loss or Compromise of Keys, Passwords, or Combinations, Changing Access Numbers or Locks Following Staff Changes

**a.** Magnetic key cards, pin codes, passwords, etc. should be kept secure from loss or theft. PIN codes and passwords should not be written down. However, any written records of PIN codes shall be kept in a secure place.

**b.** LOSS OF ID BADGE: If there is a loss of an ID badge containing your magnetic key card, then immediately notify UTPD at 956-882-7777, the Principal Investigator and EHSRM. Your magnetic key card and/or PIN number that grants access to the BSL-3 facility will be immediately terminated until magnetic key card is found or a new card is issued. An investigation and incident report will be completed.

**c.** COMPROMISED PIN CODE: If your PIN code has been compromised, immediately notify UTPD at 956-882-7777, the Principal Investigator and EHSRM. UTPD will immediately cancel the compromised PIN code and issue a new PIN code.

**d.** Keys to freezers or other equipment that stores infectious agents shall be kept in a secure location that is only known by authorized personnel. If there is a loss of a key to equipment that stores these agents, immediately notify UTPD at 956-882-4911, the Principal Investigator and EHSRM. The Principal Investigator shall inventory the biological agents in storage to make sure everything is accounted for. The equipment shall be re-keyed as soon as possible.

**e.** Any staff changes, termination or transfer, shall be reported to EHSRM and UTPD as soon as possible to ensure prompt termination of access by the UTPD.

## 8. Reporting Theft, Loss or Release of a Biological Agent

(Notification of Theft, Loss or Release)

**a.** LOSS OR THEFT OF A BIOLOGICAL AGENT: If you suspect a biological agent has been stolen, or it is lost or unaccounted for, notify UTPD (911 from any university phone, or 956-882-4911 EHSRM and the Principal Investigator as soon as possible. Give the UTPD dispatcher your name, location, what is missing and brief description of what happened. An investigation will be initiated, and an incident report must be completed even if the agent is subsequently located.

**b.** RELEASE OF A BIOLOGICAL AGENT: If there is an occupational exposure or release of an agent outside of the primary barriers of the biocontainment area, notify UTPD (911 from any university phone, or 956-882-4911, EHSRM and the Principal Investigator (call down list inside and outside laboratory) as soon as possible. Give the UTPD dispatcher your name, location, and a brief description of what happened. An investigation will be initiated, and an incident report must be completed. EHSRM will notify the appropriate State and local public health agencies.

## 9. Reporting Alteration of Inventory Records

The Principal Investigator has the primary responsibility for oversight of inventory records for his/her laboratory. All inventory recordkeeping shall be done in ink. If a mistake is made, then the entry shall be lined through and the corrected entry made above it and initialed by the person making the entry. Mistakes shall not be whited-out. Use of pencil is prohibited for recordkeeping purposes.

Any missing vials/tubes shall be immediately reported to, Dr. Richard Costello. An incident report will be initiated by EHSRM to determine if any agents are missing or unaccounted for.

## 10. Suspicious Packages

All packages, containers, etc. should be inspected by an authorized employee prior to entry into the lab or exit from the lab to ensure biological agents and toxins are properly accounted for.

## 11. Annual Review

This plan will be reviewed annually and revised as necessary. Any changes in this manual will be documented on a summary page that is signed by the Director of EHSRM and the Principal Investigators. If there are substantial changes in ACS procedures, then the Director of ACS will also review and sign on the summary page. If there are substantial changes in security procedures, then the Chief of Police, UTPD will review and sign on the summary page.

## Section 4: Biosafety and Containment Plan

### 1. Overview of Regulatory Requirements

The Biosafety and Containment Plan is designed to set site-specific policies and procedures to minimize lab accidents or exposures that would require an emergency response. This plan is designed to be commensurate with the risk posed by the biological agent, *Mycobacterium tuberculosis*.

- The UTRGV's Biological Safety manual is the primary safety manual addressing safety practices and procedures involving virulent agents at UTRGV. The Biological Safety manual can be found in the Principal Investigator's main laboratory, inside the BSL-3 facility – (bookshelf located in the corridor), through Environmental Health & Safety and Risk Management (956-665-3690) , and can be found online at: https://www.utrgv.edu/ehsrm/
- Additionally, UTRGV complies with practices and procedures for working at BSL-3 and ABSL-3 as outlined in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL – 6th Edition) and the current NIH Guidelines for Research Involving Recombinant DNA Molecules.
- The UTRGV's EREBL BSL-3 Site Specific Biosafety, Security, Incident Response and Good Laboratory Practice's Manual addresses site specific safety practices and procedures for biosafety, security, and incident response. The manual also includes an emergency call down list and ACS-specific and PI-specific Standard Operating Procedures. Currently, the only animal research being performed in the BSL-3 facility are Zika, Dengue and Chikungunya virus research conducted by Dr. John Thomas which involves mosquitos. Individuals working in the BSL-3 facility have access to this manual which can be found in the BSL-3 facility (bookshelf located in the corridor), the Principal Investigator's office and in the EREBL Environmental Health & Safety office. Copies of these manuals are also kept in the Central Campus EHSRM office in Edinburg, Texas.
- The UTRGV complies with 29 CFR 1910.1200 (OSHA Hazard Communication Act) and 29 CFR 1910.1450 (Occupational Exposure to Hazardous Chemicals in the Laboratory) and is covered in Section 6, General Safety/Chemical Safety of this manual.
- There are currently no radioactive materials used in the BSL-3 facility.

### Develop and implement a Safety Plan

Dr. Restrepo has been approved for research with clinical specimens containing *Mycobacterium tuberculosis* and strain H37Rv *of* by the Institutional Biosafety Committee (IBC) to work at biosafety level 3 (BSL-3) as recommended in the agent summaries in the publication, CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL – 6th Edition) and also in the NIH Guidelines for Research Involving Recombinant DNA Molecules, which includes both procedural and facility approval for risk assessment, physical and biological containment. Work with Mycoplasma species has also been approved by the Institutional Biosafety Committee (IBC). Mycoplasma species will also be worked with in the BSL-3 facility but may be worked with at BSL-2 containment.

2. Specific Recommendations for working with *Mycobacterium tuberculosis* (SDS for these agents can be found by contacting the EHSRM's office at 665-3690).

***Mycobacterium tuberculosis* (TB)** is a highly infectious pathogenic bacterium. The disease is acquired by inhalation of the bacterium. Primary infection may range from an asymptomatic infection to a rapidly progressive and possibly fatal disease involving the lungs and extra pulmonary tissues.

BMBL 6th ed. agent summary states:

## Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex includes *M. tuberculosis, M. bovis, M. africanum,* and *M. microti M.mungi and M.orygis* that cause tuberculosis in humans, and more recently recognized *M. caprae* and *M. pinnipedii* that have been isolated from animals. *M. tuberculosis* grows slowly, requiring three weeks for formation of colonies on solid media. The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents and allows them to stain acid-fast.

## Occupational Infections

*M. tuberculosis* and *M. bovis* infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities. The incidence of tuberculosis in laboratory personnel working with *M. tuberculosis* has been reported to be three times higher than that of those not working with the agent. Naturally or experimentally infected NHP are a proven source of human infection. Experimentally infected guinea pigs or mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols.

## Natural Modes of Infection

*M. tuberculosis* is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide. Persons infected with *M. tuberculosis* can develop active disease within months of infection or can remain latently infected and develop disease later in life. The primary focus of infection is the lungs, but most other organs can be involved. HIV infection is a serious risk factor for development of active disease. *M. bovis* is primarily found in animals but also can produce tuberculosis in humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and milk products, by handling of infected carcasses, and by inhalation. Infectious aerosols produced by coughing spread disease from person to person.

## Laboratory Safety and Containment Recommendations:

Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID50 <10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard.

BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Liquefaction and concentration of sputa for acid-fast staining may be conducted safely on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before processing.

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. Animal studies using guinea pigs or mice can be conducted at ABSL-2.111 BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. See Appendix B for additional information.

Manipulation of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria.

**Surveillance** Annual or semi-annual skin testing with purified protein derivative (PPD) of previously skin-test-negative personnel can be used as a surveillance procedure.

**Vaccines** The attenuated live BCG, is available and used in other countries but is not used in the United States for immunization

## Develop and implement a Safety Plan

Dr. Thomas has been approved for research with clinical specimens containing **Zika (*Flavivirus*) virus**, **Chikungunya** (***Alphavirus***) virus and wild type live mosquitos by the Institutional Biosafety Committee (IBC) to work at biosafety level 3 (BSL-3) as recommended in the agent summaries in the publication, CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL – 6$^{th}$ Edition) and also in the NIH Guidelines for Research Involving Recombinant DNA Molecules, which includes both procedural and facility approval for risk assessment, physical and biological containment. Work with **Sindbis virus** has also been approved by the Institutional Biosafety Committee. Sindbus virus will also be worked with in the BSL-3 facility but may be worked with at BSL-2 containment. All Chikungunya in vitro work being done in the BSL-3 facility and all animal and vector work ad in vivo analysis will be done in the BSL-3/ABSL-3 laboratory and/or insectary. Laboratory staff attend an awareness course where specific safety issues are discussed, including risk of infection, accidental exposure, proper notification of accidents, and what to do in an emergency occurs.

## ARBOVIRAL DISEASES (ZIKA, CHIKUNGUNYA DENGUE, WEST NILE VIRUS)

**Zika (*Flavivirus*)** is spread mostly by the bite of an infected Aedes species mosquito (Ae. aegypti and Ae. albopictus). Zika can be passed from a pregnant woman to her fetus. Infection during pregnancy can cause certain birth defects of the brain called microcephaly and other severe brain defects. It is also linked to other problems, such as miscarriage, stillbirth, and other birth defects. There have also been increased reports of Guillain-Barré syndrome, an uncommon sickness of the nervous system, in areas affected by Zika. Incubation period is usually 2-14 days for mosquito-borne viruses and there is no vaccine or medicine for Zika.

Clinical Features- Febrile illnesses usually lasting a week or less. Initial symptoms include fever, malaise, arthralgia, or myalgia. Occasional symptoms include nausea and vomiting, conjunctivitis, photophobia, or rash. Symptoms usually resolve within a week but may be markedly incapacitating for that time. For most of these viruses, infections are seldom fatal.

Causative agents- Single stranded ribonucleic acid (RNA) virus of various families, primarily *Flaviviridae* and *Bunyaviridae*, or double-stranded RNA viruses of the family *Reoviridae*.

Diagnosis- Arborviral infections that cause a febrile syndrome are confirmed most frequently by measurement of virus-specific antibody in serum. Acute-phase specimens should be tested for virus-specific immunoglobulin class M (IgM) antibody. For most arborviral infections IgM is detectable with in the first week after onset of illness and persists for several months, but longer persistence (years) has been documented. Therefore, a positive IgM test result may reflect a past infection. Approximately 50% of patients do not develop IgM antibodies until 2-4 weeks after illness onset. Serum immunoglobulin class G (IgG) antibody generally is detectable shortly after IgM and persists for years.

**Chikungunya** (***Alphavirus***) virus is spread to people by the bite of an infected mosquito. The most common symptoms of infection are fever and joint pain. Other symptoms may include headache, muscle pain, joint swelling, or rash. Outbreaks have occurred in countries in Africa, Asia, Europe, and the Indian and Pacific Oceans. In late 2013, chikungunya virus was found for the first time in the Americas on islands in the Caribbean. There is a risk that the virus will be imported to new areas by infected travelers. There is no vaccine to prevent or medicine to treat chikungunya virus infection.

Clinical features- Febrile disease characterized by mild to severe arthralgia or arthritis, primarily in the wrist, knee, ankle, and small joints of the extremities, lasting days to months. In many patients, onset of arthritis is followed after 1-10 days by a maculopapular rash, usually non-pruritic, affecting mainly the trunk and limbs; the rash may be pruritic with certain viruses. Buccal and palatal enanthema may occur. In infants, chikungunya viral infections typically cause vesiculobullous lesions. Rashes typically resolve within 7-10 days and can be followed by a fine desquamation. Myalgia, fatigue, headache, and lymphadenopathy are common. Conjunctivitis, paresthesia, and tenderness of palms, and soles occur in a small proportion of cases. With chikungunya, severe congenital infections, mild hemorrhagic manifestations, and rare death can occur. Mild hemorrhagic disease symptoms can also occur with Mayaro fever. Persistence of joint pains, arthritis, myalgia and/or fatigue occurs in 10%-50% of chikungunya cases.

Causative agents- ribonucleic acid (RNA) viruses of the family *Togaviridae*, genus *Alphavirus*

Diagnosis- Most commonly diagnosed by serological testing that shows immunoglobulin class M (IgM) antibodies in acute serum samples beginning at approximately 1 week after onset of illness and a rise in virus specific titers between acute and convalescent samples. IgM antibodies commonly persist for weeks to months. Diagnosis can also be made for some viruses by molecular methods such as

reverse transcription polymerase chain reaction (RT-PCR) on serum or by virus isolation from blood in the first few days of illness.

**Dengue (*Flavivirus*)** virus is spread to people by the bite of infective mosquitos, principally *Ae. Aegypti*. This day-biting species, with increased biting activity for 2 hours after sunrise and several hours before dusk. Dengue outbreaks have been attributed to *Ae. Aegypti* and to a lesser extent, *Aedes albopictus*. Patients are infective for mosquitos during their period of viremia, from shortly before, until the end of the febrile period. The mosquito becomes infective 8-12 days after the viremic blood-meal and remains so for life. Because of the approximately 7-day viremia in infected persons, bloodborne transmission is possible through exposure to infected blood, organs, or other tissues. In addition, perinatal DENV transmission occurs with the highest risk among infants born to mothers acutely ill around the time of delivery.

Clinical features- dengue is a mild to moderately severe acute febrile illness that usually follows three phases: febrile, critical, and convalescent. Patients with dengue often have sudden onset of fever, which lasts for 2-7 days and may be biphasic. Other signs and symptoms include intense headache, myalgia, arthralgia, bone pain, retro-orbital pain, anorexia, vomiting, macular or maculopapular rash, and minor hemorrhagic manifestations, including petechiae, ecchymosis, purpura, epistaxis, bleeding gums, hematuria, or a positive tourniquet test. Some patients have injected oropharynx, and facial erytherma, in the first 24-48 hours after onset. Warning signs of progression to severe dengue occur in the late febrile phase, around the time of defervescence, and include persistent vomiting, severe abdominal pain, mucosal bleeding, difficulty breathing, signs of hypovolemic shock, and rapid decline in platelet count with an increase in hematocrit (hemoconcentration).

Causative agent- Dengue viruses (DENV) are flaviviruses and include 4 types (serotypes; DENV -1, -2, -3, -4). All DENV serotypes can cause dengue and have been associated with severe dengue, including DHF/DSS with a fatal outcome.

Diagnosis- Laboratory confirmation of the clinical diagnosis of dengue can be made using a single serum specimen obtained during the febrile phase of the illness (days 0-7 after onset of fever) with diagnostic testing to detect DENV and immunoglobulin class M (IgM) anti-DENV. DENV viremia occurs 5-6 days before and after fever onset. Molecular diagnostics by nucleic acid amplification, such as by reverse transcriptase polymerase chain reaction (RT-PCR), can detect DENV ribonucleic acid (RNA) with a higher sensitivity than virus isolation by cell culture, and multiplex RT-PCR provides serotype-specific results. Molecular diagnostics are now available in many dengue endemic areas. DENV can also be detected by an immunoassay for the nonstructural protein 1 (NS1) antigen; a soluble antigen present during the viremic period. Detection of IgM anti-DENV in the febrile phase may indicate a current or recent DENV infection, or in some setting, infection with another flavivirus because of antibody cross-reactivity.

**West Nile Virus (*Flavivirus*)** is transmitted to humans primarily through the bite of infected mosquitoes, predominantly Culex mosquitoes. These mosquitoes feed mostly avidly from dusk to dawn and breed mostly in peridomestic standing water with high organic content or pools created by irrigation or rainfall. Humans usually do not develop a level or duration of viremia sufficient to infect mosquitoes. However, person-to-person WNV transmission can occur through blood transfusion and solid organ transplantation. Intrauterine transmission and probable transmission via human milk also

have been described but appear to be uncommon. Transmission through percutaneous and mucosal exposure have occurred in laboratory workers and occupational settings.

Clinical features- approximately 70%-80% of human West Nile virus (WNV) infections are asymptomatic. Most symptomatic people experience an acute systemic febrile illness that often includes headache, myalgia, or arthralgia; gastrointestinal tract symptoms and a transient maculopapular rash also are commonly reported. Less than 1% of infected people develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis. WNV meningitis is indistinguishable clinically from aseptic meningitis caused by most other viruses. Patients with WNV encephalitis usually present with seizures, mental status changes, focal neurologic deficits, or movement disorders. WNV acute flaccid paralysis often is clinically and pathologically identical to poliovirus- associated acute flaccid paralysis, with damage of anterior horn cells, and may progress to respiratory paralysis requiring mechanical ventilation. WNV-associated Guillian-Barre syndrome has also been reported and can be distinguished from WNV acute flaccid paralysis by clinical manifestations and electrophysiological testing. Cardiac dysrhythmias, myocarditis, rhabdomyolysis, optic neuritis, uveitis, chorioretinitis, orchitis, pancreatitis, and hepatitis, have been described rarely after WNV infection. Most patients with WNV nonneuroinvasive disease (West Nile fever) or meningitis recover completely, but fatigue, malaise, and weakness can linger for weeks or months.

Causative agent- WNV, of the family *Flaviviridae* and genus *Flavivirus*.

Diagnosis- Identifying anti0WNV immunoglobulin class M (IgM) antibodies in serum or cerebrospinal fluid (CSF) is the most common way to diagnose WNV infection. The presence of anti-WNV IgM usually is good evidence of recent WNV infection but may indicate infection with another closely related flavivirus. Because anti-WNV can persist in some patients for longer than 1 year, a positive test result occasionally may reflect past infection. IgM antibody to WNV develops in a majority of WNV-infected patients by the fourth day of symptom onset; 95% of infected patients develop IgM antibody within 7 days of symptom onset. Detection of WNV IgM in CSF is diagnostic of neuroinvasive disease.

**Viruses with BSL-3 Containment Recommended**

The recommendations for viruses that require BSL-3 containment are based on multiple criteria. SALS considered the laboratory experience for some viruses to be inadequate to assess risk, regardless of the available information regarding disease severity. In some cases, SALS recorded overt LAI transmitted by the aerosol route in the absence or non-use of protective vaccines, and considered that the natural disease in humans is potentially severe, life threatening, or causes residual damage.1 Arboviruses also were classified as requiring BSL-3 containment if they caused diseases in domestic animals in countries outside of the United States.

**Laboratory Safety and Containment Recommendations**

The agents listed in this group may be present in blood, CSF, urine, and exudates, depending on the specific agent and stage of disease. The primary laboratory hazards are exposure to aerosols of infectious solutions and animal bedding, accidental parenteral inoculation, and contact with broken skin. Some of these agents (e.g., VEE virus) may be relatively stable in dried blood or exudates. BSL-3 practices, containment equipment, and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animals, or arthropods.

Any respiratory protection equipment must be provided in accordance with the institution's respiratory protection program. Other degrees of respiratory protection may be warranted based on an assessment of risk as defined in Chapter 2 of this manual. All personnel in a laboratory with the infectious agent must use comparable personal protective equipment that meets or exceeds the requirements, even if they are not working with the organism. Sharps precautions as described under BSL-2 and BSL-3 requirements must be continually and strictly reinforced, regardless of whether investigational vaccines are used.

**Dealing with Unknown Arboviruses**

The ACAV has published reports documenting laboratory workers who acquired arbovirus infections during the course of their duties.6 In the first such document, it was recognized that these laboratory infections typically occurred by unnatural routes such as percutaneous or aerosol exposure, that "lab adapted" strains were still pathogenic for humans, and that as more laboratories worked with newly identified agents, the frequency of laboratory-acquired infections was increasing. Therefore, to assess the risk of these viruses and provide safety guidelines to those working with them, ACAV appointed SALS to evaluate the hazards of working with arboviruses in the laboratory setting.7,8

The SALS committee made a series of recommendations, published in 1980, describing four levels of laboratory practices and containment guidelines that were progressively more restrictive. These levels were determined after widely-distributed surveys evaluated numerous criteria for each particular virus including: 1) past occurrence of laboratory-acquired infections correlated with facilities and practices used; 2) volume of work performed as a measure of potential exposure risk; 3) immune status of laboratory personnel; 4) incidence and severity of naturally-acquired infections in adults; and 5) incidence of disease in animals outside the United States (to assess import risk).

While these criteria are still important factors to consider in any risk assessment for manipulating arboviruses in the laboratory, it is important to note that there have been many modifications to personal laboratory practices (e.g., working in BSC while wearing extensive personal protective equipment in contrast to working with viruses on an open bench top) and significant changes in laboratory equipment and facilities (e.g., BSC, PAPR) available since the initial SALS evaluation. Clearly, when dealing with a newly recognized arbovirus, there is insufficient previous experience with it; thus, the virus should be assigned a higher biosafety level. However, with increased ability to safely characterize viruses, the relationship to other disease-causing arboviruses can be established with reduced exposure to the investigators. Therefore, in addition to those established by SALS, additional assessment criteria should be considered.

One criterion for a newly identified arbovirus is a thorough description of how the virus will be handled and investigated. For example, experiments involving pure genetic analysis could be handled differently than those where the virus will be put into animals or arthropods. Additionally, an individual risk assessment should consider the fact that not all strains of a particular virus exhibit the same degree of pathogenicity or transmissibility. While variable pathogenicity occurs frequently with naturally identified strains, it is of particular note for strains that are modified in the laboratory. It may be tempting to assign biosafety levels to hybrid or chimeric strains based on the parental types but due to possible altered biohazard potential, assignment to a different biosafety level may be justified. A clear description of the strains involved should accompany any risk assessment.

Most of the identified arboviruses have been assigned biosafety levels; however, a number of those that are infrequently studied, newly identified, or have only single isolation events may not have been evaluated by SALS, ACAV, CDC, or the NIH (Table 6). Thorough risk assessment is important for all arboviral research and it is of particular importance for work involving unclassified viruses. A careful assessment by the laboratory director, institutional biosafety officer and safety committee, and as necessary, outside experts is necessary to minimize the risk of human, animal, and environmental exposure while allowing research to progress.

## Human Immunodeficiency Virus (from the BMBL 6th ed. Agent Summary)

### Occupational Infections

Data on occupational HIV transmission in laboratory workers are collected through CDC-supported national surveillance systems following a standardized case investigation protocol by state health department HIV staff with help from CDC. For surveillance purposes, laboratory workers are defined as those persons, including students and trainees, who have worked in a clinical or HIV laboratory setting anytime since 1978. Cases reported are classified as either documented or possible occupational transmission. Those classified as documented occupational transmission had evidence of HIV seroconversion (a negative HIV-antibody test at the time of the exposure which converted to positive) following a discrete percutaneous or mucocutaneous occupational exposure to blood, body fluids, or other clinical or laboratory specimens. As of 2013, confirmed HIV infections among 58 HCWs were reported, including 20 laboratory workers, of which only one involved a laboratory worker who sustained a needle exposure while working with an HIV-infected culture. There were another 49 HCWs exposed to HIV-infected blood, including four persons exposed to concentrated virus in a laboratory.

### Natural Modes of Infection

Retroviruses are widely distributed as infectious agents of vertebrates. Within the human population, spread is by close sexual contact or parenteral exposure through blood or blood products or other potentially infectious materials and from mother to child.

### Laboratory Safety and Containment Recommendations

HIV has been isolated from blood, semen, saliva, tears, urine, CSF, amniotic fluid, breast milk, cervical secretion, and tissues of infected persons and experimentally infected nonhuman primates.

Although the risk of occupationally acquired HIV is primarily through exposure to infected blood, it is also prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat, vomitus, and human breast milk. This also reduces the potential for exposure to other microorganisms that may cause other types of infections.

In the laboratory, virus should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other than intact skin) from a human (living or dead), in HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

The skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses during laboratory activities. It is unknown whether infection can occur via the respiratory tract. The need for using sharps in the laboratory should be evaluated. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other potentially infected materials.

BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids and tissues. HTLV-1 and HTLV-2 should also be handled at this level. Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BSL-2 facility, using BSL-3 practices. Activities involving large-scale volumes or preparation of concentrated HIV or SIV are conducted at BSL-3. ABSL-2 is appropriate for NHP and other animals infected with HIV or SIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2.

In addition to the aforementioned recommendations, persons working with HIV, SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.

Work with this organism should be conducted at a minimum of Biosafety Level 2 practices and containment equipment.

## 3. ***General Practices*** **(BMBL, 6th ed. – BSL-3)**

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

   **Site specific requirement:** No persons except those trained to work with BSL-3 or pathogenic agents are to enter the BSL-3 Labs area. **Exception:** Facilities Management personnel / contractors (electricians, plumbers, etc.) may enter to effect repairs after receiving proper training and wearing appropriate personal protective equipment for the agent(s) in use, and after the area has been decontaminated as much as possible and cleared by Environmental Health and Safety personnel.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

   **Site specific requirement:** Persons must remove their isolation gown, outer booties and gloves in the corridor of the BSL-3 suite, spray inner booties and gloves with disinfectant prior to entering the BSL-3 anteroom/airlock. After removal of other PPE, spray inner pair of booties and gloves with Vikron disinfectant and dispose the into the biohazard waste container. Personnel will washes their hands with soap and water or uses the automatic hand sanitizer. Then the person exits the airlock and enters the dressing room. See Appendix A, Entry/Exit Procedure for more complete information.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

**Site specific requirement:** No food or drink or smoking is allowed inside the BSL-3 facility. Makeup shall not be applied in the BSL-3 laboratories.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

**Site specific requirement:** Syringes should be handled with great care and only after adequate training.

i. After filling, excess fluid and bubbles should be expelled from syringes vertically into a plastic test tube containing a sterile cotton pledget to minimize aerosolization. This procedure should be performed in a class II biosafety cabinet.
ii. Sharps containers shall be readily available in each laboratory. All sharps including contaminated needles and syringes will be discarded in the sharp's container. **DO NOT RECAP CONTAMINATED NEEDLES.**
iii. Needles for drawing blood should also be placed in an appropriate sharps disposal container. **DO NOT RECAP CONTAMINATED NEEDLES.**
iv. Any sharps injury must immediately be reported to Environmental Health & Safety and the Principal Investigator. **Occupational exposures require immediate medical attention.** For bloodborne pathogen exposures, the *Contaminated Sharps Injury Report Form* shall be completed. This form is available on the EHSRM website at https://www.utrgv.edu/ehsrm/_files/documents/wci/wci-first-report-injury.pdf and is also in the Bloodborne Pathogen Exposure Control (appendix in this manual). The completed form may be emailed or faxed to WCI coordinator at (956) 665-2902. Contaminated sharps injuries must be reported to the Texas Department of State Health Services (Texas DSHS).

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces, for a minimum 30 minutes after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

**Site specific requirement:** Personnel working in the laboratory is responsible for decontamination of work surfaces at the completion of their work. At the end of each week, the floors of the labs shall be mopped with disinfectant. ACS is responsible for the BSL-3 corridor, the animal rooms, and the autoclave area.

8. Decontaminate, for a minimum 30 minutes, all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

**Site specific requirement:** All biohazard waste from the BSL-3 laboratories, including paper wrappers, paper towels, gloves, animal cages/bedding (none at this time), are to be placed in biohazard bags, which are closed prior to being taken to the autoclave. Waste should be autoclaved as soon as possible.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

**Site specific requirement:** UTRGV contracts with Esparza Pest Control for pest control services. For further information, please contact Facilities Management/Housekeeping. However, in the BSL-3 facility, sticky traps are placed at entrances to the BSL-3 suite and at the entrance to each laboratory and changed on a quarterly basis by ACS staff.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## **4.** *Special Practices for Biosafety Level 3*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

**Site Specific requirement: Medical surveillance**

Refer to the UTRGV's TB Exposure Control Plan for additional information.

i. Complete the TB screening questionnaire.

ii. A Tuberculin skin test (TST) is required annually for research personnel working with *Mycobacterium tuberculosis* in the EREBL BSL-3 facility. ACS personnel working within the BSL-3 facilities receive TB test prior to initial assignment to job duties, and on an annual basis. EHSRM staff occasionally enter the EREBL BSL-3 facilities, but are not actively conducting research with *M. tuberculosis*, shall receive annual TB skin testing. Anyone unable to have this test for any reason, must have a chest X-ray negative for TB prior to initial assignment to the area, and should monitor themselves for signs and symptoms of exposure thereafter. Signs and symptoms include:

- General weakness, weight loss, fever and night sweats.
- Persistent cough (> 2 weeks), chest pain upon coughing, and coughing up blood and / or mucous.

iii. Facilities Management personnel who enter the BSL-3 facility or work on any of the BSL-3 exhaust systems, shall have PPD skin testing performed prior to initial assignment to job duties, and at least annually thereafter. Personnel unable to have the skin test or blood test for any reason, shall have a chest X-Ray negative for TB, and monitor themselves for symptoms of exposure as indicated in (c)iii above.

iv. Individuals with a potential exposure to human blood, body fluids, tissues, human cell lines shall be offered the Hepatitis B Virus vaccine as outlined in the Exposure Control Plan – Appendix to this manual.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

**Site Specific requirement**: This manual is located in the corridor of the BSL-3 facility by the autoclave.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.

**Site Specific requirement:** Training to be received: (for more detailed information, see Section 8 of this manual)

- Basic Biological Safety (BSL-2) training annually
- Bloodborne Pathogen Safety training annually (clinical)
- Infectious Substance Shipping
  (Required ONLY for those who prepare and ship the package)
- Initial BSL-3 training and refresher annually
- Visitor / Facilities Mgmt/contactor (non-authorized personnel) overview training

- Agent-specific procedures/training conducted by the Principal Investigator

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated for a minimum 30 minutes, and cleaned up by staff properly trained and equipped to work with infectious material.

b. Equipment must be decontaminated, for a minimum 30 minutes, before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biological safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided, and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

## 5. Safety and Personal Protective Equipment

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC **(**preferably Class II or Class III), or other physical containment devices.

2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated, for a minimum 30 minutes, with the appropriate disinfectant before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

**Site Specific requirement:** Powered Air Purifying Respirator (PAPR) or N-95 respirators must be worn by all individuals entering the BSL-3 facility including research personnel working with the infectious agents, *Mycobacterium tuberculosis*. These units have the required eye and

face protection and are decontaminated for a minimum 30 minutes when they are removed. See Entry/Exit procedure in Appendix A for more information on the required Personal Protective Equipment. See Entry/Exit procedure in Appendix A for more information on the required Personal Protective Equipment.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:

a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

**Site Specific requirement:** Latex and nitrile gloves are available in the BSL-3 facility. MED BSL-3 entry procedure requires wearing two pairs of gloves. Outer gloves are changed when visibly contaminated or integrity has been compromised. After handling infectious agents in the biosafety cabinet, outer gloves are sprayed with disinfectant before touching anything outside the BSC.

**b.** Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

**Site Specific requirement:** PPE requirements are in effect for the entire BSL-3 suite. Therefore, PPE is not removed when exiting an individual laboratory. Gloves are sprayed with disinfectant prior to exiting the laboratory. When preparing to exit the BSL-3 suite, the outer pair of gloves are removed in the BSL-3 corridor and put into a regulated medical waste container. The inner pair of gloves are sprayed with disinfectant (Peroxigard) prior to exiting into the airlock. Inner gloves are removed in the airlock and hands are immediately washed with soap and water.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

**Site Specific requirement: Currently there are no animals in the BSL-3 facility.** However, because of site specific requirements for working in the BSL-3 facility, PAPR and other PPE is donned in the airlock prior to entry into the BSL-3 corridor and laboratories. See Entry/Exit procedure in Appendix A for more information on the required Personal Protective Equipment.

## 6. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self closing and have locks in accordance with the institutional policies.

a. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building.

b. Access to the laboratory is restricted to entry by a series of two self-closing doors.

c. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.

**Site specific requirement:** each BSL-3 lab do have hands-free sinks and a sink in the anteroom.

3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory must be sealed.

**Site specific requirement:** UTRGV BSL-3 facilities have no windows.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

**Site specific requirement:** Biosafety cabinets in the BSL-3 are located away from doors, supply vents and are in an area of the laboratory where traffic is minimized.

7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

**Site specific requirement:** Biosafety cabinets that have a vacuum line are protected by a HEPA filter.

8. An eyewash station must be readily available in the laboratory.

9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

a. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

b. The laboratory exhaust air must not re-circulate to any other area of the bldg.

c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

**Site specific requirement:** BSL-3 suite laboratories were designed with sustained directional airflow from clean areas toward the more potentially contaminated areas. The laboratories are negative with respect to the inner BSL-3 corridor. Air monitoring devices include visual ping-pong ball airflow devices. The laboratory exhaust air is not recirculated and is exhausted from the building through HEPA filters.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated in the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

**Site specific requirement**: Laboratories in the BSL-3 facility have either Class II A2 or Class II B2 biosafety cabinets in all laboratories. Ducted cabinets have HEPA filtered exhaust outside the building. All biosafety cabinets are tested and certified to NSF 49 standards annually by a certified contactor.

11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

**Site specific requirement:** The BSL-3 facility has a pass-thru autoclave for decontaminating waste. Laboratory personnel will disinfect/deactivate biological materials in accordance with institutional policy prior to disposal into a biological waste container.

12. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

**Site specific requirement:** Currently, aerosol chamber is not used.

13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

**Site specific requirement:** Agent summary statement for *M. tuberculosis* in the 6th ed. BMBL were considered in determining the required Personal Protective Equipment which is described in detail in the Entry/Exit Procedures. The BSL-3 facility (including laboratories) was built with enhancements which include, anteroom, gas tight dampers, HEPA filters with bag-in/bag-out, final HEPA filtration of laboratory exhaust. HEPA filter housing allows for leak testing and is certified annually by an NSF-49 certified contractor.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

**Site specific requirement:** Annual recertification of the facility is conducted on an annual basis by a contractor experienced in BSL-3 operations. Documentation is provided by the contractor.

## 7. GENERAL RULES FOR BSL-3 FACILITY:

Before entering the BSL-3 Labs, all personnel including visitors and contractors, are required to don scrubs. Persons must wear respiratory protection (i.e. PAPR), closed front gown and shoe covers. Latex, nitrile, or chloroprene gloves must be worn at all times while working within the BSL-3 Labs. Double gloves are required. **Disposable gloves are not to be reused. Refer to Appendix A of this manual for complete Entry/Exit procedure.**

- **ii.** Immediately upon exiting the BSL-3 Labs and while in the BSL-3 corridor, personnel must remove outer shoe covers, outer gloves and gown into the biohazard container, then spray the bottom of their inner shoe covers and inner gloves. Immediately enter the air-lock room. All personnel must discard their inner shoe covers and gloves into the appropriate biohazard bag / container. PAPR and other respirators must be chemically disinfected and placed on a charger.
- **iii.** All materials must be autoclaved or chemically decontaminated for a minimum 30 minutes, before removal from the BSL-3 Suite.
- **iv.** All cultures must be processed only within a Class II BSC. Material within this cabinet must be placed in a biohazard bag prior to removal from the unit and then autoclaved as soon as possible after removal.
- **v.** At all times, extreme care must be taken to avoid aerosolization of the infectious organisms. This is to include the discard of cultures in 10% bleach (or other appropriate

disinfectant) bath or in bags for autoclaving. All plate cultures should be sealed at the edges using scotch tape or autoclave tape and all such cultures should be maintained in the designated incubators during incubations. Centrifugation steps must be performed in the safety centrifuge cups or aerosol resistant chambers.

**vi.** Viable cultures of *M. tuberculosis* should be maintained in the biosafety cabinets or in the incubators and at no time should these cultures be stored outside of these designated areas.

**vii.** At all times have squirt bottles of 10% Bleach, Peroxigard, or other EPA registered disinfectant within the BSL-3 Labs area in the case of a spill. If using 10% Bleach, then it must be made fresh daily.

**viii.** Hemocytometers are to be loaded within the hood using 0.1 N HCl as a diluent. Discard used hemocytometers in a beaker containing 10% formalin or 1% bleach.

**ix.** Items containing biohazardous agents that are to be transported between laboratories in the BSL-3 suite must be put in secondary containers that are closed and sealed. At no time shall plates, tubes, etc. of biohazardous agents be carried out of the biosafety cabinet or incubators without secondary containment.

**x. In the event of an accident (spill) outside the containment of the biosafety cabinet:**

- If small spill, cover spill with paper towels or other adsorbent material and pour disinfectant over paper towels. Personnel should leave the BSL-3 lab and place a sign on the door to prevent anyone from entering the contaminated area. The design of the negative air pressure is such that the biological agents should be exhausted through the biosafety cabinets that contain HEPA filters. Remove contaminated PPE prior to exiting into the airlock.
- Inform the Laboratory Director or Principal Investigator, Environmental Health and Safety personnel (Responsible Official), and LAR personnel as listed in Appendix C: *Call Down List for Emergencies.* **THIS MUST BE DONE IMMEDIATELY TO ENSURE THAT PROPER MEASURES ARE TAKEN.** If none of these persons are available, then inform the UTRGV Police Dispatcher.
- Be sure that the details of the accident are reported to the physician-in-charge at the chosen medical provider so that an appropriate medical evaluation for *M. tuberculosis* exposure will be undertaken.
- Complete Incident Report and First Report of Injury or Illness forms and send to the WCI Risk Manager in the Environmental Health and Safety office (email or fax 956-665-2699).

**xi. BROKEN GLASS – BIOHAZARDOUS OR NON-BIOHAZARDOUS**

Any broken non-biohazardous glass in the BSL-3 facility will be picked up with tongs or a dust pan and treated as biohazardous and placed in a puncture-resistant container. This container shall be autoclaved prior to removal from the BSL-3 facility.

## K. DECONTAMINATION AND STERILIZATION

**General Procedures:**

**i.** Biohazardous materials or contaminated items should be autoclaved or chemically disinfected as soon as possible.

**ii.** All materials and contaminated items should be placed in an appropriately marked place, or decontaminated for a minimum 30 minutes at the close of each workday. This will minimize the exposure hazard to firemen, disaster crews, or maintenance personnel in the unlikely event of a disaster.

**iii.** All autoclaves should be monitored for operating efficiency on a monthly basis using commercially available biological indicators (bacterial spore). The results from the biological indicators shall be recorded in the appropriate departmental manual. Results should be reported to the supervisor or the safety manager for the department.

**iv.** All laboratory areas working with biohazardous materials should have leak proof, covered containers for decontaminating items.

**v.** All floors, laboratory benches, and other surfaces in areas where biohazardous materials are handled should be chemically decontaminated, for a minimum 30 minutes, as often as deemed necessary by the supervisor, but minimally after any spill, and at the end of each working shift. The supervisor may choose the chemical decontaminant to be used.

**vi.** Upon completion of operations involving plating, pipetting, centrifugation, and similar procedures with biohazardous materials, the surrounding area **must** be chemically decontaminated for a minimum 30 minutes.

**vii.** Floors in the Biosafety Level 3 facility are to be mopped weekly with an appropriate disinfectant. If work has been done in the BSL-3 laboratory that week, then the lab should also be mopped. Water used to mop floors **must** contain a chemical disinfectant (i.e. Century Q, an EPA registered disinfectant, used at a dilution of ½ oz. per gallon of water). Each laboratory is responsible for mopping inside their lab. ACS is responsible for mopping the animal rooms, autoclave room and the corridor. Mop bucket will be stored in the janitor's closet.

**viii.** Stock solutions of suitable chemical decontaminates **must** be maintained in each laboratory for decontamination purposes. Before work is started, appropriately diluted agents **must** be available at the workstation for immediate use in case of a spill.

**Sterilization.**

**ix.** **Autoclaves.** Our autoclaves are designed to generate steam under pressure (121$^{o}$C/15 lbs.) for sterilization. The autoclaves must not be overloaded. If the autoclaves are overloaded, they may not effectively sterilize the items being treated. Items are placed in a pan or on the tray and 3 liters of deionized water added to the bottom of the autoclave. To start the autoclave cycle, press the reset button, set the exhaust toggle to the appropriate setting (fast for dry items or slow for liquids), and turn the timer to the appropriate time. The time required will vary depending upon the application. Clean instruments or pipette tips will be sterilized in approximately 15-20 minutes. Directions on time will be given on the label of microbiological media. These times will range from 15 -25 minutes. If an item is not labile to autoclaving, the autoclaving procedure should be extended to 30 minutes. To sterilize contaminated equipment, media, or supplies or other heavily soiled items, especially if the soil is of proteinaceous nature, autoclaving should be for longer periods of time. The reason is that soil may protect the microorganism from the lethal effects of the wet heat. Because of this, an exposure time of 45-60 minutes for soiled items is not unreasonable. Pathological Waste is to be autoclaved for a minimum of 2 hours.

x. **Dry Heat.** The use of dry heat for sterilizing glassware or other heat-resistant equipment is less efficient than autoclaving and requires a longer exposure time with higher temperatures. It may be possible to sterilize these items by exposing them to 160ºC for 3-4 hours. These conditions also render glassware endotoxin-free.

xi. **Chemical disinfection.** There are several acceptable chemical methods for decontaminating biologicals in our laboratories. These are discussed below.

- Peroxigard – This disinfectant is routinely used in cleaning and disinfecting in industrial, life science research facilities, vivariums, laboratories, and other animal housing facilities. This disinfectant is effective against viruses, bacteria, and fungi. The active ingredients for Peroxigard include 0.5% Hydrogen Peroxide, water, surfactant blend (proprietary), solvent (proprietary), Phosphoric Acid, Peroxide Stabilizer (proprietary), Corrosion Inhibitor Blend (proprietary).
- Vikron S – This disinfectant is routinely used in cleaning and disinfecting industrial, animal, and agricultural facilities. The disinfectant is effective against viruses, bacteria, and fungi. The active ingredients for Vikron S include Potassium peroxymonosulfate 21.41%, Sodium chloride 1.50%, Other ingredients 77.09%, equivalent to 9.75% available chlorine. The broad spectrum efficacy of Vikron S has been proven effective against: 61 strains of virus, 33 strains of bacteria, and 7 strains of fungi.
- Clidox - For samples that are potentially biohazardous that will be autoclaved and discarded we are currently using Clidox. The active ingredients are chlorine dioxide and the working dilution for all our applications is 1:10:1 (base:water:activator) . This dilution is stable for 7 days. The manufacturer states that this preparation is biocidal for mycobacteria, fungi, bacteria, and viruses. Exposure time is recommended that surfaces be wet for at least 10 minutes. Typical uses for this item are for swabbing work surfaces prior to and after use and Clidox can be placed in waste discard carboys and trays for decontamination of pipettes, tips, loops, and surgical instruments.
- LpHse – This disinfectant is used in the disinfectant trays that hold forceps. Working dilution is ½ oz. per gallon of water. Undiluted LpHse is used for the sink traps.
- Century Q 256 – This disinfectant is routinely use for mopping the floors. Working dilution is ½ oz. per gallon of water.
- Sodium hypochlorite. This disinfectant is standard household sodium hypochlorite (bleach) in a 5.25% solution. The working dilution is a 1% final concentration (1:5 dilution in water) and must be prepared fresh daily. Contact time is recommended for at least 10 minutes. Typical uses for this disinfectant is in waste discard containers and in containers for decontaminating surgical instruments, homogenizers, and hemacytometers and coverslips used for counting cells from all human sources or from infected animals. Concentrated stock bleach can also be used in discard waste containers so that the final dilution with samples does not exceed 1% final bleach**. <u>This solution should not be autoclaved.</u>**
- Formalin. Formalin is typically a 37% solution of formaldehyde. A working dilution is 10% (1:10 dilution of the 37% stock) and contact time is

recommended for at least 10 minutes. Typical uses for this disinfectant are for decontaminating surgical instruments, homogenizers, and hemacytometers and coverslips, which are used for counting cells from all human sources, or from infected animals. **This solution should not be autoclaved**.

- LpHse – This disinfectant is used in the disinfectant trays that hold forceps. Working dilution is ½ oz. per gallon of water. Undiluted LpHse is used for the sink traps.
- Century Q 256 – This disinfectant is routinely use for mopping the floors. Working dilution is ½ oz. per gallon of water.
- **Roccal II**. Roccal II is a 10% solution of dimethyl benzyl ammonium chloride. A working dilution of 15 ml to 4 liters of water is recommended. This compound is used for providing a decontaminant for water baths and inside the jackets of the $CO_2$ incubators.

**L. General precautions:**

**a.** Most of the manipulations of microbial cultures common in research laboratories, release aerosols of viable organisms. The release of viable aerosols during routine laboratory manipulations must be considered when evaluating the individual degree of risk. Keeping in mind the four main factors governing infections: dosage, virulence, portal of entry, and state of resistance.

**i. Protective clothing (BSL-3)**

1. All personnel will wear at a minimum, scrubs, closed front gown, respiratory protection, shoe covers, and gloves before entering the area.
2. Scrubs. Disposable scrubs and shoe covers are donned in the men's or women's changing rooms.
3. Gowns and shoe covers. In the airlock, don closed-front wrap around gowns or Tyvek coverall, that are made of water-resistant material and totally cover the front of the worker, creating a barrier to splashing of biohazardous material. At the end of each workday, each gown will be placed in the autoclave bag for decontamination. If wearing a non-disposable gown and the gown becomes contaminated, the surface should be wetted with an appropriate disinfectant and the gown should be removed and immediately autoclaved. A second pair of water-resistant shoe covers are used to cover the shoes. This outer pair of shoe covers are removed in the corridor and discarded to ensure that no material biological material leaves the BSL-3 suite.
4. Respiratory Protection. Respiratory protective devices are available for use in our BSL-3 lab. The Power Air Purifying Respirator (PAPR) is the respirator of choice for the BSL-3 facility. All employees must have an initial medical evaluation / questionnaire prior to wearing a respirator. Persons wearing a PAPR (RACAL HOOD) do not require fit-testing. Facial hair may impair the function of any mask. Personnel using other respirators (North or P100 particulate filter) must be fit-tested and approved before being permitted to perform work in the BSL-3 lab.

a. Power Air Purifying Respirator (PAPR): This is the preferred respirator for working in the *M. tuberculosis* laboratory. The highest level of protection is provided by the Powered Air Purifying Respirator (PAPR), outfitted with HEPA or P-100 filters. This respirator can normally be used one complete 6-hour shift without loss of charge if batteries are fully charged. This PAPR

blows positive pressure HEPA filtered air across the user's breathing zone, and is designed for biological agent/particulate work in the BSL-3 area. It is not designed for use with hazardous chemicals or gases. No fit testing is required when using a PAPR, and moderate facial hair is allowed.

b. Moldex 8000 Series Respirator: The Moldex 8000 respirator with a P100 particulate filter provides a higher level of protection for the employee. It must be fit-tested for each individual to ensure that the employee can properly place the respirator on their face. If a half face respirator is worn, safety glasses must also be worn in animal rooms.

5. Protective laboratory clothing (including scrubs) will not be worn outside the BSL-3 containment facility.

**i. Decontamination (BSL-3)**

All materials and equipment must be decontaminated. for a minimum 30 minutes, prior to removal from the facility. Laboratory notes of experiments should not be removed from the area. If work requiring biocontainment is being done, cultures and other material, that are labile to autoclaving, may be removed from the area only if appropriate measures are taken to insure against contamination of the container by the BSL-3 agent. Secondary containers should be sprayed with disinfectant prior to taking out of containment.

- All working surfaces will be decontaminated, for a minimum 30 minutes, at the end of each workday using the decontaminant specific to the infective agent. The choice of decontaminant is the responsibility of the laboratory director or principal investigator assigned to the project.
- It is the responsibility of each Principal Investigator and the research staff to mop the floor with disinfectant at least once a week if work is being done in the lab. ACS will be responsible for mopping the common area and animals rooms.

**ii. Emergency action in event of spill or other release of BSL-3 agent**. The person discovering the condition will:

- For small spills, cover with paper towels, saturate with disinfectant. If outer gloves are contaminated, change outer gloves. Leave the BSL-3 laboratory and notify others in the work area to leave.
- Alert personnel listed in Appendix C: *Call Down List for Emergencies* (located by phone in the BSL-3 corridor) giving as much detail about the incident as is available.
  - **a.** Upon notification of a release of any BSL-3 agent (spill outside of containment), the laboratory director will :

1. Notify EHSRM Safety personnel listed in Appendix C: *Call Down List for Emergencies*. The UTRGV *Emergency Response and Evacuation Plan will* be initiated if the situation warrants. The laboratory director in conjunction with EHSRM will perform an assessment and determine the best decontaminant and procedure to decontaminate the area. For a large spill, room decontamination may need to be performed by an outside contractor.

**b.** Complete an Incident Report and forward it to the Biosafety Officer as soon as possible.

**c.** A summary of this procedure will be visibly posted within the BSL-3 Lab.

**L. Biohazards containment equipment (BSL-3):**

**i. Class II Biological Safety Cabinets (BSC)**

1. A suitable, ventilated safety cabinet containing a high efficiency particulate air (HEPA) filter is recommended for carrying out all procedures with biohazardous materials. Procedures that must be carried out in such containment equipment include opening of test tubes, flasks, and bottles, using pipettes, making dilutions, inoculating and autopsying animals, grinding tissue, blending cultures, opening lyophilized material from ampoules or any other process that may potentially generate aerosols.
2. The supervisor is responsible for ensuring the proper use of containment equipment. Tubes containing biohazardous materials must be manipulated with care. Studies have shown that simple procedures such as removing a tube cap or transferring an inoculum can create a potentially hazardous aerosol. Manipulation of biohazardous material must be conducted in safety cabinets.
3. These units are certified annually to current NSF 49 field testing requirements by an NSF accredited contractor.
4. Refer to Appendix B – *Safe Operation, Use, and Maintenance of a Biological Safety Cabinet (BSC)* for specific procedures.

**ii. Mouse isolator units** (Note: <u>no</u> animal work is currently performed)

1. This is a self-contained unit designed to hold cages of experimentally infected animals. The unit is on continuously when animals are in the room. It draws room air over the cages and filters any potential aerosol through the HEPA filter.
2. This unit must be certified annually.

**iii. Small Animal Cage Changing Stations**

1. This negative airflow unit is equipped with a prefilter and exhaust is HEPA filtered and is used for changing cages of infected and noninfected animals.
2. This unit is certified semi-annually.

**M. CDC/NIH Biosafety Level 3 recommendations.** Procedures for working in the BSL-3 facility follow the recommendations in the CDC-NIH publication *Biosafety in Microbiological and Biomedical Laboratories, 6$^{th}$ Ed.* It can also be viewed on the

CDC website at Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition | CDC Laboratory Portal | CDC

N. **Infected Animals**. All animal infections will be performed within a biological safety cabinet or utilizing a self-contained aerosol exposure chamber (with HEPA filtered exhaust) within the BSL-3 labs. Infected animals will be housed in appropriate cages within the HEPA animal racks of the BSL-3 animal rooms. Dirty cages, lids, and water bottles will be autoclaved before dumping of used litter and removal from the BSL-3 facility for cleaning. After euthanasia, all infected animals will be autoclaved before disposal.

O. **Facilities Maintenance**. All personnel performing routine repairs within the BSL-3 facility should provide one day notice before entering the facility. Routine maintenance should be scheduled for the early in the workday to allow for overnight air exchange to minimize risk of airborne microorganisms. No work will be performed until maintenance workers have left the facility. All tools should be decontaminated, for a minimum 30 minutes, before leaving the facility. Workers will follow entry / exit procedures in Appendix A: *Access (Entry/Exit) Procedures for Facilities Maintenance / Contractors / Visitors*.

P. **Housekeeping.** Within each of the BSL-3 laboratories, routine housekeeping will be performed by research personnel and will include daily wipe-down of active work surfaces and minimum weekly mopping of the floor with a germicidal solution. Hallways and common use areas will be maintained by LAR personnel.

Q. **Cage Washer:** After cages are autoclaved out of the BSL-3 facility, cages are washed in a cage washer. The mechanical cage washer must have a final rinse temperature of at least 180°F. (BMBL p. 86)

R. **Sink traps:** A hands free sink is provided in each animal room and he sink trap is filled with the appropriate disinfectant. (BMBL p. 77) Sink traps in the animal rooms are checked on a daily basis by ACS personnel and refilled as needed as part of their maintenance checklist. After use of the sink, research staff shall check the sink traps in their laboratories and refill with disinfectant as needed.

**SPECIFIC RECOMMENDATIONS FOR WORKING WITH SAMPLES FROM HUMANS**

**A. Introduction:**

**i.** Any sample from patients or healthy donor (controls) should be considered infectious and **standard / universal precautions** used at all times. Potential organisms that could be present include: *Mycobacterium tuberculosis* (potentially drug resistant strains), hepatitis viruses, and the human immunodeficiency virus-I (HIV-1). Infection with HIV-1 can result from direct contact with blood or other biological specimens that contain HIV-1. Therefore, all processing and cultures must be performed in strict adherence to the guidelines listed below and the revised guidelines set forth by the Centers for Disease Control and Prevention (see Appendix D, copied from MMWR, Vol. 37(24); 377-388, June 1988).

**ii.** There are to be no deviations from these recommended protocols.

**iii.** Assume standard blood and body fluid (universal) precautions for all biological samples and take appropriate action. All blood, body fluids and tissue from patients must be considered infective.

**B. Prevent Hand to Mouth Contamination:**

**i.** Do not eat, drink, smoke, or apply cosmetics in the laboratory work areas.

**ii.** Store and consume food only in designated areas.

**iii.** Do not pipette by mouth; use mechanical pipetting devices.

**iv.** Wash your hands frequently and **ALWAYS** before leaving the laboratory.

**v.** Limit the movement of hazardous materials to another department, provide appropriate containment measures and label the container/material carefully. Potentially hazardous fluid cultures or powders that are kept in glass vessels should be transported, incubated, and stored in easily handled non-breakable leak-proof pans, trays, pails, carboy holders, or containers that are large enough to contain all of the fluid or powder in case of leakage or breakage of the glass vessel. All inoculated Petri plates or other inoculated solid media should be transported and incubated in leak-proof containers with an appropriate label.

**vi.** All infected samples and material will be decontaminated, for a minimum 30 minutes, as quickly as possible using one of the appropriate methods described in part 5 (Decontamination and Sterilization) of this section.

**C. Phlebotomy precautions: (Phlebotomy is performed outside of the BSL-3 facility)**

i. Latex, nitrile, or chloroprene gloves, a protective visor (or a mask and protective eye wear), and a lab coat or gown must be worn.

ii. Immediately after performing the venipuncture, the needle and Vacutainer syringe are to be placed directly into a puncture-resistant container for disposal. Do not under any circumstances attempt to remove, bend, or recap the needle. In the event that blood or any other bio-fluid from the patient splashes onto you, immediately flood the area with water and then wash thoroughly with copious amounts of soap and water.

iii. The specimen(s) is to be transported immediately to the BSL-3 Labs and placed within a Class II BSC within that laboratory until processed.

D. **Disinfectant:**

i. At all times have full squirt bottles of disinfectant within the BSL-3 Labs area in the case of a spill. If using bleach, 10% bleach (1:10 dilution) shall be made fresh daily.

E. **Specimen processing:**

**i.** Specimens are to be processed only within the BSL-3 Labs and within the Class II BSCs.

**ii.** Two pairs of latex, nitrile, or chloroprene examination gloves must be worn at all times. Both pairs must be changed immediately if contaminated and discarded in a biohazard leak-proof container within the biosafety hood.

**iii.** A disposable or autoclavable liquid resistant gown must be worn at all times to avoid contamination of skin or clothing. All personnel must wear a mask and a protective face shield at all times.

**iv.** It is recommended that an absorbent mat or underpad be placed on the work surface within the biosafety cabinet. This is to be discarded in the biohazard bag after completing the specimen processing.

**v.** Disposable plastic tubes and pipettes are to be used. Glass tubes, glass pipettes, and needles are to be avoided at all times.

**vi.** Liquid waste and contaminated plastic ware should be placed within a puncture-resistant container with disinfectant.

**vii.** All pipetting should be done using a hand-held automatic pipetting device. There is to be no mouth pipetting under any circumstance.

**viii.** Hemacytometers are to be loaded within the hood using 0.1 N HCl as a diluent. Discard used hemacytometers in a beaker containing 10% formalin or 1% bleach.

**ix.** Any spill is to be flooded immediately and thoroughly with disinfectant. Care must be taken to discard all towels and wipes in the biohazard bag and to change gloves after the decontamination process.

**x.** All cells and cell cultures from patients or healthy donors are to be maintained in the biosafety hoods or in the incubators and at no time should these cultures be stored outside of these designated areas.

**xi.** After completing the specimen processing, remove the biohazard bags and containers and autoclave immediately. Wipe down the interior surface of the biosafety hood with disinfectant.

**xii.** Immediately after exiting the BSL-3 Labs and while within the air-lock entry room, all personnel must discard their lab coats, mask, and gloves into the proper biohazard bag. Personnel must then wash their hands thoroughly before leaving the BSL-3 lab.

F. **Cell culture precautions:**

**NOTE**: Tissue culture assays for lymphocyte proliferation are extremely biohazardous because the T cells have been stimulated with mitogens and antigens, which results in an increased replication of HIV- I. It is imperative, therefore, that extreme care be exercised in harvesting these mononuclear cell cultures. Specific guidelines are as follows:

i. The MASH harvester must be used within the Class II bio-cabinet. This is to include the vacuum pump in order to avoid aerosolization of infectious particles through the pump exhaust.

ii. The harvester should be rinsed with 10% formalin before and after use.

iii. A vacuum trap filter should be placed between the pump and the MASH unit waste bottle.

iv. After harvesting, the cellulose acetate papers should be dried and transferred to screw-capped vials containing liquid scintillation fluid. This entire procedure is to be performed within the Class II bio-cabinet.

G. **In the event of an accident:**

**a.** All personnel should leave the BSL-3 lab. Place signs on the doors to prevent anyone from entering the contaminated area.

i. Follow procedures as outlined in L, ii of this section.

ii. Refer to the UTRGV Exposure Control Plan and Post Exposure Policy for specifics on reporting, treatment, and prophylactic procedures following a potential bloodborne pathogen exposure.

## Section 6: General Safety and Hazardous Materials Management Plan

**Purpose:** The following section describes procedures and behaviors for working in the laboratory to ensure that all workers are at minimal risk for exposure to potential hazards. Primary potential hazards found in the laboratories include: biological (covered in the previous section), chemical, radioisotope, cryogenic and electrical.

### 1. GENERAL SAFETY IN THE LABORATORY

All workers should be familiar with the following procedures and, if any technique is not fully understood, it is the worker's responsibility to inform the supervisor to obtain adequate training. This section applies to the 1st floor BSL-3 laboratory settings except where specifically noted otherwise.

### 2. CHEMICAL SAFETY: HAZARD COMMUNICATION & CHEMICAL HYGIENE PLAN:

UTRGV complies with 29 CFR 1910.1200 (OSHA Hazard Communication Act) and 29 CFR 1910.1450 (Occupational Exposure to Hazardous Chemicals in the Laboratory)

**Standard Operating Procedures for Laboratory Chemicals**

A. **Chemical Procurement.** Personnel who receive chemical shipments shall be knowledgeable of the proper procedures for receipt. Chemical containers should be checked upon receipt for proper label, if not properly labeled contact the Laboratory Director. All chemical shipments should be dated when received, when the chemical is opened for use and an expiration date if applicable.

B. **New Hazardous Chemicals.** Any new chemicals received which are classified as hazardous must be added to the hazardous chemical inventory list and a copy of the SDS must be obtained from the manufacturer or from the SDS Pro database.

C. **Chemical Storage.** Received chemicals shall be immediately moved to the designated storage area within the section. Large glass containers of hazardous materials shall be placed in carrying containers for corrosives or safety cans for flammables. The storage area shall be well illuminated. Large bottles should be stored no more than three feet from ground level. Hazardous chemicals shall be segregated by hazard classification and compatibility with flammable and corrosives signs posted in storage area.

- Storage of chemicals at the lab bench or other work areas shall be limited to the amount necessary for operation. The container size shall be the minimum convenient. Chemicals in the work place shall not be exposed to sunlight or heat.
- Stored chemicals shall be examined at least annually by the section supervisor for replacement, deterioration, label clarity, and container integrity. The inspection should determine whether any corrosion, deterioration, or damage has occurred to the storage facility as a result of leaking chemicals.

D. **Chemical Handling.** Each laboratory employee with the training, education and resources provided by supervision, shall develop and implement work habits to minimize personal and coworkers exposure to the chemicals in the laboratory. Based on the realization that all chemicals inherently present hazards in certain conditions, exposure to all chemicals shall be minimized. General precautions which shall be followed for the handling and use of all chemicals are:

- Skin contact with all chemicals shall be avoided.
- All employees shall wash all areas of exposed skin prior to leaving the laboratory.
- Mouth suction for pipetting or starting a siphon is prohibited.
- Eating, drinking, smoking, gum chewing, or application of cosmetics in areas where laboratory chemicals are present shall be avoided. Hands shall be thoroughly washed prior to performing these activities.
- Storage areas, refrigerators, glassware or utensils used in laboratory operations shall not be used in the storing or handling of food or beverages.
- Risk determinations shall be conservative in nature.
- Any chemical mixture shall be assumed to be as toxic as its most toxic component.
- Substances of unknown toxicity shall be assumed to be toxic.
- Laboratory employees shall be familiar with the symptoms of exposure for the chemicals with which they work and the precautions necessary to prevent exposure.
- The intent and procedures of this Chemical Hygiene Plan shall be continuously adhered to.
- In all cases of chemical exposure, neither the OSHA Permissible Exposure Limits (PELs) nor the Threshold Limit Values (TLVs) of the American Conference of Governmental Industrial Hygienists (ACGIH) shall be exceeded.
- The engineering controls and safety equipment in the laboratory shall be tested and inspected at least annually by Institutional Safety & Facilities Management personnel.
- Specific precautions based on the toxicological characteristics of individual chemicals shall be implemented as deemed necessary by the laboratory director.

E. **Work Area and Workplace Chemical Lists.** The workplace is defined as the laboratories at the MED BSL-3 and each section of the laboratory is defined as a work area.

- A **work area chemical list** containing all chemicals in a particular section or room will be developed. The section supervisor when notified by the Laboratory director or Principal Investigator will update this list annually. The work area chemical list shall contain the chemical identification and hazards associated with the chemical as well as quantities on hand in the lab. A copy of the work area chemical list shall be put in the safety section of the appropriate section's procedure manual. The original list will be kept on permanent file by the Laboratory Director, or Principal Investigator.
- **Workplace Chemical List.** The Laboratory director will combine all the Work Area chemical lists and develop a work place list for the laboratories. The workplace chemical list will include the chemical identification, hazards associated with the chemical and the sections (lab numbers) where the chemical may be found. The Laboratory director will keep the original list on permanent file. A copy will be submitted to the Environmental Health and Safety Office. Additional copies will be placed in the front of each SDS Binder and in the laboratory safety manual. The Laboratory director will update this list annually.

F. **Laboratory Equipment and Glassware**. Each employee shall keep the work area clean and uncluttered. All chemicals shall be properly labeled. At the completion of each workday or operation, the work area shall be thoroughly cleaned, and all equipment properly cleaned and stored. In addition, the following procedures shall supply to the use of laboratory equipment:

1. All laboratory equipment shall be used only for its intended purpose.
2. All glassware will be handled and stored with care to minimize breakage. All broken glassware will be immediately disposed of in containers designated for broken glass. All broken glass exposed to infectious waste shall be dispose of in the large sharp containers provided.
3. Labels shall be attached to all chemical containers, identifying the contents and related hazards.
4. Waste receptacles shall be identified as such.
5. All laboratory equipment shall be inspected annually and replaced or repaired as necessary**.**

G. **Personal Protective Equipment.**

1. Safety goggles or face shields have been provided for each section of the laboratory and shall be kept in an easily accessible place within section. These items shall be worn during chemical transfer and handling operations as procedures dictate.
2. Sandals, perforated shoes, shorts, and bare feet are prohibited in the laboratory.

3. Chemical resistant aprons shall be used when working with corrosive chemicals.
4. Appropriate chemical-resistant gloves shall be worn at all times when there may be skin contact with chemicals. Each section is provided with nitrile gloves, contact your supervisor when new ones are needed. Used gloves shall be inspected and washed prior to re-use. Damaged or deteriorated gloves will be immediately replaced. Gloves shall be washed prior to removal from the hands.
5. Thermal-resistant gloves shall be worn for operations involving the handling of heated materials and exothermic reaction vessels. Thermal-resistant gloves shall be non-asbestos and shall be replaced when damaged or deteriorated.

H. **Personal Work Practices.** Laboratory supervision must ensure that each employee knows and follows the rules and procedures established in this plan. All employees shall remain vigilant to unsafe practices and conditions in the laboratory and shall immediately report such practices and/or conditions to the laboratory director. The supervisor must correct unsafe practices and/or conditions as promptly as possible. Encourage safe work practices in coworkers by setting the proper example. Horseplay is strictly forbidden. Seek information and advice from knowledgeable persons, standards and codes about the hazards present in the laboratory. Plan operations, equipment and protective measures accordingly.
1. Long hair and loose-fitting clothing shall be confined close to the body to avoid being caught in moving machine/equipment parts.
2. Use only those chemicals appropriate for the ventilation system.
3. Avoid unnecessary exposure to all chemicals by any route.
4. Do not smell or taste any chemicals.
5. Use engineering controls in accordance with directions.
6. Inspection personal protective equipment prior to use, and wear appropriate protective equipment as procedures dictate and when necessary to avoid exposure.

I. **Labeling.** All containers in the laboratory shall be labeled in accordance with GHS labeling requirements. This includes chemical containers and waste containers. The label shall be informative and durable. The label must contain the chemical identification, physical and health hazards, route of entry and target organs affected. Additional items that should be put on the container are date of acquisition, date open and expiration if applicable.
1. Portable containers shall be labeled by the individual using the container.
2. Exemptions for labeling requirements shall be made for chemical transfers from a labeled container into a container that is intended only for the immediate use by the employee who performed the transfer.
3. The labeling program shall be reviewed annually when the chemical list for the section is updated. All chemicals shall be checked for properly labeling by the section supervisor to ensure that labels have no been defaced, removed and contain the necessary information listed in the first paragraph.

4. Any new chemical shall be checked by the section supervisor for proper labeling.
5. Contact the Laboratory Director or Principal Investigator if the manufacturer's label is insufficient. A TDSHS/OSHA compliant replacement label will be made.

J. **Safety Data Sheets (SDS).** Each chemical used in the laboratory must have a SDS kept on file.
   1. These SDS will be collected and compiled into binders by the Laboratory director. These copies will be available in the bookshelf in the Autoclave room.
   2. The staff that have access to computer may access SDS online via Environmental Health and Safety website address, http:www.utrgv.edu/ehsrm.edu
   3. All SDS received on chemicals in the laboratory should be forwarded to the Laboratory Director.

K. **Instructions for Handling of Hazardous Chemicals.** Written instructions will be developed by the section supervisor for handling all hazardous chemicals such as carcinogens, corrosives and flammable chemicals. These instructions shall include the storage and handling instructions, the type of protective equipment needed and disposal and spill procedures to be used (Refer to the UTRGV *Hazard Communication Lab Safety Maual* for guidance). These will be reviewed annually and updated when necessary by the section supervisor.

L. **Housekeeping.** Each laboratory worker is directly responsible for the cleanliness of his or her workspace, and jointly responsible for common areas of the laboratory. Laboratory management shall insist on the maintenance of housekeeping standards. The following procedures apply to the housekeeping standards of the laboratory:
   1. All spills on lab chairs or floors shall be immediately cleaned and properly disposed of.
   2. The lab chairs shall be kept clear of equipment and chemicals except those necessary for the work currently being performed.
   3. The work area shall be cleaned at the end of each operation.
   4. All apparatus shall be thoroughly cleaned and returned to storage upon completion of usage.
   5. All floors, aisles, exits, fire extinguishing equipment, eyewashes, showers, electrical disconnects and other emergency equipment shall remain unobstructed.
   6. All labels shall face front. Chemical containers shall be clean, properly labeled and returned to storage upon completion of usage.
   7. All chemical wastes will be disposed of in accordance with procedures listed in the UTRGV *Chemical Safety Handbook.*

M. **Safety and Emergency Equipment.** An updated list of telephone numbers of emergency personnel, supervisors and other workers will be kept on file in the laboratory.
   1. All laboratory personnel will be trained in the proper use of fire extinguishers when hired at New Employee Orientation.

2. All employees who might be exposed to chemical splashes shall be instructed in the location and proper usage of emergency shower and eyewash. The eyewash stations shall be inspected periodically by the laboratory director. The safety shower is inspected annually by Facilities Management.
3. Location signs for safety and emergency equipment have been posted. Instructions for accidents involving corrosives will be posted in those sections handling corrosive materials.
4. A spill kit containing absorbent pads, booms, and a universal absorbent is located in each laboratory.

N. **Laboratory Chemical Fume Hood. The following guidelines should be followed.**
   1. Confirm adequate hood ventilation performance prior to opening chemical containers inside the hood. An inward flow of air can be confirmed by holding a piece of paper at the face of the hood and observing the movement of the paper. Minimize interference with the inward flow of air into the hood.
   2. The fume hood should be operating 24/7 when it is not in active use if hazardous chemicals are contained inside the hood. On an annual basis, the ventilation system shall be inspected according to the manufacturers' instructions to ensure the system is working properly and has adequate air balance.

O. **Storage Cabinets.** Storage cabinets for flammable and hazardous chemicals will be ventilated as needed.

P. **Employee Information and Training.** All employees will be apprised of the hazards presented by the chemicals in use in the laboratory. Each employee shall receive training at the time of initial assignment to the laboratory and at a regular frequency as determined by the Laboratory director.
   1. **Hazard Communication & Safety Awareness.** All employees shall complete this course given by Environmental Health and Safety at the time of new hire orientation. The Safety Dept. maintains a record of this training for at least 5 years.
   2. **Specific Hazardous Chemical Training.** This training shall include methods of detecting the presence of a hazardous chemical, physical and health hazards of chemicals in the lab, and measures employees can take to protect themselves from these hazards. The training shall present the details of the Chemical Hygiene Plan, and shall include: the contents of the OSHA laboratory standard, and its appendices; the location and availability of the Chemical Hygiene Plan; the permissible exposure limits for OSHA regulated substances or recommended exposure values for other hazardous chemicals not regulated by OSHA which are present in the laboratory; and location and availability of reference material on chemical hygiene. This training shall be conducted by the Laboratory Director, Principal Investigator, or Area Supervisor and documented on the form (or similar form) to the one located in the UTRGV Hazard Communication Lab Safety Manual. Training records are retained at least 5 years.

3. **Laboratory Safety Awareness / Hazardous Waste Generator.** All new laboratory employees must attend this course as soon as possible after hiring. The course is offered monthly by the Environmental Health and Safety Department. The Safety dept. maintains a copy of this training for 5 years.

Q. **Medical Consultations and Examinations.** An opportunity to receive medical attention is available to all employees who work with hazardous chemicals in the laboratory. The opportunity for medical attention will be made available to employees under the following circumstances: whenever an employee develops signs or symptoms associated with a hazardous chemical to which the employee may have been exposed in the laboratory, Medical surveillance programs will be established where exposure monitoring reveals an exposure level above the action level for an OSHA regulated substance for which there are exposure monitoring and medical surveillance requirements. Whenever an event takes place in the laboratory such as a spill, leak, explosion or other occurrence resulting in the likelihood of a hazardous exposure the employee will be provided an opportunity for medical consultation for the purpose of determining the need for medical examination.

1. These medical consultations and examinations shall be administered through a Medical Provider under the direct supervision of a licensed physician. A list of recommended providers is available.

**R. Chemical Hygiene Responsibilities**

1. **Laboratory Director / Principal Investigator** shall: work with administrators and other employees to develop and implement appropriate chemical hygiene policies and practices, monitor procurement and use of chemicals in the lab, including determining the facilities and training levels are adequate for the chemicals in use, perform regular chemical hygiene and housekeeping inspections including inspections of emergency equipment, maintain current knowledge concerning the legal requirements of regulated substances in the laboratory, review and improve the Chemical Hygiene Plan on an annual basis, ensure that workers know and follow the chemical hygiene rules, determine the proper level of personal protective equipment is available and in working order, ensure that appropriate training has been provided to employees, monitor the waste disposal program.
2. **Laboratory Workers** are individually responsible for planning and conducting each laboratory operation in accordance with the Chemical Hygiene Plan and developing good personal chemical hygiene habits.

S. **Recordkeeping.** The immediate supervisor will conduct accident investigations with assistance from other personnel as deemed necessary. An incident report must be filled out as promptly as possible for accidents and exposures. The same form will be used for both accidents and exposures. The incident report and First Report of Injury or Illness form will be filled out by the employee and their immediate supervisor then submitted to the Laboratory Director.

1. Medical records for employees exposed to hazardous chemicals and harmful physical agents will be maintained for the duration of employment plus 30 years per 29 CFR 1910.1020.
2. Chemical inventory lists will also be kept on file for 30 years.
3. Records of inspections will be kept on file 3 yrs.
4. Chemical safety training records will be kept at least 5 years.

T. **Chemical Spills, Releases, and Disposal.** Spills and Releases should be handled immediately by laboratory personnel. Personal protective equipment such as nitrile or other appropriate gloves and goggles must be worn while cleaning up a spill. A respirator should be worn if there is a possibility of harmful vapors being produced. A spill kit has been developed for the laboratory and is located in the chemical cabinet. The kit contains nitrile gloves, goggles, soda-sand bucket, scoopers, and waste disposal bottles. Once the substance has been absorbed, scoop up contents and place into a closed container. Label the container with a chemical waste label that has been properly filled out. For dry material, scoop up contents, place into a closed container, and label properly. If the release or spill of a hazardous agent is major and cannot be contained, then contact the UTRGV Environmental Health and Safety office, or after normal hours, contact the UTRGV Police Dispatcher.
    **1.** A Spill/Release Report Form must be filled out as promptly as possible for all spills and releases and returned to the Laboratory Director / Principal Investigator. Contact the Laboratory Director for the form.

U. **Controlling Chemical Waste.** Less hazardous chemicals should be substituted wherever possible to minimize disposal problems. Hazardous substances should be purchased and stored in minimal amounts. Laboratory personnel should work towards reducing the volume of hazardous waste generated when every possible. Waste containers shall be removed from the laboratory and brought to the storage area as promptly as possible once full. All waste containers must be labeled properly. The label shall include the chemical identification and words "hazardous waste", approximate % of each component, accumulation start date, laboratory section, and technician's initials. Refer to the UTRGV Hazard Communication Lab Safety Manual .

V. **Disposal of Chemical Waste.** The method of disposal used depends upon the type, volume and concentration of waste generated. Safety Data Sheets, the UTRGV *Chemical Safety Handbook,* and other reference materials should be consulted in determining the preferred method of disposal. All hazardous chemicals should have a written procedure that contains a disposal method, which can be found in the safety section of each section's procedure manual. Methods that may be used to manage and dispose of chemical waste generated in the laboratory include the following:
    1. Disposal into the sanitary sewer system. Please refer to the UTRGV Hazard Communication Lab Safety Manual.
    2. Disposal with Normal Trash. Please refer to the UTRGV Hazard Communication Lab Safety Manual.

3. Neutralization and Dilution of Chemicals. Many acids and alkaline materials can be neutralized, properly diluted and disposed of safely in the sanitary sewer system.
4. Evaporation. Volatile chemicals should not be placed in a fume hood to evaporate.
5. Waste for Pick-up & Disposal: Hazardous chemicals, large quantities of chemicals, or chemicals that cannot be disposed of by one of the above methods, will need to be disposed of through the contracted waste disposal firm. For hazardous waste needing to be disposed of through the waste disposal firm, contact the UTRGV Environmental Health and Safety office, at 956-665-3690. The outside of any chemical containers that are picked up from the BSL-3 labs must first be chemically disinfected. UTRGV has a designated storage area for hazardous waste. The Environmental Health and Safety Department will come to the laboratory collection area, pick up the hazardous waste and hold the waste properly within the designated storage area until transfer for disposal.

**3. RADIOACTIVE MATERIALS: OPERATING PROCEDURES FOR THE HANDLING AND USE OF RADIOISOTOPES**

**a. UTRGV *Radiation Safety Handbook.*** Refer to this document for general university policies and procedures.

b. **Personnel and Training.** All personnel working with radioisotopes must receive the laboratory in service on the proper methods for handling and use of radioisotopes before performing any experiments. In addition, before independent experiments are performed, personnel must attend the Radiation Safety course provided by the UTRGV, Division of Radiation Safety, or provide documentation from another institution of equivalent training.

c. **Personnel Work Practices.** Laboratory supervision must ensure that each employee knows and follows the rules and procedures established in this plan.

- Eating, drinking, smoking, chewing gum, or applying make-up is prohibited in the laboratory.
- Mouth pipetting is prohibited.
- Proper attire must be worn during the performance of experiments. This includes the use of gloves, gown or lab coat, and face shield or safety goggles.
- All employees must use proper techniques and work with diligence to ensure that all experiments are performed with minimal exposure to themselves and other in the laboratory
- All work will be performed on the plastic-backed underpad to aid clean up should a spill occur. When possible work should be performed in the biological safety cabinet.

**d. Record Keeping.** Records shall be kept on each chemical form of isotope and include the receiving report, dates of use in experiments, and disposal. Upon receipt each order of isotope will be given a unique identification number and a wipe test will be performed to ensure that no leaks occurred during shipping. The usage report will contain the activity of isotope used

per experiment and the estimated activity going to either the dry or liquid waste.

e. **Storage.** Each chemical isotope will have its own designated storage area for source vials and waste storage within the laboratory. In addition, temporary waste storage areas will be designated within the laboratory. Each Isotope will have a designated drum for long-term storage in the covered radiation storage facility to hold for decay or removal by a commercial waste company.

f. **Waste Disposal.** Refer to the UTRGV *Radiation Safety Handbook*. Disposal is divided into liquid and solid forms and handling varies depending upon the isotope. All liquid waste will be treated to kill any microorganisms with either final concentration of 1% bleach or formalin and will be stored in plastic bottles. Solid waste is bundled and secured within an underpad that is then placed in a plastic disposal bag. Solid $^{3}H$ waste will be placed in the 55-gallon waste drum within the covered radiation storage area. All other isotopes will be held in 55-gallon waste drums until 10 1/2 lives have past. Thereafter, the waste will be repackaged and disposed of through the hazardous waste handler for the university.
    - Contact the Environmental Health and Safety Dept. Environmental Protection Division for pick up of radioactive waste materials by completing an online pickup request.

g. **Wipe Tests.** For each week an experiment is performed, wipe tests will taken on the designated areas to ensure that no isotope is contaminating routine work areas.

h. **Radioactive spills.** Should a spill occur, notify the Laboratory Director or Principal Investigator, Institutional Safety personnel, and LAR personnel as listed in Appendix C: *Call Down List for Emergencies.* If you are unable to contact any of the individuals listed, then contact the UTRGV Police dispatcher. Secure the area and prevent entry by unnecessary personnel. Liquid spills should be contained by the underpad that can be carefully folded and placed in an appropriate waste container. Decontaminate the area with the Rad-Con decontaminating detergent and perform wipe tests immediately to ensure adequate removal of activity.

- **COMPRESSED GASES**. Some general rules for handling large cylinders of compressed gas are:
    - **Transporting cylinders.** Always transport cylinders using a hand truck to which the cylinder is secured.
      Leave valve cap on cylinder until cylinder is ready for use, at which time the cylinder should have been secured by a support around the upper 1/3 of its body. Disconnect hose or regulator, shut off valve, and replace cap before the cylinder is completely empty to avoid the possibility of developing a negative pressure. Place "empty" sign or label on cylinder.
    - **Securing cylinders.** Chain or secure cylinders at all times even when empty.

- **Routine Checks.** Always check cylinders for composition of contents before connection.
  i. Never force threads - if regulator does not thread readily, something is wrong.
  i. Only use compressed gasses with a pressure-reducing regulator.
- **Storage.** Compressed gases and flammables should not be stored together.

- **Employee Action:**

**i.** Read this manual and adhere to the guidelines it contains. Request that your supervisor explain anything you do not understand. Discuss with your supervisor the hazards involved in your work and his recommendations on how to recognize potential risks and to minimize risks and ask for additional material to read.

**ii.** Immediately notify the supervisor in case of any accident, injury, exposure, or illness and perform immediate first aid, clean wound and apply antiseptic agent.

**iii.** If the accident is serious and medical assistance is required, dial appropriate emergency numbers found in Section 2 (Emergency Preparedness & Life Safety) of this manual, or in the UTRGV Post Exposure Policy.

## 4. PROTECTIVE CLOTHING (Biological Agent LABORATORIES)

a. When working in the BSL-3 laboratories personal protective equipment should be used at all times.

b. Many of the chemicals used can be absorbed through the skin. Therefore, it is incumbent upon the worker to read the **Safety Data Sheet (SDS).** A hard binder is located in the bookshelf in the inside Molecular Mycobacteriology Lab. SDS are also available on line via the *chemicalsafety.com* program available on the Environmental Health and Safety web page at the following website address, https://www.utrgv.edu/ehsrm/programs/lab-safety/sds/index.htm. Review and become familiar with the proper use, handling, and disposal of workplace chemicals. When a potential exposure is apparent, the use of latex, nitrile, or chloroprene gloves in the handling of these materials is required.

c. Some chemicals present a respiratory hazard in the process of weighing for preparation of solutions. Two acceptable approaches can be used to minimize exposure.
   i. The preferred choice is to weigh items in the laboratory chemical fume hood, or a class II, type B2 BSC (100% exhaust).
   ii. The second approach is for the employee to use a disposable, surgical mask to prevent inhalation while weighing the powders.

d. To protect eyes during any procedure that may cause splashing of chemicals, infectious agents, or during the use of ultraviolet light, a face shield or eye goggles must be used.

## Section 7: Incident Response Plan:

### 1. Overview and Coordination with entity-wide plans:

The UTRGV has procedures and emergency response plans for various types of incidents that may occur on the UTRGV campus. These plans are coordinated with entity-wide plans and UT system- wide plans for business continuity. These plans are as follows:

- Spill Prevention Control and Countermeasures Plan (SPCC)
- Hazardous Waste Contingency Plan
- Emergency Response Action Plan (ERP)
- Pollution Prevention Plan
- Laboratory Animal Emergency Plan
- UT System Mutual Aid Agreement

### 2. Incident Response Plan and the UTRGV's Emergency Response and Evacuation Plan.

The following procedures cover the plans for response and/or evacuation to be followed in the event of an emergency. The following list of contact numbers include the UTRGV EHSRM office, UTRGV Police (Campus Police which provides security), UTRGV Environmental Health and Safety and the building Facilities Management. The call down list that is posted in all BSL-3 laboratories and at the entrance to the BSL-3 facility can be found in appendix C of this manual. The Appendix C call down list is also available to UTPD, ACS, EHSRM and Facilities Management.

**Emergency Response Phone Numbers**

**Dr. Richard Costello, ……..……work (956-665-3690) or cell (956-457-2357)**
**Javier Garcia, BS work (956-665-2052)**
**Emergencies (campus Police from on campus phone) ..... 24911**
**Emergency (Off campus) ..... 911**
**Campus Police**…….**956-882-4911**
**Building Trouble Calls (Business and non business Hours)** .....…….**956-665-2748**
**Poison Center** ..... ..**1-800-POISON-1**
**UTRGV EHSRM Office Business Hours (Hazardous Materials)…….956-381-3690**

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For more detailed information, employees should refer to the UTRGV *Emergency Response and Evacuation Plan (EREP).* See the last Appendix in this manual. An emergency will be defined as severe weather and other natural disasters (*EREP* p. 14), workplace violence (HOP), bomb threats (*EREP* p.25-27), suspicious packages (EREP p. 26), the unexpected release of biological, chemical, or radiological agents (*EREP* p. 29, and in this section), fire, gas leak, explosion, power outage, electrical problems, airflow problems (EREP p. 4), or any other event that might result in the exposure of personnel to possible injury.

# PROCEDURES FOR REPORTING FIRE OR OTHER EMERGENCIES

The University of Texas Rio Grande Valley Department (UTRGVPD) is notified of emergencies in two ways:

1) Direct notification by an employee, student, or visitor; or

2) The UTRGV PD communication center via *on-campus* 956-882-4911 or 911 call.

When notified of an emergency by a UTRGV employee, student, or visitor, the UTRGV guard or officer on site will immediately notify the UTRGVPD communication center. For fire and medical emergencies, the dispatcher will notify fire and medical personnel.

Immediately after being notified of an emergency, the UTRGVPD dispatcher will then call the appropriate UTRGV emergency contact personnel based on the type of emergency.

(In the BSL-3 facility, EHSRM personnel will assess the situation if there is a spill or release of a biological agent and notify the UTPD of the situation. In coordination with EHSRM, UTPD will provide site security and Control. If assistance is required of the Fire Dept or Hazmat, UTPD will notify them of the situation.)

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1. **General procedures, irrespective of the emergency are as follows, and can be remembered with acronym - R.A.C.E.**

   **R**eact - Notify others in your area.

   **A**ctivate - Activate the nearest fire alarm pull station or call 911.

   **C**ontain - Close all the doors upon exit

   **E**vacuate - Evacuate and assemble at a known location with your workgroup.

2. **Specific Emergency Response Procedures for spills and other emergencies in the BSL-3 Facility**

**a. Biological spill.** In the BSL-3 facility, personnel should leave the BSL-3 labs immediately. Press the biological alarm to notify other personnel to leave the BSL-3 facility. Place signs on the doors to prevent anyone from entering the contaminated area. Contaminated PPE should be removed and affected person(s) shall shower prior to exiting the BSL-3 facility. The design of the negative air pressure is such that biohazardous agents should be exhausted through the Class II biosafety cabinets, which contain HEPA filters. Notify the first available person for each department (Microbiology, EHSRM, & ACS) listed in Appendix C: *Call Down List for Emergencies.* **THIS MUST BE DONE IMMEDIATELY TO ENSURE THAT PROPER MEASURES ARE TAKEN.** If none of these persons are available, then contact the UTRGV Police Dispatcher (956) 665-7151. Be sure that the accident is reported to the physician-in-charge at the chosen Medical Provider so that appropriate medical evaluation will be undertaken. Should a Biological spill occur after hours, the appropriate supervisor should be notified and proper Incident

Report and First Report of Injury or Illness form should be filled out and faxed to the WCI Risk Manager in the Environmental Health and Safety office at (956)665-3690 as soon as possible? Seek medical treatment.

**b. Chemical spill.** The major types of chemical spills will involve acids, caustics, or solvents. A portable spill kit to contain these types of spills is located in the chemical cabinet area. General procedures for minor spills (less than 1 liter) will involve the following:

- **i.** Put on face shield or eye goggles, appropriate type of gloves and, if necessary water-resistant gown.
- **ii.** Take the spill kit to the affected area. Ensure that you have the appropriate absorbent agent.
- **iii.** Place the absorbent agent over the spill and allow for the appropriate contact time.
- **iv.** Scoop the absorbed spill and place into an appropriate waste receptacle.
- **v.** If the spill is major (> than 1 liter), or cannot be contained, evacuate the area and contact the UTRGV Environmental Health & Safety office immediately. After normal hours contact U.T. Police Dispatcher who will notify appropriate Environmental Health and Safety personnel.

**c. Radioactive spill.** Should a spill occur, notify the UTRGV safety office and the UTRGV Environmental Health and Safety (Radiation Safety Division), the Principal Investigator, and ACS personnel as listed in Appendix C: *Call Down List for Emergencies.*

- **i.** Secure the area and prevent entry by unnecessary personnel.
- **ii.** Wear appropriate PPE (gloves and water-resistant gown.)
- **iii.** Absorb the spill into a blue pad and place it into the radioactive waste container.
- **iv.** Decontaminate the entire area with an appropriate detergent followed by 70% alcohol.
- **v.** Perform wipe tests immediately to ensure adequate removal of activity. Repeat decontamination if necessary.

**d. Fire.** The following are rules that should be emphasized or may be in addition to procedures listed in the UTRGV *Physical Safety Standards Handbook.*

- **i.** All employees must learn the location of fire extinguishers, blankets (if equipped) and pull station alarms.
- **ii.** Fire extinguishers can be used to extinguish small fires.
- **iii.** Flammable liquids will be stored and labeled properly, and the supply will not exceed the quantity necessary to perform routine work. The supply will not exceed 60 gallons/3000 square feet of laboratory space in a safety cabinet, 25 gallons in safety cans, and 10 gallons may be stored on open shelves.
- **iv.** Household refrigerators will not be used to store flammable liquids.
- **v.** Learn the emergency exit routes and conduct routine fire drills. Environmental Health and Safety conducts a facility-wide drill annually.

**e. Electrical Hazards.** Experiments performed in laboratories often require the use of electrical equipment that can deliver high voltage shocks if not used or maintained properly. In addition to shock, other hazards associated with electricity are resistive heating and ignition of flammables.

- **i.** Any electrical equipment that appears to be malfunctioning should be turned off, unplugged, and must be checked by an authorized biomedical maintenance technician before use.

**ii.** During routine maintenance, the equipment should be unplugged, or if power is required, a second person will be available to disable the circuit and assist in case of an accident.

**iii.** Never handle electrical equipment with wet hands or while in contact with water (wet floor).

**iv. Electrical injury:**

a. Rescue the victim from the circuit promptly and safely. If the victim remains in contact with the circuit, the power source or breaker should be turned off before attempting to rescue the victim. If this is not possible, rubber gloves or other insulators should be used to free the victim.

b. If a live wire is lying across a victim and the power cannot be readily turned off an insulated device should be used to lift the wire from the victim.

c. If the victim is not breathing, immediately apply an approved form artificial respiration. If the circulation appears to have stopped, apply an approved method of cardiopulmonary resuscitation (CPR). Personnel providing this assistance must be certified in CPR.

**f. Thermal Hazards.** Personnel may come into contact with material that is extremely hot or cold during the routine performance of duties.

**i.** In all instances of extreme thermal contact, appropriate use of non-asbestos gloves should be used to protect against possible injury.

**ii.** Do not attempt to handle items out of the freezer or liquid nitrogen with wet hands. If a high level of dexterity is required, wear latex gloves.

**g. Emergency Contact Numbers after Normal Business Hours**. Should an emergency occur in laboratories outside normal business hours, please contact the UTRGV Police Dispatcher (911 or ext. 956-882-4911). If possible, also call the first available person listed in Appendix C: *Call Down List for Emergencies.*

***h.*** **Security Breach:** Due to the nature of the agents that are being researched in the BSL-3 facility, a breach of security and/or information is treated as a very serious matter. The agent, *Mycobacterium tuberculosis* can cause serious life-threatening illness due to an inhalation exposure. If the door to the BSL-3 facility is open or not secure, do not enter the facility. Immediately notify the UTPD and the Director of EHSRM or first available person on the EHSRM call down list posted on the door to the facility. (Due to the security in place, if doors are open for more than 45 seconds, the UTPD dispatch will receive an alarm and notify the RO). The RO and EHSRM in conjunction with the UTPD will determine if any labs security has been breached. UTPD will provide a secure perimeter around the facility. The Principal Investigators will inventory agents to ensure that there are no missing specimens. If research personnel **enter** a lab and find that freezers are unlocked, this shall be immediately reported to the PI and the RO. An incident report will be filed and the PI shall inventory all stocks to ensure all are accounted for. If any are missing, follow procedure in Section 11 of this manual, *Notification of Loss, Theft or Release.*

**i. Loss, Theft or Release** of a biological agent must be reported as soon as the loss is discovered. Notify UTRGV EHSRM and UTPD so the incident can be investigated.

## 3. Procedures for Employees Performing Rescue or Medical Duties, Emergency Medical Treatment and First Aid

**IN THE EVENT OF A MEDICAL EMERGENCY, FOLLOW THESE PROCEDURES: (Specific information in EREP and in appendix of this manual)**

- **a.** Using a campus telephone, call 911 without delay for medical assistance.
- **b.** Administer first aid and cardiopulmonary resuscitation (CPR), if properly trained to do so, or summon a person with the appropriate training.
- **c.** Avoid moving injured persons unless it is absolutely necessary for safety reasons.
- **d.** Try to find out what happened and check for medical ID tags.
- **e.** Follow universal precautions: treat all blood and body fluids as if they are infectious.
- **f.** Use a first aid kit, if available.

## 4. Personal Protective Equipment and Emergency Equipment

a. Personal Protective Equipment for responders to emergencies (ie. spills) is located in the 1st floor of the Medical building in the Environmental Health and Safety Storeroom.
PPE includes: tyvek suits, respirators, shoe covers, gloves, hair bonnets
Emergency equipment includes spill kit, scoops, adsorbent pads and booms

b. Emergency Equipment in the BSL-3 facility:
Spill kits located in each of the BSL-3 laboratories
Fire extinguishers and safety shower in the BSL-3 corridor
Fire suppression system
Alarm pull station in the corridor
Biological alarm in the corridor

## 5. Site Security and Control

In the BSL-3 facility, UTRGV EHSRM personnel will assess the situation if there is a spill or release of a biological agent and notify the UTPD of the situation. In coordination with the UTRGV EHSRM, UTPD will provide site security and Control. If assistance is required of the Fire Dept or Hazmat, UTPD will notify them of the situation.

**6. Procedures for Emergency Evacuation.** In the event of an emergency evacuation (fire alarm sounding), quickly remove PPE and exit the BSL-3 facility. The emergency exit may be used if necessary. Exit the building and everyone will meet at the designated Emergency Meeting Place for a head count and assessment of the situation!!! For more information on a specific type of emergency – (EREP p. 5-6– appendix in this manual)

## 7. Decontamination

If there is a spill or release of a biological agent, that results in contamination of the individual, follow procedures for notification of the Principal Investigator and EHSRM. Individuals shall remove all PPE according to procedures. Place all contaminated PPE in autoclave bag. If there is contamination on scrubs, remove scrubs and place in biohazard bag. Shower with soap and water. Do not exit the facility with contaminated PPE unless there is a situation that is immediately

dangerous to life. All individuals that have been potentially exposed to an infectious agent must immediately report to occupational medicine for evaluation and treatment.

## 8. Plan Review

The Incident Response Plan is reviewed annually and revised as necessary.

# Section 8: Training Requirements

1. **Overview:** Training must be provided on biosafety and security to:
   - Each individual with approved access to the BSL-3 facility before he/she has such access.
   - Each individual not approved for access before he/she works in or visits areas where agents or toxins are handled or stored. The training must cover the risks posed by the agent or toxin and be specific to the work assignment of that individual.
   - Refresher training must be provided annually.
   - A record of training must be maintained and contain the name of the individual, date of the training, description of the training provided and the means to verify that the training was understood by the employee.
2. **Minimum Educational Requirements:**

   Minimum Educational Requirements to enter a biological agent or toxin room or lab:
   - Research personnel: Bachelor of Science degree in appropriate discipline, or six months of supervised work experience.
   - Environmental Health & Safety personnel: Bachelor of Science Degree
   - ACS, Facilities Management, & Visitors: G.E.D. or H.S. diploma
3. **Required Training Courses for Individuals entering biological agent rooms:**

**BIOLOGICAL SAFETY & BLOODBORNE PATHOGEN TRAINING**

a. BASIC BIOLOGICAL SAFETY: In addition to training received via departmental standard operating procedures, Research, ACS, and Environmental Health and Safety personnel shall attend the ***Basic Biological Safety*** offered by Environmental Health and Safety prior to initial assignment to job duties, and within one year of their previous training, thereafter.

b. BASIC BLOODBORNE PATHOGENS: Research, ACS, Environmental Health and Safety, and Facilities Management personnel shall attend the ***Basic Bloodborne Pathogens Training Course*** offered by Environmental Health and Safety prior to initial assignment to job duties, and within one year of their previous training, annually thereafter.

**BSL-3 / BIOLOGICAL AGENT TRAINING**

**Initial EHSRM BSL-3 Specific Training**

The required training must provide information so that each individual approved for access to the BSL-3 facility understands the hazards of the biological agents present. This initial training shall fulfill this requirement. The laboratory Principal Investigator will collaborate with the UTRGV and EHSRM to provide this initial training that will cover not only agent specific hazards, but also laboratory security, safety, containment issues, incident response plans.

**EHSRM BSL-3 Annual Refresher Training**

This training will consist of the same content as described in the initial training and will be offered on an annual basis, or prior to the implementation of any new assignments involving new exposure situations or as regulation change would warrant.

**BSL-3 Laboratory Training for Facilities, Contractors, and Visitors**

Visitors and contractors will be required to sign in and wear a visitor's or contractor's badge. **Facilities Mgmt. personnel, contactors, and visitors shall be escorted at all times by an authorized individual.** They shall be informed of the hazards of the agent, biosafety, security, relevant emergency procedures, and entry/exit procedures including donning and doffing personal protective equipment prior to entering the laboratory.

**Infectious Substance / Diagnostic Specimen Shipping Training (if necessary)**

If a biological agent or toxin is required to be transferred, all personnel involved in the classification, packaging, labeling, and/or documentation of the shipment shall attend this training course prior to transfer. DOT and IATA Dangerous Goods Regulations will be covered to ensure compliance with applicable shipping requirements.

*No individuals shall be permitted to access the laboratory where biological agents are handled or stored without receiving the required training as stated above.

** UTRGV keeps a record indicating the topics covered in the training, the identity of the individual trained, the date of the training, the trainer's name and title. Individuals are given an opportunity to ask questions. A training certificate will be issued by EHSRM confirming that the individual has received and understood the training. The UTRGV EHSRM department will keep a copy of the training certificate for each individual trained, although it is strongly recommended that the individual also retain a copy of the training certificate.

## 4. Additional Required Training Courses

**a. Basic Laboratory / Hazard Communication.** All new employees must attend this course as soon as possible after hiring. The course is offered monthly by the Environmental Health and Safety Department. The Safety dept. maintains a copy of this training for 5 years.

**b. Specific Hazardous Chemical Training.** This training shall include methods of detecting the presence of a hazardous chemical, physical and health hazards of chemicals in the lab, and measures employees can take to protect themselves from these hazards. The training shall present the details of the Chemical Hygiene Plan, and shall include: the contents of the OSHA laboratory standard, and its appendices; the location and availability of the Chemical Hygiene Plan; the permissible exposure limits for OSHA regulated substances or recommended exposure values for other hazardous chemicals not regulated by OSHA which are present in the laboratory; and location and availability of reference material on chemical hygiene. This training shall be conducted by the Laboratory Director, Principal Investigator, or Area Supervisor and documented on the form (or similar form) to the one located in the *Chemical Safety Handbook*. Training records are retained at least 5 years.

**e. Radiation Safety.** This course is required for all employees that will use radioactive materials during the course of their job duties.
**f. Laser Safety Course:** This course is required for all employees that will use equipment with Lasers during the course of their job duties.

**g. Site Specific Standard Operating Procedure - Training on site specific and agent specific procedures shall be conducted by the Principal Investigator or the Laboratory Supervisor. Personnel shall not work unsupervised in the BSL-3 facility until proficiency in the safe conduct of site-specific procedures is documented.**

### 5. Review of Training Documentation:

- Environmental Health and Safety will review all training documentation every year.
- The Principal Investigator will review all training documentation every year.
- The ACS Facility Manager will review training documentation every year.

## Section 9: TRANSFER OF BIOLOGICAL AGENTS

### 1. Overview:

A biological agent may only be transferred to individuals or entities authorized to receive such materials. Transfer may require CDC or APHIS approval prior to the transfer. A permit from the USDA may be required.

**Definitions:**

**a. Diagnostic specimens.** Any specimen classified as a diagnostic specimen under the current Department of Transportation (DOT) regulations 49 CFR part 173.134 shall be shipped according to current ICAO / IATA packaging instruction 650.

**b. Infectious substances.** *M. tuberculosis and bovis* cultures are classified as Risk Group 3 agents and are handled at BSL-3. Cultures must be shipped as Infectious Substances under DOT 49 CFR part 173.134. Packaging and labeling used shall conform to current DOT regulations for Class 6.2 materials, and ICAO / IATA packaging instruction 602.

- **Training.** Employees packing and shipping these substances must have training prior to shipping these agents, and required DOT training for shipping class 6, division 6.2 materials within 3 years of previous training date. If shipping by air, IATA requires training every 2 years.
- **Documentation:** Certificates of training should be kept on file by the Principal Investigator and a copy sent to EHSRM dept. A copy of the shipper's Declaration for Dangerous Goods should also be kept of file.

## Section 10: RECORDKEEPING

### 1. Inventory Records

Inventory records of all biological agents and toxins will be maintained a minimum of 3 years. These records must include:

- **i.** Name, characteristics, and source data (strain designation, GenBank accession number, or other designation). Subcultures shall be accounted for in the same manner as the original culture.
- ii. The quantity acquired from another individual or entity (# of tubes/containers, volume or mass, the source, and date of acquisition.
- **iii.** Where stored (building, room, freezer)

  The quantity, volume, or mass disposed of or destroyed, method of destruction and date of this action. These records of destruction shall be kept with the Principal Investigator's inventory records.
- iv. Any agent or toxin that is lost, stolen, or unaccounted for; and
- v. A written explanation for any discrepancies (incident report to be filed)

### 2. Access Approval Records

A current list of all individuals that have been granted access to the BSL-3 facility are maintained by the UTPD and EHSRM.

### 3. Records of Entry into Biological Agent Rooms

Entry into Biological agent rooms are maintained in two formats: electronic and paper

1. Electronic records: The BSL-3 suite and each room have magnetic keycard / keypad access for authorized personnel. Each time an authorized individual swipes his/her card or enters their PIN number, an electronic log of the entry time is recorded. These electronic records of entry are maintained by the UTPD and are reviewed by EHSRM Director on a periodic basis.

2. Paper records: A sign in/sign out log sheet is kept on a clipboard at the entrance to the BSL-3 facility. This log sheet is used by all non-authorized personnel (Facilities, contractors, visitors) who enter the BSL-3 facility. The log sheet contains the name of the individual and their escort, date and time of entry and time of exit, and the reason for entry. Completed log sheets are kept by EHSRM.

### 4. Records – Security, Biosafety, Incident Response, Training

Training records are maintained by EHSRM. Campus UTPD reports of incidents involving security breaches or incident response will be maintained by UTPD. EHSRM follow-up incident reports shall be maintained by EHSRM.

### 5. Records and Databases

Records and databases must be accurate, have controlled access, and the authenticity be verified. Written Records created by the Principal Investigator and laboratory staff (inventory, experimental results, etc.) must be maintained in a legible manner and secured from unauthorized personnel. The Principal Investigator shall provide routine oversight of these records to ensure that the records are accurate. Paper records maintained by the PI and EHSRM are to be stored in a locked cabinet inside the Environmental Health and Safety and Risk Management Office.

## 6. Record Retention

Paper and electronic records and databases must be maintained per record retention schedule. For most records, not including research data, the record retention is for three years.

# Appendix A:

### UTRGV Edinburg Research Education Building Biosafety Level 3 Facility
### Access (Entry / Exit) Procedures for Facilities Maintenance / Contractors / Visitors

**Pre-Entry Procedure:**

1. Complete BSL-3 Work Order or contact Principal Investigator (P.I.) or Research Associate (see appendix C: Call Down List) for room(s) or equipment to be worked in, or repaired, at least one (1) day prior to performing maintenance in any part of the BSL-3 suite, air handling / exhaust system, or emergency power system. Contact Environmental Health, Safety & Risk Management (956-665-3690) or UTRGV Safety personnel if you cannot reach the individual researcher, and for all air system (supply or exhaust) or power shut downs / interruptions.
2. Active work must be stopped and minimal surface decontamination performed plus at least two (2) hours of air changes in any room or area prior to the scheduled maintenance. The morning of the scheduled maintenance, a sign shall be posted on the entrance door to the BSL-3 suite stating "Closed for Maintenance".

**Entry Procedure:**

Note: All Facilities Management staff or contractors must be escorted by a member of the Research, LAR, or Environmental Health, Safety & Risk Management (EHSRM) staff. Prior to entry, all personnel and contractors must notify the Principle Investigator (PI) or Research Associate, sign in on the white board and sign in on the clipboard when initially entering the BSL-3 facility. **Visitors and contractors must be accompanied at all times.** The staff member escorting the individual must also sign in on the log sheet. Street clothes or uniforms are not allowed to be worn into the BSL-3 area.

1. Enter DLAR area and enter room 1.400.36 (women's) or 1.400.40 (men's) changing / dressing room. Change into scrubs.
2. After donning required scrubs, shoes covers and securing personal items, move across inner access corridor to 1.500.2 (door to BSL-3 suite changing room & airlock), sign in on sheet next to door (Date, Name, Purpose, and Time In), and then enter BSL-3 airlock with escort.
3. Put on the following personal protective equipment (PPE) prior to entering the BSL-3 suite:
    a. Reusable wrap-around gown (a disposable gown, labcoat, or coverall may be substituted)
    b. Disposable water resistant shoe covers
    c. Disposable bouffant cap (optional)

d. Respirator (ie. North, P100 particulate filters) or HEPA filtered supplied air (Powered Air Purifying Respirator which is commonly known as a PAPR)- Tyvek hood.
e. Latex, choloroprene, or nitrile protective e gloves (double glove)

**Note:** Employees wearing respirators are required to have a medical evaluation and fit testing prior to wearing the respirator – no interfering facial hair allowed.
Personnel wearing a PAPR are not required to have a medical evaluation or fit testing. Some facial hair is allowed.

**Note: Any tools or equipment brought into the BSL-3 area must be decontaminated prior to removal from the BSL-3 suite. Only take in what you need to complete the job.**

4. After your escort checks your PPE for correctness, enter the BSL-3 inner corridor through double door.
5. Complete assigned work / task in the BSL-3 suite as required. Notify responsible Researcher and Environmental Health, Safety & Risk Management of any delays or problems.

**Exit Procedure:**

1. In the BSL-3 corridor, remove your PPE as follows:
    a. Remove and discard water resistant shoe covers into medical (biohazard) waste container
    b. Remove and discard protective gloves into medical (biohazard) waste container
    c. Remove reusable wrap-around gown and place in receptacle for autoclaving

2. Move through door into airlock, remove PPE as follows:
    a. Remove and discard bouffant cap (if wearing one) into medical waste container
    b. Decontaminate PAPR hood and power supply, plug power supply into outlet
    c. Remove inner pair of gloves and place in medical waste container
    d. Spray inner pair of shoe covers with disinfectant

3. Go to hands free sink and immediately wash hands for 15 seconds with disinfectant soap and water.

4. Exit room 1.500.2 and **record Time Out on sheet.**

5. Erase “Closed for Maintenance from white board and write “OPEN” at entrance to the BSL-3 suite.

6. Move across access corridor into appropriate 1.400.36 (women’s) or 1.400.40 (men’s) changing / dressing room. Remove scrubs and place in designated receptacle. Showering is optional. Put on personal clothes.

7. Exit changing / dressing and exit DLAR.

8. Notify P.I., Research Associate, EHS&RM staff at UTRGV that the work is complete and sign out on the white board.

# APPENDIX B:

## University of Texas Rio Grande Valley Biosafety Level 3 Facility
## Safe Operation, Use, & Maintenance of a Biological Safety Cabinet (BSC)

### GENERAL CONSIDERATIONS

Class I, II, or III BSCs may be utilized in the BSL-3 area (refer to figure 1). It is important to note that Class I BSCs only protect the worker and outside environment, and not the product. Therefore, a Class I BSC should not be used for any application (tissue culture, rDNA, etc.) where airborne contamination of the product is a concern.

Procedures to be carried out in a BSC include: opening of test tubes, flasks and bottles, tissue culture work, culturing of infectious agents transmitted via the airborne route (i.e. M. tuberculosis), or any other task that may cause aerosolization of an infectious agent. Many biosafety cabinets in the BSL-3 suite are type B2 units connected to the HEPA filtered building exhaust system, however several type A2 (A/B3) BSCs vented to the room are in use, and one class I unit. The building exhaust fan should always be on and running and is backed up by a redundant system should the primary unit fail. The Class II, type A2 cabinet recirculates 70% of the air inside the cabinet and is not suitable for working with open flames, flammables, or toxic chemicals, except in trace amounts.

The effectiveness of any BSC is a direct function of the carefully engineered directional laminar airflow, both inward and downward, through one or more High Efficiency Particulate Air (HEPA) filters. **Anything that disrupts the airflow pattern will reduce the effectiveness of the BSC.**

- Avoid the following when working in the BSC
  - The rapid movement of equipment or arms in and out of the sash opening
  - People walking behind you disrupting air flow
  - Opening the laboratory door
  - Down drafts from the ventilation system

### OPERATING PROCEDURES

1. Starting Up the BSC – Refer to figure 2
   a. Turn off the UV light if so equipped
   b. Ensure that the BSC certification sticker is current (BSL-3 = 6 months; BSL-2 = 1 yr of sticker date)
   c. Turn on the fluorescent light
   d. Check the grille for obstructions and remove / relocate
   e. Check that the BSC blower fan is running (alarm should sound if not working). If no airflow is detectable then do not proceed any further – contact Environmental Health & Safety (956-665-3690).
2. Wipe down

a. Disinfect all interior surfaces with appropriate disinfectant for agent being used

3. Material & Equipment
    a. Wipe off each item needed for your procedures and place in the cabinet
    b. Place only those items needed to perform the procedures
    c. Do not place equipment or material over the front intake grille
    d. Do not block the rear exhaust opening
    e. Refer to figure 3 for the following
        i. Arrange materials in such a way as to minimize movement of contaminated items over clean items
        ii. Keep clean items out of the primary work area, contaminated items to the rear of the work area – work from, "clean to dirty" as illustrated
        iii. Set up a biohazard bag inside the cabinet to collect medical waste that is generated
4. Air Purge
    a. Always allow 2-3 minutes of air changes before beginning work if unit was not running
5. Safe Work Practice Controls
    a. Hold open tubes and bottles as vertical as possible
    b. Use mechanical pipetting devices – do not mouth pipette
    c. Use horizontal pipette discard pans (figure 3) containing appropriate disinfectant inside the BSC – do not use a vertical discard container on floor outside the cabinet
    d. It is strongly recommended you do not flame items inside the cabinet, it should not be necessary and creates air turbulence which can compromise sterility
    e. Ensure protection of the building's vacuum system from hazardous agents by placing a HEPA filter between the vacuum trap and the source valve inside the cabinet (figure 4)
    f. Immediately clean up any spills inside the cabinet – wait 3-5 minutes if possible prior to resuming your work
    g. When adding or removing items from the BSC, move your arms in and out of the cabinet slowly
    h. Perform all work and keep all items at least 6 inches inside the sash opening, trying to work in the center work area as much as possible. The window sash should be in the appropriate down position (8-10 inch opening)
    i. Tie off the autoclave medical waste bags before they are full and place to be sterilized as appropriate for the biohazardous agent Risk Group
6. Terminal Purge and Wipe Down
    a. Allow 2-3 minutes of air changes before removing materials
    b. Disinfect all materials as they are removed from the cabinet
    c. Disinfect all interior surfaces with an appropriate disinfected for biohazardous agent being used
7. Shutdown
    a. Turn off the lights
    b. Blower fan should remain on
    c. If the unit is equipped with UV lights, turn on only after all work is finished and other personnel have vacated the lab

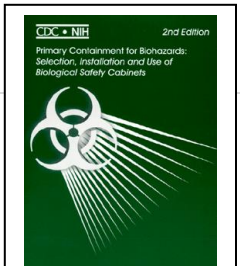



**FIGURE 1: Choosing the correct Biosafety Cabinet**

| Bio Risk Assessment | Protection Provided | | | BSC Class |
|---|---|---|---|---|
| | Personal | Product | Environ-mental | |
| BSL 1-3 | YES | NO | YES | I |
| BSL 1-3 | YES | YES | YES | II (A1, A2, B1, B2) |
| BSL 4 | YES | YES | YES | III (B1, B2) |

**FIGURE 2: Starting up a Class II BSC**

A. Front opening
B. Sash
C. Exhaust HEPA filter
D. Supply HEPA filter
E. Neg. pressure exhaust plenum
F. Blower

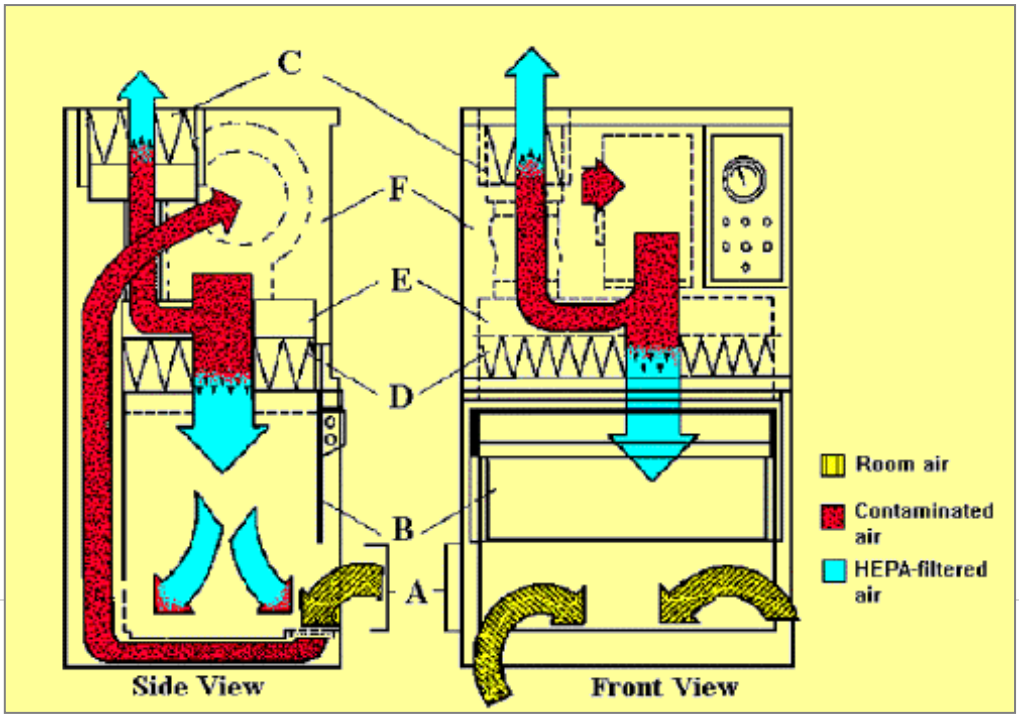
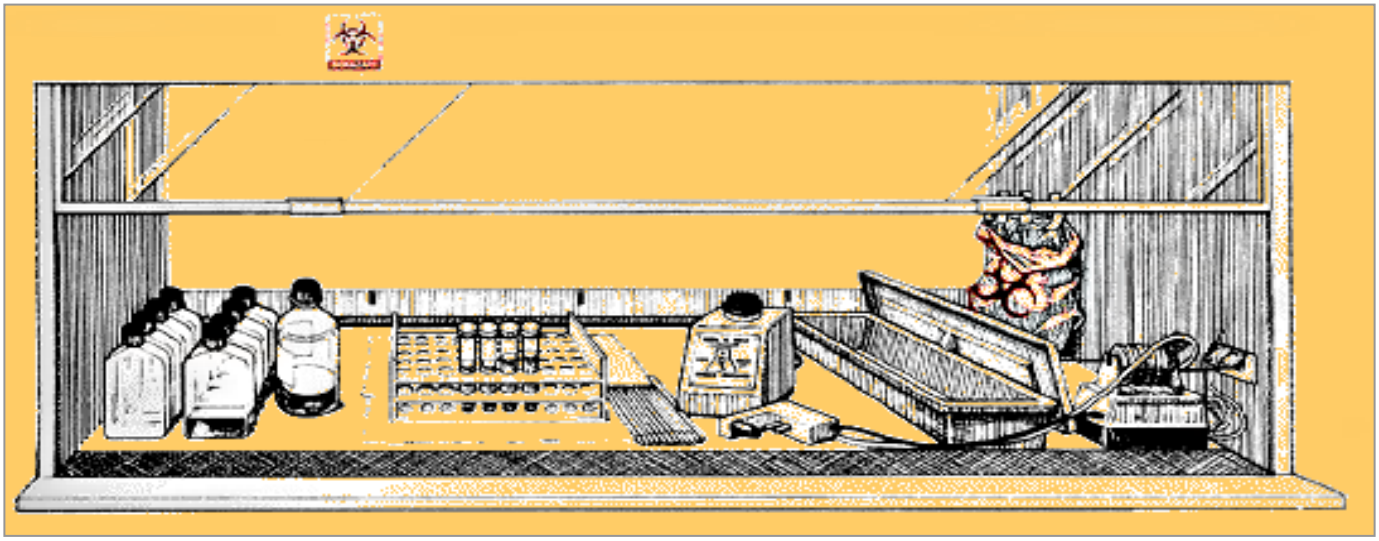
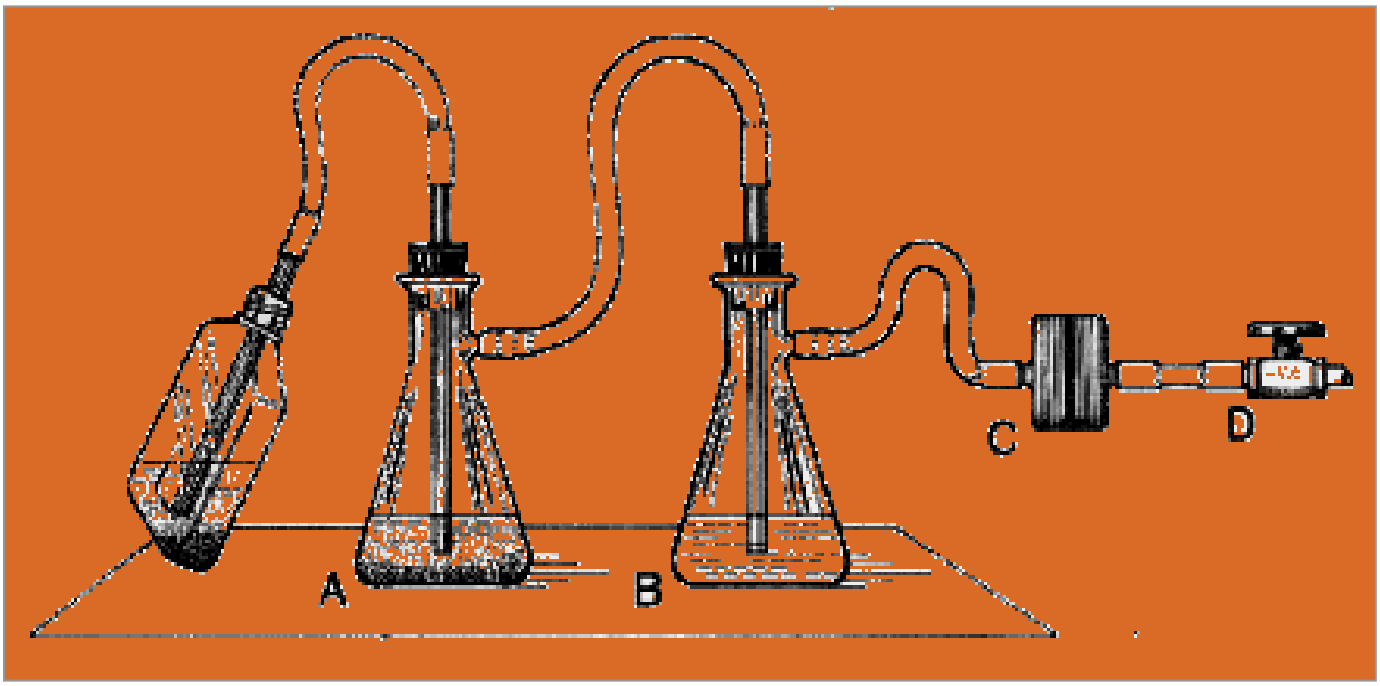


**FIGURE 3: Proper set-up inside the Class II BSC**



**Typical layout (right handed) for working "clean to dirty" within BSC. Clean cultures (left) can be inoculated (center). Dirty pipettes can be discarded in shallow sharps pan and other contaminated materials placed in biohazard bag (right).**

**FIGURE 4: Proper set-up for suctioning**




**A. Suction flask with decon solution**
**B. Overflow flask**
**C. In-line HEPA filter**
**D. Vacuum valve**

# Appendix C:

## University of Texas Rio Grande Valley Biosafety Level 3 Facility
## Call Down List for Emergencies

In the event one of the following, or other emergency situation occurs:

**Air Supply / Exhaust / Power Interruption; or Fire; / Biological or Chemical spill**

Contact the ****** individual, or first available person, listed from the Department, Environmental Health, Safety and Risk Management & Laboratory Animal Resources. **For all air supply/exhaust problems immediately contact the Control Room Operator anytime, day or night, at 956-665-2796.**

**Departmental Personnel**

| Name | Office Phone | Emergency Phone | Cell Phone | Response Time (from home) |
|---|---|---|---|---|
| Dr. Blanca Restrepo | N/A | 956-664-2124 | 956-279-3841 | 1 hour |
| Dr. Christopher Vitek | 956-665-2845 | 772-766-0360 | 772-766-0360 | 20 minutes |
| Dr. John Thomas | 956-665-7147 | | | |

**Facilities Management Personnel**

| Name | Office Phone | Emergency Phone | Cell Phone | Response Time (from home) |
|---|---|---|---|---|
| John McDonald** | 956-665-6451 | 956-605-0100 | 956-605-0100 | 15 minutes |
| Facilities | 956-665-2796 | N/A | | N/A |

**Environmental Health, Safety & Risk Management Department Personnel**

| Name | Office Phone | Emergency Phone | Cell phone | Response Time (from home) |
|---|---|---|---|---|
| Richard Costello | 956-665-3690 | 956-457-2357 | 956-457-2357 | 10 minutes |
| Javier Garcia | 956-665-2052 | 956-874-7769 | 956-874-7769 | 15 minutes |

**Laboratory Animal Resources Personnel**

| Name | Office Phone | Emergency Phone | Cell Phone | Response Time (from home) |
|---|---|---|---|---|
| Cordelia Rasa | 956-665-6436 | 956-313-5964 | 956-313-4319 | 10 minutes |
| Dr. Sander Hacker | 210-567-6166 | 210-288-2819 | 210-288-2819 | 4 hours |

ABSL-3
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| BSL-3 Phones | Extension |
|---|---|
| BSL-3 Hallway | 956-665-2456 |
| 1.500.4 | 956-665-2461 |
| 1.500.6 | 956-665-2471 |
| 1.500.12 | 956-665-2457 |
| 1.500.30 | 956-665-2477 |

# Appendix D:

**MMWR, June 24, 1988 / 37(24); 377-388 –** ***Perspectives in Disease Prevention and Health Promotion Update: Universal precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Bloodborne Pathogens in Health-Care Settings***

## For

**EREBL BSL-3**
**Site Specific Biosafety, Security & Good Laboratory Practices Manual**

The MMWR can also be accessed online from the CDC website @
http://www.cdc.gov/mmwr/preview/mmwrhtml/00000039.htm