2.0 GENERAL GUIDELINES FOR HANDLING BIOLOGICAL AGENTS

2.1 THE SEVEN BASIC RULES OF BIOSAFETY

The most common means of exposure can be essentially eliminated as occupational hazards by following these seven basic rules of biosafety.

- Do not mouth pipette.
- Manipulate infectious fluids carefully to avoid spills and the production of aerosols and/or droplets.
- Restrict the use of needles and syringes to those procedures for which there are no alternatives; use disposable or needle-locking syringes to avoid self-inoculation; and dispose of “sharps” in leak and puncture-resistance containers.
- Use protective laboratory coat, gloves, and eye protection.
- Wash hands following all laboratory activities, or contact with infectious materials/animals.
- Decontaminate work surfaces before and after use, and immediately after spills.
- Do not eat, drink, store food, or apply cosmetics in the laboratory.

These procedures are targeted at minimizing overt occupational exposures and constitute basic essentials of good laboratory practice.
2.2 **Personal Hygiene**

Whenever handling biological material, wash your hands thoroughly:
- after working with any biohazards or animals
- after removing gloves, lab coat, and other contaminated protective clothing
- before eating, drinking, smoking, or applying cosmetics
- before leaving the laboratory area

You should always keep your hands away from your face while handling biological material.

*Do not:*
- eat
- drink
- smoke
- apply cosmetics in the work area

2.3 **Clothing (Primary Barriers) and Personal Protective Equipment**

- **Clothing:** clothes should be comfortable and practical for the type of work conducted. No open toe shoes will be worn in the lab. Hair will be properly held back as to not require to be brushed back by the hand
- **Gloves and Lab Coat:** are NOT worn outside the laboratory area.

**BSL 1**
- It is recommended that lab coats or gowns be worn to prevent soiling of street clothes.
- Water proof bandage and double gloves will be worn if skin on hands is broken or if a rash is present.
- Gloves must be worn to protect hands from exposure to hazardous materials.
- Protective eyewear (if splashes are anticipated) should be worn.
- Use of contact lenses is not recommended; if contact lenses are used, laboratory eye protection or face protection must be worn.

**BSL 2**
- Protective laboratory coats, gowns, smocks or uniforms designated for lab use are worn when in the laboratory.
- Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Double gloving is recommended when handling infectious material.
- Face protection is used for anticipated splashes or sprays of infectious or other hazardous materials when biohazardous material is manipulated outside the Biological Safety Cabinet (BSC).

**BSL 3**
- Solid front or wrap-around gowns are used when in the lab; re-usable gowns are decontaminated (autoclaved) prior leaving the lab to be laundered. Disposable gowns are autoclaved prior to leaving the lab. Gowns are changed when contaminated or damaged.
- Double gloves must be worn when handling infectious materials, infected animals and contaminated equipment, two pairs of gloves is highly recommended.
• Respiratory and face protection are used in rooms containing infected animals or if determined by the Institutional Biosafety Committee.

2.4 Handling Procedures

Always
• Use mechanical pipetting devices and cotton-plugged pipettes.
• Perform all procedures with a minimum of aerosol production.
• Add a disinfectant to water baths used with infectious substances.
• Use trunnion cups with screw caps or sealed rotors for centrifuging procedures. If safety cups/sealed rotors are not available, run centrifuge inside BSC.
• Inspect centrifuge tubes for cracks.
• Use secondary leak-proof containers when transporting samples, cultures, inoculated plates, or other containers of biohazardous materials.
• Place all containers on a lab cart for transport between laboratories.
• Label containers indicating contents, date and name of researcher.

2.5 Handwashing

Handwashing is an extremely important procedure for preventing exposure to and dissemination of infectious agents. Unless microbial contamination is routinely removed, exposure via contact with mucous membranes, inoculation through skin, or ingestion becomes inevitable.

Laboratory personnel should wash their hands:
• when coming on duty
• on leaving the laboratory for whatever reason
• when hands are obviously soiled or after cleaning a spill
• before and after completion of a task in a biological safety cabinet, even if gloves are worn
• before touching the face or mouth
• when removing gloves for any reason
• upon completion of duty

2.6 Protocol for Handwashing

A protocol for handwashing is as follows:
• Turn on faucets and wet hands with tepid water.
• Dispense nonantiseptic soap or antiseptic compound into a cupped hand.
• Spread soap or compound around both hands and between fingers. If needed, add a little more water to facilitate spread and lathering.
• Wash hands for about 30 seconds. Vigorously rub both sides of hands starting from a few inches above the wrist, extending downward between the fingers and around and under the fingernails.
• Rinse thoroughly under the tepid running water. Rinsing should start above the wrist area and proceed to the tips of the fingers. Note: if faucets are not knee- or foot-operated, do not turn off water (do not touch faucet handles) yet.
• Dry hands thoroughly with paper towels. If faucets are hand operated, turn them off now, using a dry paper towel to protect clean hands.
2.7 **Syringes**

Avoid using syringes and needles whenever possible. If a syringe is necessary:
- use the needle-locking type, or a disposable syringe-needle unit
- place disposable syringes directly into sharp container
- non-disposable sharps & Luer-lock (glass) syringes must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving
- do not place syringes in pans containing pipettes or other glassware requiring sorting
- do not recap needles
- dispose of needles in leak proof, puncture resistant containers specifically designed for sharps disposal and in accordance with Chapter 10 - Disposal of Hazardous Materials

2.8 **Controlling the Biohazard Area**

- Keep laboratory doors closed.
- Laboratory doors are locked when lab is unoccupied.
- Limit access into the laboratory during procedures involving biohazardous agents.
  A sign will be posted that includes the universal biohazard symbol when infectious materials or infected animals are present in the laboratory or animal room. This warning sign must identify the agent and indicate requirements for entry (such as immunizations or respiratory protection), emergency contact information, and the approved biosafety level for the laboratory.
- Have a HEPA filter and vacuum trap will be used on laboratory vacuum lines.

2.9 **Housekeeping**

- Decontaminate work surfaces:
  - daily and
  - after each spill of biological material using appropriate disinfectant
- Decontaminate all potentially contaminated equipment used with an experiment.
- To decontaminate or sterilize materials at a site away from the laboratory, transport in a closed leak-proof container.
- Dispose of contaminated wastes according to UTMB Policy for recycle and disposal of chemicals and biohazardous material. (See section 7.2 - “Waste Disposal”).
- Keep books and journals only in clean areas of the laboratory.
- All equipment must be completely decontaminated prior to sending the equipment for routine maintenance or repair work.

2.10 **Containment**

Containment defines the safe methods for controlling infectious agents where they are being handled. The purpose of containment is to reduce exposure to, and prevent the escape into the environment of, potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.
Laboratory practice and techniques include the following:

- Strict adherence to standard microbiological practices and procedures (Biosafety in Microbiological and Biomedical Laboratories)
- Awareness of any potential hazards and training and proficiency in the practices and techniques associated with the materials being handled
- Use of appropriate safety equipment for the specific procedure
- The laboratory supervisor must be trained in laboratory techniques, safety procedures, and hazards associated with handling potentially infectious agents.

Safety equipment

- includes primary barriers between the infectious agent and the worker
- includes biological safety cabinets, safety centrifuge cups, personal protective clothing
- is most effective when used with good laboratory techniques

Facility design

- provides secondary barrier against potential exposure
- includes engineering features allowing protection of laboratory personnel, the work area, or the environment while handling hazardous materials
- most effective when combined with good laboratory technique and safety equipment

2.11 Biosafety Levels

Four biosafety levels are described by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) to recommend laboratory practices, safety equipment, and facilities appropriate for the potential hazards posed by the laboratory activity and the microorganism involved.

The practices, safety equipment, and facility design for each biosafety level and animal biosafety level are fully described in the U.S. Department of Health and Human Services’s Biosafety in Microbiological and Biomedical Laboratories Manual (See Appendix C of BMBL 5th Ed).

**Biosafety Level 1** - practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans.

**Biosafety Level 2** - practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials.

**Biosafety Level 3** - practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents relate to auto inoculation, ingestion, and exposure to infectious aerosols.
Biosafety Level 4 - practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents, which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Additionally, agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and auto inoculation.

2.12 Universal Precautions/Standard procedures

Laboratories in healthcare and research facilities may handle human specimens. Human blood, blood products, human tissue and biological body fluids (urine, feces etc.) are to be handled as infectious or potentially infectious. Specimens may contain multiple infectious etiologic agents. To minimize personal exposure to specimens of an unknown nature, all personnel in laboratories will observe Centers for Disease Control (CDC) guidelines for universal/standard precautions when handling all specimens of tissue, blood and body fluid. This means that all human material will be considered to be infectious and will be handled as potentially hazardous. (Appendix G)