Biological Safety Manual (BSL-2)

Environmental Health and Safety and Risk Management

Revised July 2022

Safety
Is In Everybody's Job Description

1 Responsibilities

1.1 Introduction

The National Institutes of Health (NIH) and Centers of Disease Control and Prevention (CDC) define biohazards (biological hazards) as "infectious agents presenting a risk or potential risk to the well-being of man, or other animals, either directly through infection or indirectly through disruption of the environment." Proper handling and disposal of biohazardous materials greatly reduces the potential for exposure to infectious or harmful agents. General information and guidelines are presented in this chapter. Details for the safe use of specific biological agents and recombinant DNA (rDNA) may be obtained from the Environmental Health and Safety Department.

1.2 Environmental Health, Safety and Risk Management

UTRGV Environmental Health and Safety (EHSRM) Department will:

- develop and implement university environmental health and safety programs and standards for the safe use of biological materials and infectious materials at UTRGV,
- provide consultation and technical information for handling biological agents,
- review proposals and protocols for the use of hazardous biological agents, rDNA and discuss specific procedures and recommendations with the Biological Safety Committee as necessary,
- ensure that biological safety cabinets are certified on annual basis,
- conduct general biological safety trainings on bi-monthly basis and upon request
- review and approve purchases of items such as:
    - biological infectious/toxic agents
    - controlled or regulated substances/items
    - biosafety cabinets and other relevant potentially hazardous equipment
- survey laboratories for compliance with approved standards and policies of UT-Rio Grande Valley, CDC, and NIH,
- provide assistance or advice on decontamination of facilities and equipment,
- and assist in the development of safety plans and training programs.
- EHSRM subcribes to the American National Standard for Occupational Health and Safety Management Systems to be applied to all workplaces. Reducing risk to the lowest possible level is the foundation for protecting workers from hazards that may be present in the workplace. This standard utilizes a hierarchy of controls (elimination, substitution, engineering controls, warning signs, administrative controls and PPE) that are applied to risk reduction and determine the most effective and feasible method to reduce the risk associated with a hazard.

1.3 Laboratory Management

Management (principle investigators and faculty) will

- adhere to established biological safety guidelines and polices,

- ensure that laboratory personnel receive university mandated health and safety training as well as specific training in the hazards and safe handling procedures of biological agents as per protocol,
- encourage employees to report any changes or suspected changes in their health status,
- advise the Environmental Health and Safety Department of any significant changes in the protocol for the use of hazardous biological agents.

1.4 Staff, Students and Visitors

Staff and students will:

- adhere to established biological safety guidelines and policies,
- inform immediate supervisor of any unsafe practices or conditions in the work area,
- report to supervisor any change or suspected change in their health status if there is a possibility it may be work-related,
- follow proper chemical and biological waste disposal procedures as defined by EHSRM,
- report all biological spills and accidents to their supervisor.

1.5 Instituional Biological Safety Committee (IBC)

The IBC committee will:

- establish policies that support university environmental health and safety programs and standards developed by the Environmental Health and Safety Department,
- review Notification of Use applications and registration documents for research involving biological agents or rDNA molecules for the proposed purpose,
- approve, recommend changes, or deny the intended use of the biological agent or rDNA molecules
- ensure that there are no undue hazards to the participants, other staff members, or the public,
- verify the adequacy of the technical procedures performed in order to minimize occupational exposure,
- review the qualifications of the applicant undertaking the proposed research,
- monitor programs for the control and safe use of hazardous biological agents including rDNA, oncogenic viruses and infectious agents,
- review all applications for use of radiation and lasers to ensure compliance with federal, state, and local regulations,
- review and approve the use of chemicals listed within each protocol and ensure compliance with chemical hygiene practice and use.

1.6 Biosafety levels

Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have defined four biosafety levels, recommending laboratory practices, safety equipment, and facilities appropriate for the potential hazards posed by the laboratory

activity and the microorganisms involved for each one. The practices, safety equipment, and facility design for each biosafety level and animal biosafety level are fully described in Appendix C of this document.

1.6.1 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 well-defined and well-characterized strains of viable microorganisms NOT known to cause disease in healthy adult humans and that present minimal potential hazard to laboratory personnel and the environment.

1.6.2 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. Important differences from BSL-1 is 1) laboratory personnel receive specific training in handling infectious agents are are supervised by a principal investigator/laboratory supervisor competent inhandling infectious agents and associated procedures. (2) Access to the laboratory is restricted when work is conducted. (3) All procedures in which infectious aerosols or splashes may be created are conducted in Biosafety Cabinets or other physical containment equipment. 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.

1.6.3 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 autoinoculation, ingestion, and exposure to infectious aerosols.

1.6.4 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 and that are frequently fatal. 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 autoinoculation.

2 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:

1. Do not mouth pipette.
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols and droplets.
3. Restrict the use of needles and syringes to those procedures for which there are no alternatives; use needles, syringes, and other "sharps" carefully to avoid self-inoculation; and dispose of sharps in leak- and puncture-resistant containers
4. Use protective laboratory coat and gloves.
5. Wash hands following all laboratory activities and following contact with infectious materials.
6. Decontaminate work surfaces before and after use, and immediately after spills.
7. Do not eat, drink, store food, apply cosmetics, or smoke in the laboratory.

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

2.2 Personal hygiene and 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
- before leaving the laboratory for whatever reason
- when hands are obviously soiled
- after working with or touching potentially hazardous/biohazardous materials
- before and after completion of a task in a biological safety cabinet, even if gloves are worn
- after removing gloves, lab coat, and other contaminated protective clothing
- before contact around one's face or mouth
- upon completion of duty
- before eating, drinking, smoking, or applying cosmetics

You should always keep your hands away from your face while handling biological material. While in the work area, do not eat, drink, smoke, or apply cosmetics.

A protocol for handwashing is as follows:

1. Turn on faucets and wet hands with tepid water.
2. Dispense nonantiseptic soap or antiseptic compound into a cupped hand.

3. Spread soap or compound around both hands and between fingers. If needed, add a little more water to facilitate spread and lathering.
4. Wash hands for about 20 seconds. Vigorously rub both side of hands starting from a few inches above the wrist, extending downward between the fingers and around and under the fingernails.
5. 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.
6. Dry hands thoroughly with paper towels. If faucets are hand-operated, turn them off now, using a dry paper towel to protect clean hands.

2.3 Clothing

Biosafety Level 1 (BSL1):

- Lab coats or gowns are recommended to be worn to prevent soiling of street clothes.
- Gloves are worn if skin on hands is broken or if a rash is present.
- Protective eyewear is used if splashes are anticipated.
- Contact lenses are not recommended to be used; if contact lenses are used, laboratory eye protection or face protection must be worn.
- Potentially contaminated protective clothing must not be worn outside the laboratory area.

Biosafety Level 2 (BSL2):

- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.
- Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces, or equipment.
- 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.

Biosafety Level 3 (BSL3):

- Solid-front or wraparound gowns are used when in the lab; reusable gowns are decontaminated (autoclaved before leaving the lab to be laundered).
- Gloves must be worn when handling infectious materials, infected animals, and contaminated equipment.
- Respiratory and face protection are used in rooms containing infected animals.

2.3.1 Procedures for removal of protective clothing

1. While wearing double gloves doff gown.
2. Doff outer pair of booties from the back.
3. Doff outer pair of gloves while not contaminating inner gloves.
4. Disinfect inner gloves and booties using an EPA registered disinfectant and enter anteroom.

5. Doff remaining gloves using “Beak Method”, remove booties and dispose into Biohazard Box.
6. Remove head covering from the peak and dispose into Biohazardous Box or remove PAPR and disinfect using EPA registered disinfectant and charge battery unit.
7. Spray bottom of shoes with EPA registered disinfectant
8. Wash hands for 20 seconds before leaving anteroom.

2.4 Glove use

Gloves are an integral piece of personal protective equipment (PPE) for hand protection at UTRGV. Gloves are in common use in research, healthcare, animal, groundskeeping, and mechanical areas requiring attention to glove types and glove materials.

2.4.1 General recommendations

- Gloves should be worn when handling hazardous biological material or when protection of the biological material from contamination is required.
- Gloves chosen should be of a material known to be resistant to permeation by the agent for the duration of use, while allowing sufficient dexterity.
- Inspect gloves for discoloration, small holes or tears before use.
- Remove gloves before touching objects such as doorknobs, elevator buttons, telephones, or computers. When transporting biohazdous items personnel will use one gloved-hand method.
- Reusable gloves should be washed and inspected before and after each use.

Note: Wearing the wrong type of glove could be more hazardous than wearing no gloves, by giving a false sense of protection, holding the hazardous agent in prolonged contact with the skin, or creating new hazards by decreasing dexterity.

2.4.2 Glove materials and types

The most common glove materials used when handling biologicals:

- latex – low-protein, powder free
- polyvinyl chloride (PVC)
- nitrile
- vinyl gloves are NOT recommended

2.4.3 Glove selection

When selecting gloves, consider the type of work and type of hazard. Important parameters for gloves for physical protection of the hands include material strength, dexterity, permeation, abrasion, and heat or cold resistance. When working with biologicals it is important to also consider the chemicals that are being used to choose the correct glove material for both biological and chemical protection. This may involve glove changes to provide the best protection for the particular job. Latex gloves are often worn with actual or potentially infectious material. Problems encountered include reaction and/or allergies to both the powder and the latex material itself. Gloves are available that are powderless and of other materials.

The following factors should be considered when selecting gloves:

- Chemicals will ultimately penetrate a glove, and may do so without evidence of damage to the material.
- Although gloves may protect again a biological hazard, they may not protect against a chemical or a mixture of chemicals.
- Temperatures above room temperature decrease breakthrough time of most materials.
- Thicker materials or multiple layers are usually better for combined biological and chemical use.
- Contaminated gloves must be discarded after use or thoroughly decontaminated before reuse.

Note: Disposable gloves are designed for single use. **Do not** reuse these gloves. Dispose of them in the appropriate container after use.

2.5 Handling procedures

Always

- use mechanical pipetting devices and cotton-plugged pipettes
- perform all procedures with a minimum of aerosol production or splashes
- add a disinfectant to water baths used with infectious substances
- use trunnion cups with screw caps for centrifuging procedures
- inspect the tubes for cracks
- use secondary leak-proof containers when transporting samples, cultures, inoculated petri plates, or other containers of biohazardous materials
- place all containers on a lab cart for transport between laboratories
- label containers indicating contents

2.6 Syringes/Sharps Control

Avoid using syringes and needles whenever possible and use safer medical devices (eg, shielded needles). If a syringe is necessary,

- take care to prevent injuries when using needles, scaples, lancets, and other sharp instruments or devices.
- use the needle-locking type, or a disposable syringe-needle unit
- place used reususable syringes and needles (eg, biopsy needles) in a dedicated and labeled sharps container or pan of disinfectant without removing the needle for transport to a reprocessing area.
- do not place syringes in pans containing pipettes or other glassware requiring sorting
- do not recap needles
- dispose of needles, syringes, scalpel blades, lancets, and other sharps in leak proof, puncture resistant containers specifically designed for sharps disposal

- sharps containers should be easily accessible and located as close as is feasible to the immediate areas wehere sharps are used or are reasonably anticipated to be found.
- clinical specimens submitted in syringes with needles should NOT be accepted for testing. Speciments collected with needle and syringe (eg, synovial fluid, would aspirate) should be transferred to a separate sterile container for transport to the laboratory.
- if it is necessary to submit the specimen in the syringe, the needle should be replaced with a sterile Luer lock tip cap at the place of collection to prevent specimens contained in syringes from leaking during transport.

2.7 Controlling the biohazard area

- Keep laboratory doors closed while experiments are in progress.
- Keep laboratory doors locked when vacant.
- Limit access to the laboratory during procedures involving biohazardous agents – allow entry only to persons informed of the potential hazards.
- Post a warning sign 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 "Immunization Required" or "Respirator Required") and the approved biosafety level for the laboratory.
- Have a suitable trap on laboratory vacuum lines.

2.8 Housekeeping

The principal investigator or laboratory supervisor must ensure that the laboratory has adequate procedures for routine care, cleaning, and disinfection of environmental surfaces and frequently touched items (eg, telephone, keyboards, doorknobs) that may become contaminated and that these procedures are being followed.

- Decontaminate work surfaces
  - Daily (before and after leaving for work)
  - after each spill of biological material
- 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 Environmental Health and Safety disposal procedures regarding chemicals and biohazardous material.
- Keep books and journals only in clean areas of the laboratory.
- All equipment must be completely decontaminated prior to being sent for routine maintenance, repair works or surplus.

2.9 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 to prevent the escape into the environment of, potentially hazardous agents. The three elements of containment are laboratory practice and technique, safety equipment, and facility design.

2.9.1 Laboratory practice and techniques

- Adhere strictly to standard microbiological practices and procedures.
- Be aware of any potential hazards and be trained and proficient in the practices and techniques associated with the materials being handled.
- Use appropriate safety equipment for the specific procedure.
- Ensure that the laboratory supervisor is trained in laboratory techniques, safety procedures, and hazards associated with handling potentially infectious agents.

2.9.2 Safety equipment

- Includes primary barriers between the infectious agent and the worker.
- Includes biological safety cabinets, safety centrifuge cups, and personal protective clothing.
- Is most effective when used with good laboratory technique.

2.9.3 Facility design

- Provides secondary barrier against potential exposure.
- Includes engineering features which allow handling of hazardous materials without endangering laboratory personnel, the work area, or the environment.
- Is most effective when combined with good laboratory technique and safety equipment.

2.10 Universal precautions

Clinical laboratories in healthcare and research facilities must handle human specimens without full knowledge of diagnosis. Specimens may contain multiple infectious etiologic agents. To minimize personal exposure to specimens of an unknown nature, all personnel in laboratories will observe CDC guidelines for universal 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. Clinical laboratory protections for workers is discussed in more detail in section 13 starting on page 43 of this manual.

3 Transportation and Transfer of Biological Agents

3.1 Introduction

Biological agents include infectious agents of humans, plants, and animals, as well as the toxins that may be produced by microbes and by genetic material potentially hazardous by itself or when introduced into a suitable vector. “Etiologic agents” and “infectious substances” are closely related terms that are found in the transfer and transportation regulations. Biological agents may exist as purified and concentrated cultures but may also be present in a variety of materials such as body fluids, tissues, soil samples, etc. Biological agents and the materials that are known or suspected to contain them are recognized by federal and state governments as hazardous materials and their transportation and transfer is subject to regulatory control. Transportation refers to the packaging and shipping of these materials by air, land, or sea, generally by a commercial conveyance. Transfer refers to the process of exchanging these materials between facilities.

3.2 Transportation

Regulations on the transportation of biological agents are aimed at ensuring that the public and the workers in the transportation chain are protected from exposure to any agent that might be in the package. Protection is achieved through the requirements for (a) rigorous packaging that will withstand rough handling and contain all liquid material without leakage to the outside, (b) appropriate labeling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents, (c) documentation of the hazardous contents of the package in case such information should be necessary in an emergency situation, and (d) training of workers in the transportation chain to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

3.3 Regulations

- Public Health Service 42 CFR Part 72, *Interstate Transportation of Etiologic Agents*. This regulation is in revision to harmonize it with the other US and international regulations. A copy of the current regulation may be obtained from the Internet at: http://www.cdc.gov/od/ohs.
- Department of Transportation 49 CFR Parts 171-178, *Hazardous Materials Regulations*. This regulation applies to the shipment of both biological agents and clinical specimens. Information may be obtained from the Internet at: http://www.dot.gov.rules.html.
- United States Postal Service 39 CFR Part 111, *Mailability of Etiologic Agents*. Codified in the Domestic Mail Manual 124.38: Etiologic Agent Preparations. A copy of the Domestic Mail Manual may be obtained from the Government Printing Office by calling 202-512-1800 or from the Internet at: http://www.access.gpo.gov.

- Occupational Health and Safety Administration (OSHA) 29 CFR Part 1910.1030, *Occupational Exposure to Bloodborne Pathogens*. This standard provides minimal packaging and labeling requirements for transport of blood and body fluids within and outside the laboratory. Information may be obtained from your local OSHA office or from the Internet: http://osha.gov
- *Dangerous Goods Regulations* (DGR), International Air Transport Association (IATA). These regulations provide packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These are the regulations followed by the airlines. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air which is provided by the International Civil Aviation Organization (ICAO). A copy of the DGR may be obtained by calling 800-716-6326 or through the Internet at: http://www.iata.org, or http://www.who.org.

3.4 Shipment of hazardous agents

The transportation of hazardous agents is strictly regulated. Failure to adhere to applicable regulations can result in fines and/or punitive actions against the university and the transporter. In addition to violating state and federal transportation laws, personal liabilities can be associated with failure to follow the appropriate shipping and handling requirements. The International Air Transport Association (IATA) and the Department of Transportation (DOT) regulations regarding the shipment of hazardous materials state that "no person may offer or accept a hazardous material for transportation in commerce unless…the hazardous material is properly classed, described, packaged, marked, labeled and in condition of shipment" (HM-171.2).

No UTRGV employee will be permitted to ship chemicals without having completed certified IATA (HighQ LLC module) and DOT training. Since the definition of hazardous materials is very broad, any such shipments must be reviewed by Director of the Environmental Health and Safety Department prior to shipment. EHSRM staff have successfully completed the training course certifying them to carry out these responsibilities.

3.5 Packaging requirements for transport of biological agents and clinical specimens

Figure 1 below shows the generalized "triple" packaging (primary receptacle, watertight secondary packaging, durable outer packaging) required for a biological agent of human disease or materials that are known or suspected to contain them. This packaging requires the "Infectious Substance" label shown in Figure 2 on the outside of the package. This packaging must be certified to meet rigorous performance tests as outlined in the DOT, USPS, PHS, and IATA regulations. Clinical specimens with a low probability of containing an infectious agent are also required to be triple packaged, but performance tests require only that the package shall not leak after a four-foot drop test. DOT, PHS, and IATA require a "Clinical Specimen" label on the outside of the package.

3.6 Transfer

Regulations on the transfer of biological agents are aimed at ensuring that the change in possession of biological materials is within the best interests of the public and the nation. These regulations require documentation of the personnel, facilities, and justification of need for the biological agent in the transfer process and subsequent approval of the transfer process by a federal authority. The following regulations fit in this category:

3.6.1 Importation of etiologic agents of human disease

42 CFR Part 71 *Foreign Quarantine*. Part 71.54 *Etiologic Agents, Hosts and Vectors*. This regulation requires an import permit from the Centers for Disease Control and Prevention for importing etiologic agents of human disease and any materials, including live animals or insects, that may contain them. An application and information on importation permits may be obtained by calling 404-718-2077 or on the Internet at: http://www.cdc.gov/od/eaipp/importApplication/. E-mail: ImportPermit@cdc.gov. Fax: 404-471-8333.

3.6.2 Importation of etiologic agents of livestock, poultry, and other animal diseases

9 CFR Parts 92, 94, 95 96, 122 and 130. These regulations require an import permit from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services to import or domestically transfer etiologic agents of livestock, poultry, other animals, and any materials that might contain these etiologic agents. Information can be obtained at 301-851-3300 option 3, or email at OV@aphis.usda.gov.

3.6.3 Importation of plant pests

7 CFR Part 330 *Federal Plant Pest Regulations*; General; Plant Pests; Soil; Stone and Quarry Products; Garbage. This regulation requires a permit to import or domestically transfer a plant pest, plant biological agent, or any material that might contain them. Information can be obtained by calling 301-851-2046 or 887-770-5990 or Email: plantproducts.permits@.usda.gov

3.6.4 Transfer of Select Biological Agents of Human Disease

42 CFR Part 73.16 *Additional Requirements for Facilities Transferring or Receiving select agents*. Facilities transferring or receiving select agents must be registered with the CDC and each transfer of a select agent must be documented. Information can be obtained on the Internet at:
https://www.selectagents.gov/compliance/guidance/transfer/index.htm

3.6.5 Interstate Shipment of Etiologic Agents of Human Disease

Department of Transportation 49 CFR Parts 171-178. This regulation states that exporters of a wide variety of etiologic agents of human, plant, and animal diseases, including genetic material, and products which might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoT Hazardous Materials Information Center at 1-800-467-4922 or through the Internet at https://www.phmsa.dot.gov/

3.6.6 Packaging and labeling

Figures 1 and 2 illustrate the packaging and labeling of infectious substances and clinical specimens in volumes of less than 50 milliliters in accordance with the provisions of subparagraph 72.3(a) of the regulation on Interstate Shipment of Etiologic Agents (42 CFR, Part 72). A revision is pending that may result in additional package labeling requirements, but this has not been issued in final form as yet.

For further information on any provision of this regulation contact:

Centers for Disease Control and Prevention
Attn: IDSS Help Desk
1600 Clifton Road N.E.
Atlanta, GA 30333
Telephone: 1-855-612-7575

Note that the shipper's name, address, and telephone number must be on both the outer and inner containers. The reader is also advised to refer to additional provisions of the Department of Transportation 49 CFR, Parts 171-180, Hazardous Materials Regulations.

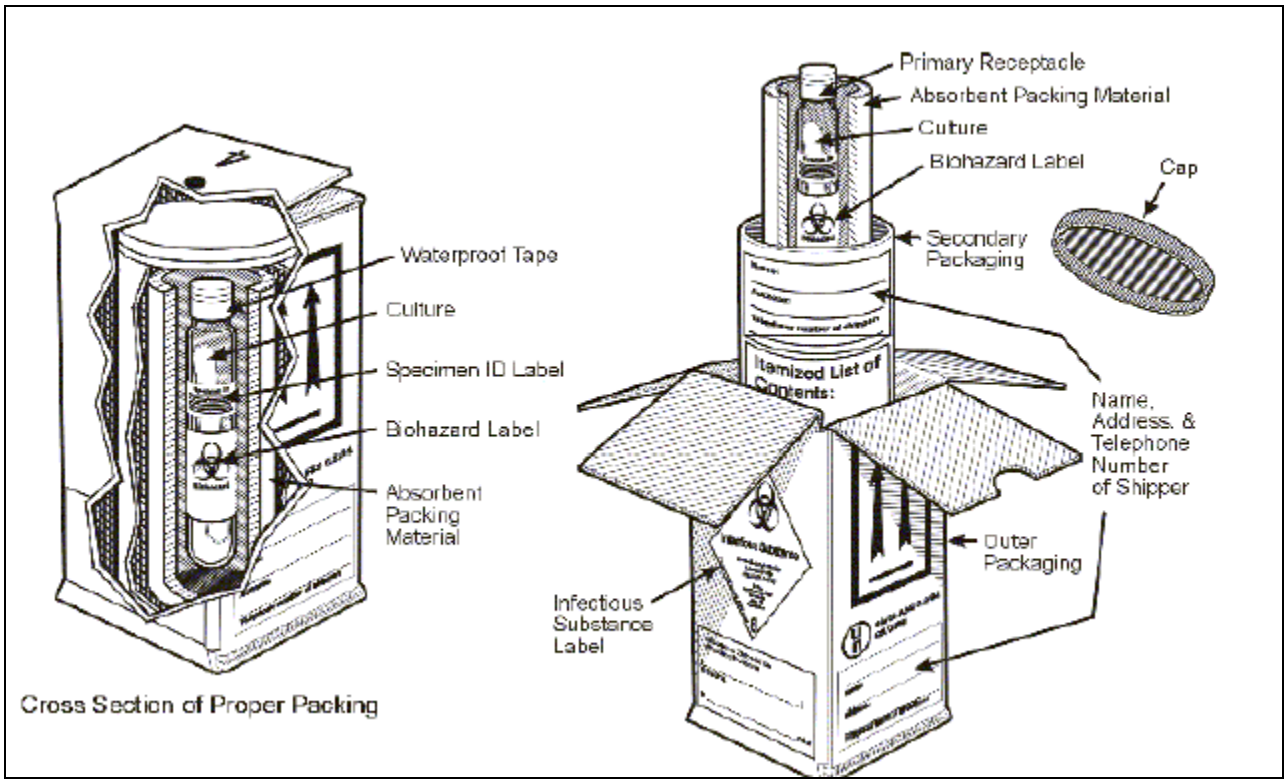

Figure 1: Packing and labeling of infectious substances

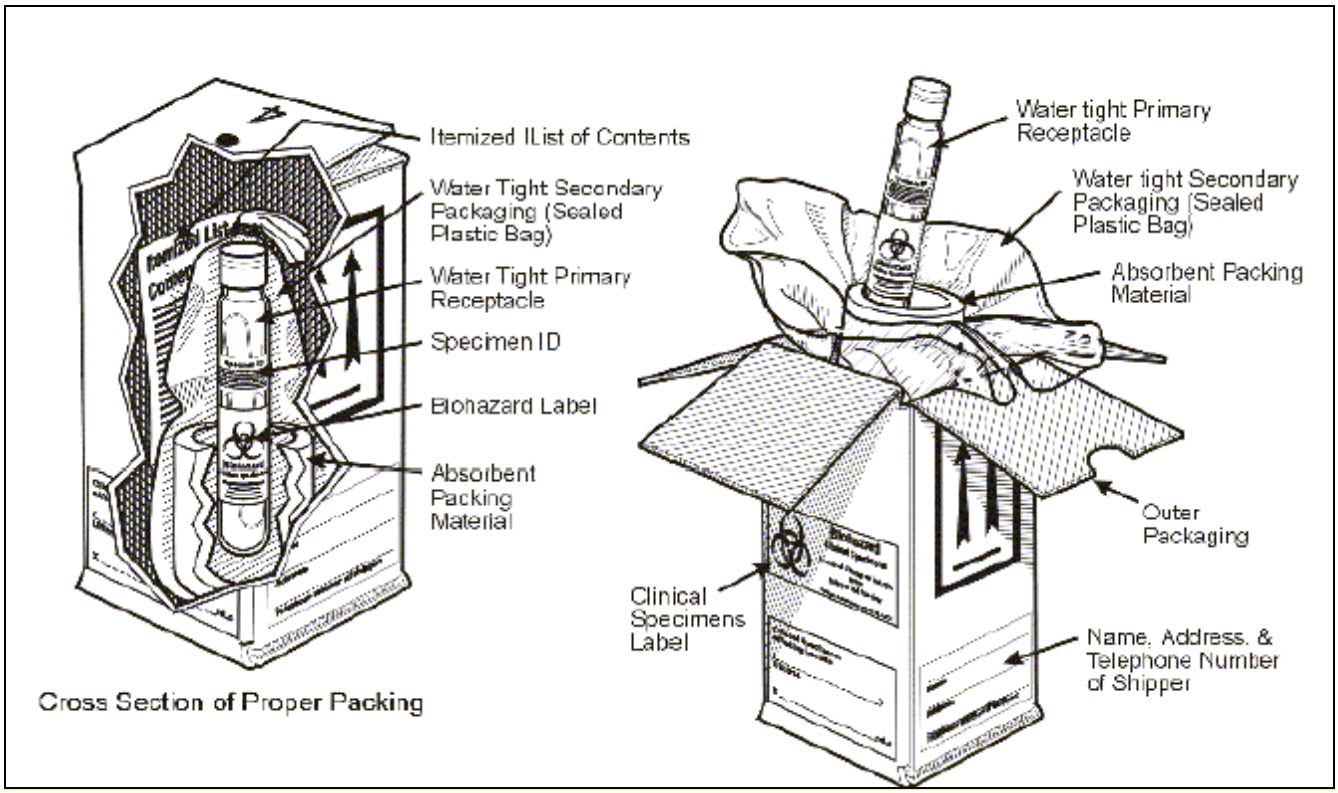

Figure 2: Packing and labeling of clinical specimens

4 Aerosols

4.1 Introduction

The word “aerosol” refers to the physical state of liquid or solid particles suspended in air. The production of aerosols while handling infectious agents may present a serious risk of exposure.

4.2 Risks

Aerosol particles one to five microns in size present the greatest hazard to the laboratory worker because:

- small particles readily penetrate and remain in the respiratory tract if inhaled
- many routine laboratory procedures create aerosols in this size range
- they may remain suspended in air for long periods of time

Aerosols can settle on equipment normally considered to be clean. Skin contamination from aerosols or from handling contaminated equipment may result in infection through ingestion, contact, or skin abrasions. It is also possible for aerosol particles to be spread by the building ventilation system. Risks associated with aerosols can be reduced or eliminated by the use of good technique in a biological safety cabinet.

4.3 Aerosol production

Use of the following pieces of equipment may lead to the production of aerosols:

- centrifuge
- blender
- shaker
- magnetic stirrer
- sonicator
- pipette
- vortex mixer
- syringe and needle
- freeze-dried sample
- vacuum-sealed ampoule
- grinder, mortar, and pestle
- test tubes and culture tubes
- heated inoculating loop
- separatory funnel

4.4 Minimizing aerosol production

To reduce or minimize aerosol production, follow these guidelines:

- Use a biological safety cabinet or a chemical fume hood when performing activities that may produce aerosols.
- Keep tubes stoppered when vortexing or centrifuging.

- Allow aerosols to settle for one to five minutes before opening centrifuge, blender, or tubes that have been mixed.
- Place a cloth soaked with disinfectant over the work surface to deactivate possible spills or droplets of biohazardous agents.
- Reconstitute or dilute contents of an ampoule slowly.
- When mixing two solutions, discharge the secondary fluid down the side of the container or as close as possible to the surface of the primary solution.
- Allow inoculating loop or needle to cool before touching biological specimens.
- Perform centrifugation using balanced trunnion cups with disinfectant added between the tube and the cup.
- Wrap the relevant item with disinfectant-soaked gauze when:
    - removing the needle from the rubber stopper of a test tube or vial
    - breaking the cap on an ampoule
    - removing stoppers or plugs from tubes
    - expelling air or surplus solution from a syringe
- Do not
    - mix a solution by flushing with a pipette or syringe
    - blow or force liquids out of a pipette
    - use a hypodermic and syringe as a substitute for mechanical pipetting devices when transferring infectious fluids

5 Disinfection and Sterilization

5.1 Definitions

Decontamination: The application of microbiocidal steam, gas, solid (granular), or liquid chemical agents in situations in which microbes may be protected from contact by extraneous matter. The destruction of or removal of microorganisms to some lower level, but not necessarily total destruction. Sterilization, disinfection, and antisepsis are forms of decontamination.

Sterilization: The total destruction of all living organisms by processing in steam sterilizers (autoclaves) or with ethylene oxide autoclaves.

Disinfection: The destruction of all non-spore-forming organisms that could pose a potential hazard to humans or compromise the integrity of the equipment. Disinfection implies the use of antimicrobial agents on inanimate objects (floors, bench tops, equipment, etc.).

Antisepsis: The application of a liquid antimicrobial chemical to living tissue, either human or animal. The objective is to prevent sepsis by either destroying potentially infectious organisms or inhibiting their growth and multiplication.

5.2 General procedures

- Frequently disinfect all floors, cabinet tops, and equipment where biohazardous materials are used.
- Decontaminate all infectious materials and contaminated equipment before they are washed, stored, or discarded.
- Use autoclavable or disposable materials whenever possible. Keep reusable and disposable items separate.
- Minimize the amount of materials and equipment present when working with infectious agents.
- Sterilize, properly store, or dispose of all biohazardous materials at the end of each day.
- Be aware that agar and other materials may interfere with the germicidal actions of chemical disinfectants, thus requiring higher concentrations or longer contact time.
- Ensure sterilization by using suitable indicators with each autoclave load.
- Use holding containers clearly marked with signs such as "NON-INFECTIOUS" or "BIOHAZARDOUS: TO BE AUTOCLAVED."

5.3 Choosing a method

The choice of method for sterilization or disinfection will depend on two factors:

- the target organism (the biological agent)
- the characteristics of the materials or areas to be cleaned

5.4 Selecting a disinfectant

Use the following table to aid in the selection of an appropriate disinfectant:

Substance	Description
Alcohols	Ethyl or isopropyl alcohol at 70-80% concentration is a good general-purpose disinfectant; not effective against bacterial spores.
Phenols	Effective against vegetative bacteria, fungi, and lipid-containing viruses; unpleasant odor; toxic by skin contact.
Formaldehyde	At a concentration of 5-8% formalin, good disinfectant properties against vegetative bacteria, spores, and viruses; irritating odor; carcinogen.
Quaternary ammonium compounds	Cationic detergents which are strongly surface active; extremely effective against lipoviruses; not effective against bacterial spores; may be neutralized by anionic detergents (soaps).
Chlorine	Low concentrations (50-500 ppm) active against vegetative bacteria and most viruses; higher concentrations (2500 ppm) required for bacterial spores; corrosive to metal surfaces; must be prepared fresh; laundry bleach (5.25% chlorine) may be diluted and used as a disinfectant.
Iodine	Recommended for general use; effective against vegetative bacteria and viruses; poor activity against bacterial spores.

Table 1: Descriptions of commonly used disinfectants

5.5 Sterilization methods

5.5.1 Wet heat (steam)

This method requires approximately 15psi pressure with a chamber temperature of at least 250°F (121°C). The cycle time begins when the materials being sterilized reach the predetermined temperature. Then the length of time is dependent upon the volume size of the load (usually 30-60 minutes). Steam sterilization effectiveness is measured with a *Bacillus stearothermophilus* biological indicator.

5.5.2 Dry heat

This is less effective than steam, and requires more time (two to four hours) and a higher temperature (320-338°F or 160-170°C). Dry-heat sterilization can be monitored with a *Bacillus subtilis* biological indicator.

5.5.3 Biological test packs

Biological test packs for sterilizers are available through the laboratory supervisor or biology safety officer.

5.5.4 Ethylene oxide gas (EO)

Ethylene oxide sterilization is not available at UTRGV. Contact Environmental Health and Safety for additional information if needed.

6 Biological Safety Cabinets

Note: Sections relevant to Biosafety Levels 3 and 4 (specifically, this chapter) are not currently in use at UTRGV and will be reviewed by the IBC committee as these procedures become necessary for laboratory compliance.

6.1 Overview

A biological safety cabinet (BSC) is used as a primary barrier against exposure to biohazardous or infectious agents, as it surrounds the immediate workspace involving the agent. However, total containment is not provided by primary barrier equipment, and aerosols can escape. **A primary barrier such as a biological safety cabinet merely complements careful work practices.**

Biological safety cabinets contain high-efficiency particulate air (HEPA) filters, which have 99.97% to 99.99% efficiency for 0.3-micron particles. These cabinets operate with laminar air flow – the movement of air with uniform velocity in one direction along parallel flow lines, either horizontally or vertically.

6.2 Primary containment: biological safety cabinets

Biological safety cabinets are among the most effective and the most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Classes I, II, III) have performance characteristics and applications which are described here and in further detail in the appendix. Properly maintained Class I and II BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate- and high-risk microorganisms (agents at Biosafety Levels 2 and 3). Both Class I and Class II BSCs have inward face velocities (75-100 linear feet per minute) that provide comparable levels of containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs also protect the research material itself through HEPA filtration of the air flow down or across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the community, and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

6.3 Safety rules for use of Classes I and II cabinets

6.3.1 Completion of a job

When finished using a biosafety cabinet, make sure that:

- all equipment which has been in direct contact with the research agent is enclosed and the surface decontaminated prior to removal from the cabinet
- waste containers are covered
- the cabinet is allowed to operate for five minutes with no activity in order to purge airborne contaminants from the work area
- interior work surfaces are decontaminated

- you thoroughly wash your hands and arms with warm, soapy water

6.3.2 Biohazardous spills in the cabinet

If a biohazardous spill occurs inside the cabinet:

- Take decontamination steps while the cabinet is operating to prevent the escape of contaminants.
- Wearing gloves, spray or wipe walls, work surface, and all affected apparatus with an appropriate disinfectant detergent.
- If the spill is large (puddles), flood the work surface with disinfectant and allow to stand 15 to 30 minutes before removing it.
- If a drain system is involved, consult the BSC manufacturer's specific instructions regarding decontamination.
- Wipe the area clean with water followed by 70% ethanol or cavicide.

After a spill is decontaminated, the cabinet must be thoroughly cleaned and dried. Residual materials can support the growth and multiplication of microorganisms, and can jeopardize the product protection normally provided by biological safety cabinets.

6.3.3 Points to remember

- The biological safety cabinet is not a substitute for good laboratory practice.
- Aerosols can escape.
- The airflow is disrupted by
    - rapid movement of hands or arms
    - opening doors to the room
    - persons walking past the cabinet
- Decontaminate the cabinet before and after each use.

6.4 Class I

Class I BSCs are currently being manufactured on a limited basis; many have been replaced by Class II BSCs.

The Class I biological safety cabinet (Fig. 3) is a negative-pressure, ventilated cabinet usually operated with an open front and a minimum face velocity at the work opening of at least 75 linear feet per minute (lfpm). All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside. The Class I BSC is designed for general microbiological research with low and moderate-risk agents, and is useful for containment of mixers, blenders, and other equipment. These cabinets are not appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet. The Class I BSC can also be used with an installed front closure panel without gloves, which will increase the inward flow velocity to approximately 150 lfpm. If such equipped cabinets are ducted to the outside exhaust, they may be used for toxic or radio-labeled materials used as an adjunct to microbiological research. Additionally, arm-length rubber gloves may be attached to the front panel with

an inlet air pressure release for further protection. In this configuration, it is necessary to install a make-up air inlet fitted with a HEPA filter in the cabinet.

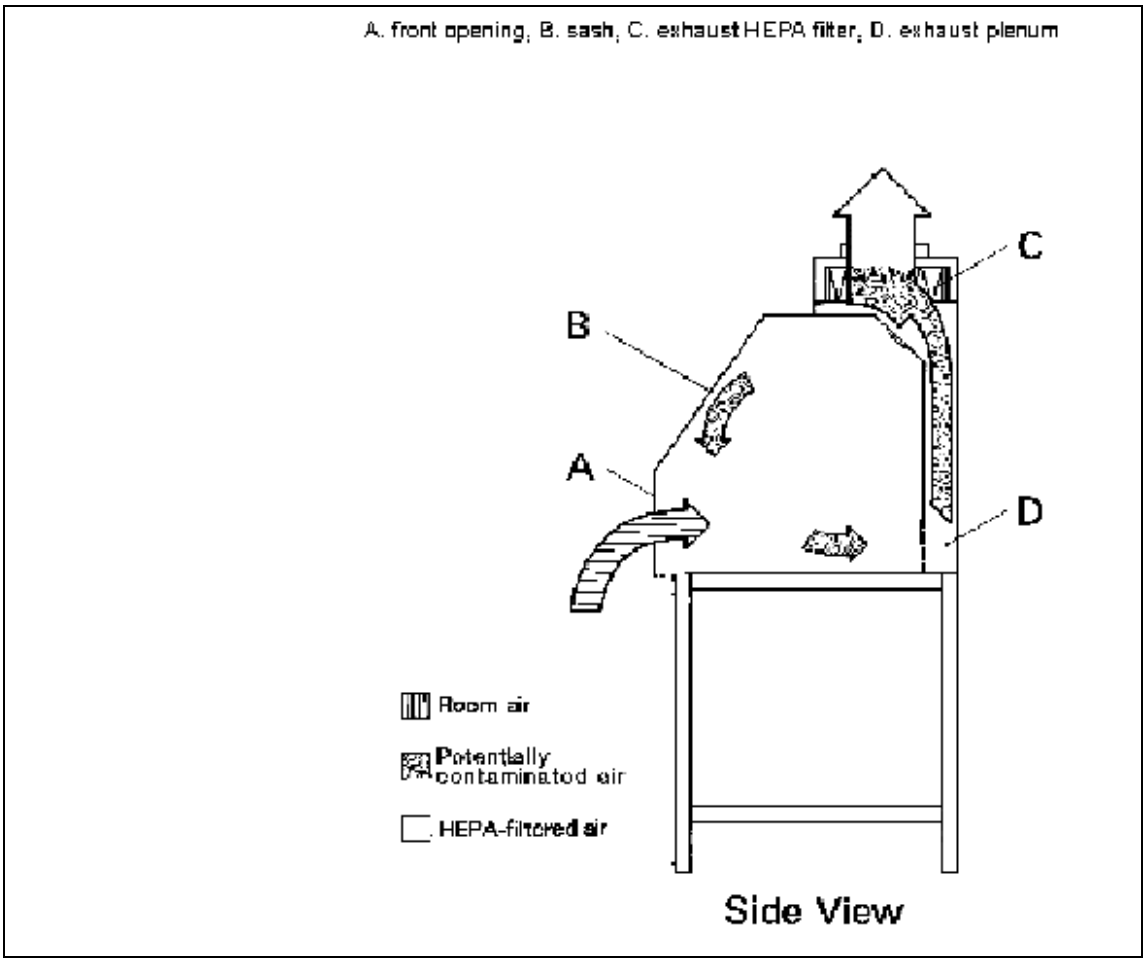

Figure 3: Class I biological safety cabinet

6.5 Class II

The Class II Biological Safety Cabinet (Fig. 2) is designed with inward air flow at a velocity to protect personnel (75-100 lfpm), HEPA-filtered downward vertical laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Design, construction, and performance standards for Class II BSCs, as well as a list of products that meet these standards, have been developed by and are available from the National Sanitation Foundation, Ann Arbor, Michigan (see publications list, Appendix E). Utilization of this standard and list should be the first step in selection and procurement of a Class II BSC.

Class II BSCs are classified into types A and B, based on construction, air flow velocities and patterns, and exhaust systems. Basically, type A cabinets are suitable for microbiological research in the absence of volatile or toxic chemicals and radionuclides,

since air is recirculated within the cabinet. Type A cabinets may be exhausted into the laboratory or to the outdoors via a "thimble" connection to the building exhaust system.

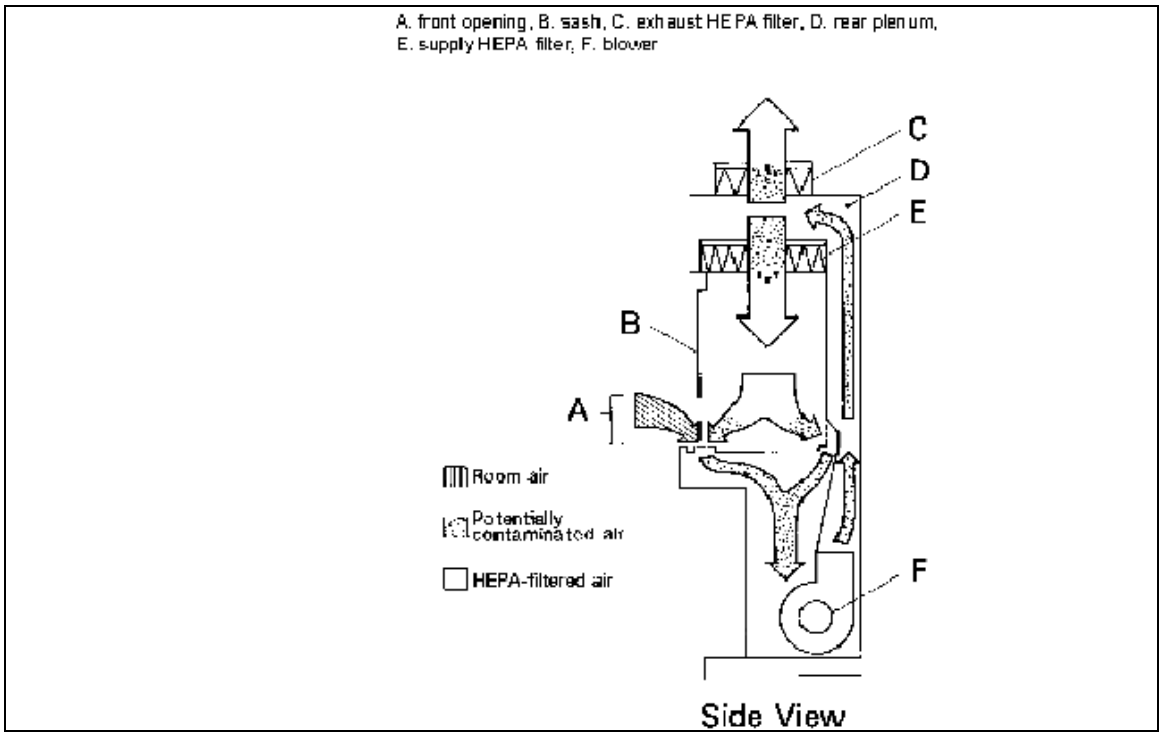

Figure 4: Class II, type A biological safety cabinet

Type B cabinets are further sub-typed into types B1, B2, and B3. A comparison of the design features and applications are presented in Figures 5, 6, and 7, respectively. Type B cabinets are hard-ducted to the building exhaust system and contain negative pressure plena. These features, plus a face velocity of 100 lfpm, allow work to be done with toxic chemicals or radionuclides.

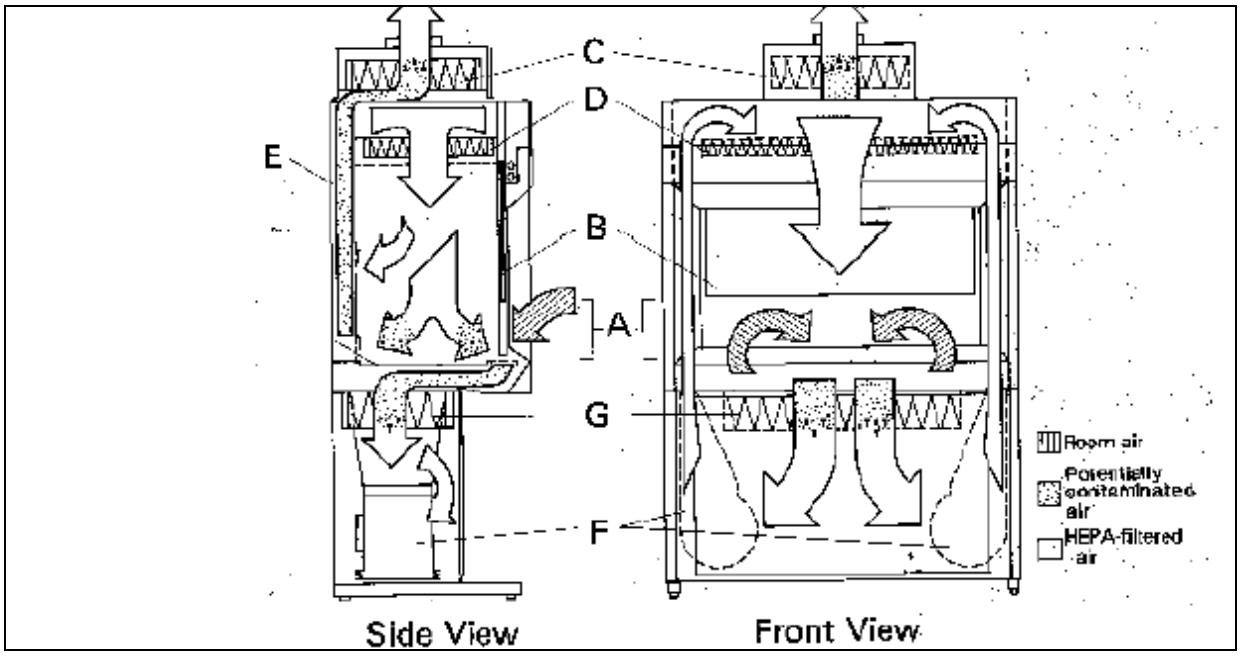

Figure 5: Class II, type B1 biological safety cabinet (classic design).
A: front opening, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: negative pressure exhaust plenum, F: blower, G: additional HEPA filter for supply air.
Note: The cabinet exhaust must be connected to the building exhaust system.

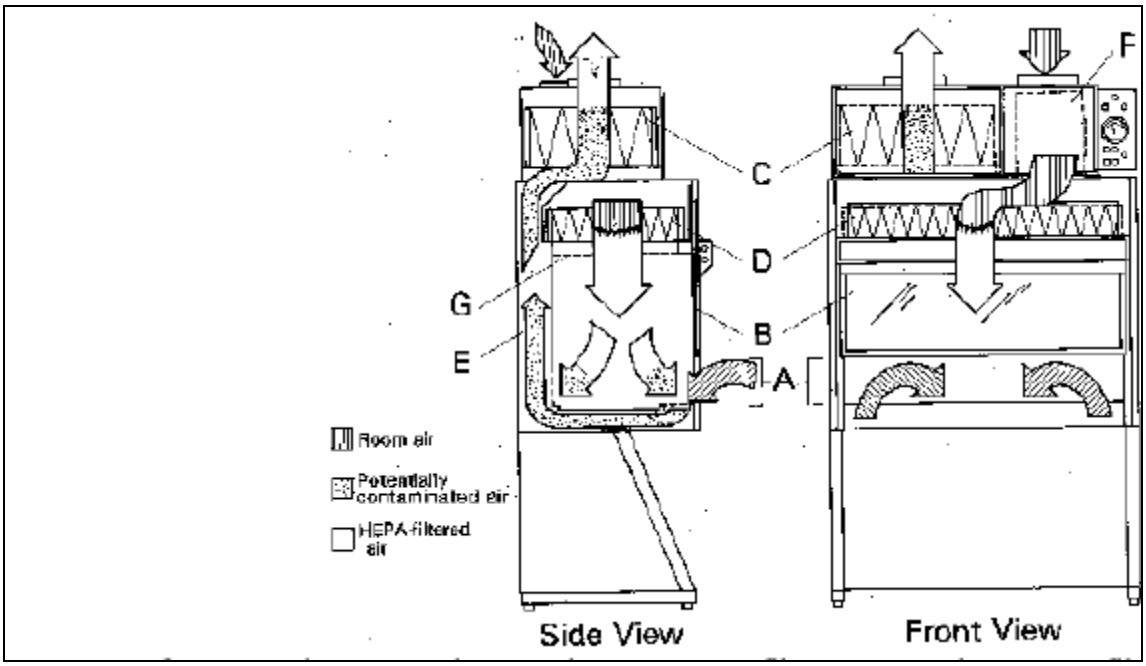

Figure 6: Class II, type B2 biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: negative pressure exhaust plenum, F: filter screen.
Note: The carbon filter in the building exhaust system is not shown.
The cabinet exhaust must be connected to the building exhaust system.

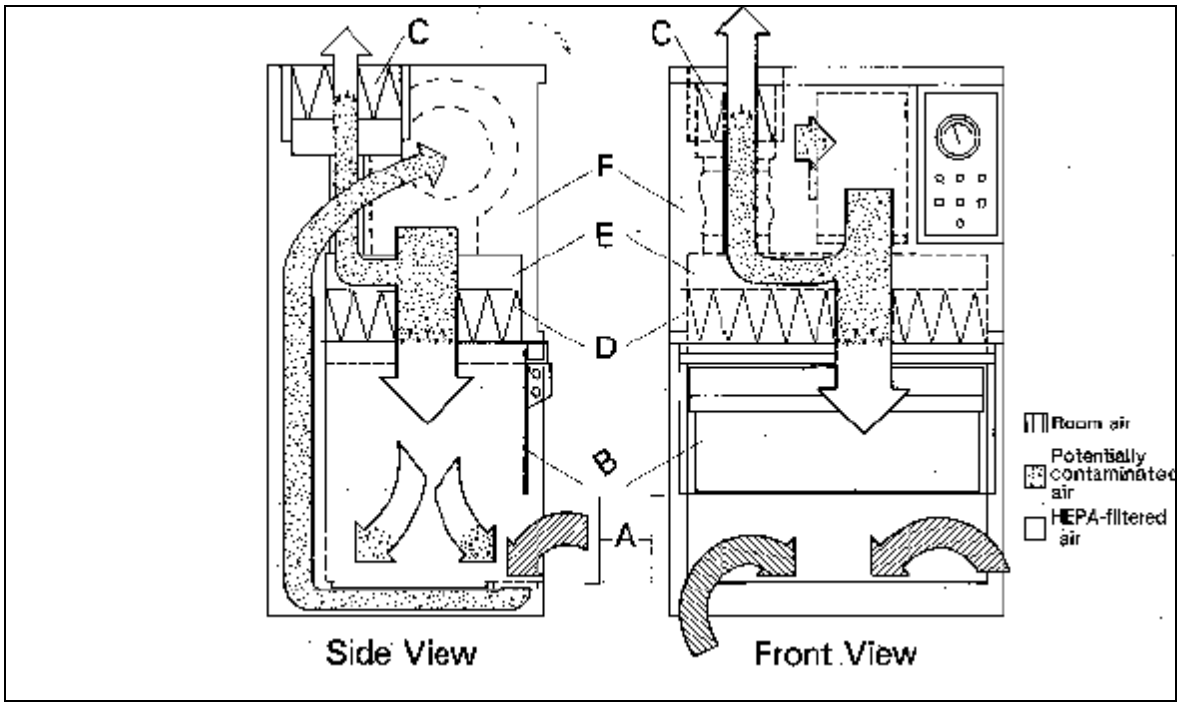

Figure 7: Table-top model of a Class II, type B3 biological safety cabinet.
A: front opening, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: positive pressure plenum, F: negative pressure plenum.
Note: The cabinet exhaust must be connected to the building exhaust system.

It is imperative that Class I and II biological safety cabinets be tested and certified *in situ* at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not replace the critical certification prior to use in the laboratory.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the biological safety cabinets. Of particular note are activities that may disrupt the inward directional airflow. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber, opening and closing doors to the laboratory or isolation cubicle, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in use have been demonstrated to cause the escape of aerosolized particles from within the cabinet. Class I and II cabinets should be located away from traffic patterns and doors. Air flow from fans, room air supply louvers, and other air-moving devices can disrupt the airflow pattern at the face of the cabinet. Strict adherence to recommended practices for the use of BSCs and their proper placement in the laboratory are as important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

6.6 Class III

The Class III biological safety cabinet (Fig. 8) is a totally enclosed, ventilated cabinet of gas-tight construction and offers the highest degree of personnel and environmental protection from infectious aerosols, as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4 containment.

All operations in the work area of the cabinet are performed through attached arm-length rubber gloves or half-suits. The Class III cabinet is operated under negative pressure. Supply air is HEPA filtered and the cabinet exhaust air is filtered through two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside of the facility.

All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system. The Class III cabinet must be connected to a double-doored autoclave and/or chemical dunk tank used to sterilize or disinfect all materials exiting the cabinet, and to allow supplies to enter the cabinet. Several Class III cabinets are therefore typically set up as an interconnected system.

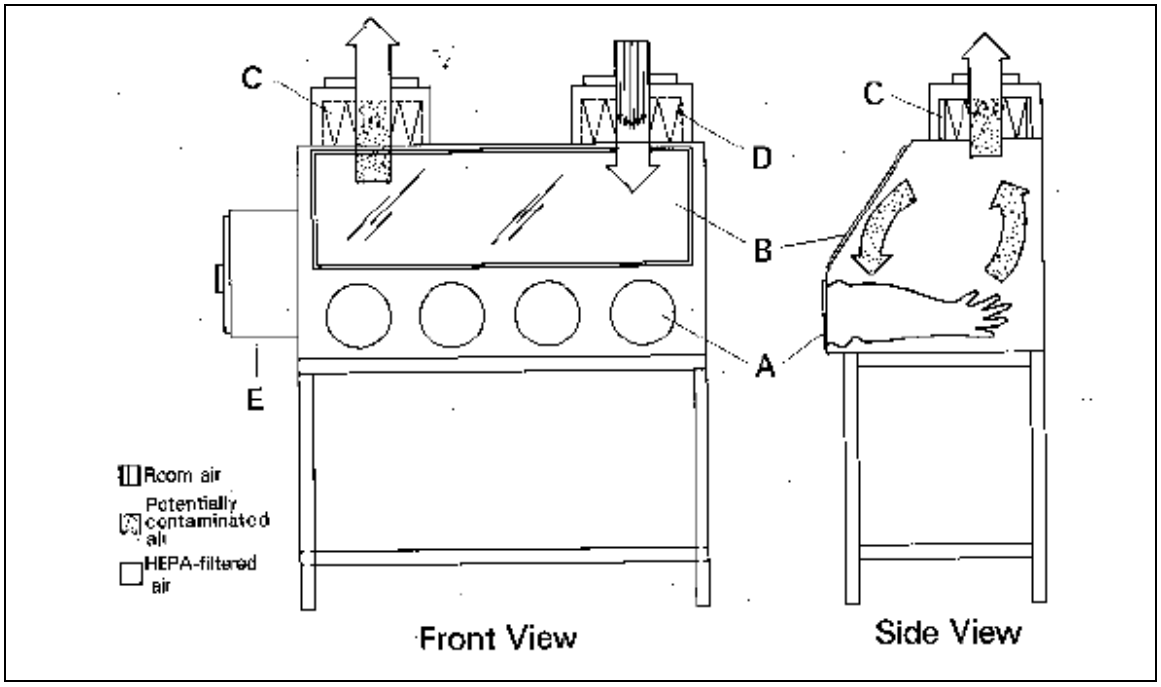

Figure 8: Class III biological safety cabinet.
A: glove ports, with O-ring for attaching arm-length gloves to cabinet, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: double-ended autoclave or pass-through box.
Note: A chemical tank may be located beneath the work surface of the BSC with access from above. The cabinet exhaust must be connected to the building exhaust system.

6.6.1 Positive-pressure personnel suit

Personnel protection equivalent to that provided by Class III cabinets can also be obtained with the use of a one-piece, ventilated suit worn by the laboratory worker when working with biosafety level 3 or 4 agents in a "suit area" and using Class I or II BSCs. The personnel suit is maintained under positive pressure with a life-support system to prevent leakage into the suit. In this containment system, the worker is isolated from the work materials.

The personnel suit area must be essentially equivalent to a large Class III cabinet. The area is entered through an air-lock fitted with airtight doors. A chemical shower is provided as a "dunk tank" to decontaminate the surfaces of the suit as the worker leaves the area. The exhaust air from the suit area is filtered through two HEPA filter units installed in series. The entire area must be under negative pressure.

As with Class III BSCs, the gloves of the personnel suit are the most vulnerable component of the system, as they are subject to punctures by sharps or animal bites.

6.6.2 Other devices

Horizontal laminar flow "clean benches" are used in clinical, pharmaceutical, and laboratory facilities strictly for product protection. This equipment must never be used for handling toxic, infectious, radioactive, or sensitizing materials, since the worker sits in the immediate downstream exhaust from the clean bench. Vertical laminar flow benches may be useful for certain manipulations of clean materials (e.g. pouring agar plats) but should not be used when working with infectious materials.

6.7 Comparison of BSCs

Type	**Face velocity (lfpm)**	**Airflow pattern**	**Radionuclides / toxic chemicals**	**Biosafety levels**	**Product protection**
Class I* open front	75	In at front; rear and top through HEPA filter	No	2,3	No
Class II type A	75	70% recirculated through HEPA; exhaust through HEPA	No	2,3	Yes
Class II, type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Yes (Low levels / volatility)	2,3	Yes
Class II, type B2	100	No recirculation; total exhaust via HEPA and hard ducted	Yes	2,3	Yes
Class II, type B3	100	Same as IIA but plena under negative pressure to room and exhaust air is ducted	Yes	2,3	Yes

Class III	N/A	Supply air inlets and exhaust through two HEPA filters	Yes	3,4	Yes

Table 2: Comparison of biological safety cabinets

* Glove panels may be added and will increase face velocity to 150 lfpm; gloves may be added with an inlet air pressure release that will allow work with chemicals/radionuclides.

6.8 Certification

Never use a biological safety cabinet unless it has been certified to meet minimum safety specifications (e.g. NIH-03-112 or NSF Standard No. 49). Every biological safety cabinet will be certified by qualified personnel at the following times:

- when newly installed
- after filter replacement
- after the cabinet has been moved
- annually

EHSRM maintains an inventory of biological safety cabinets and schedules the annual certification inspections through an outside contractor.

7 Procedures for Centrifugation

7.1 Low-speed centrifugation

All low-speed centrifugation must be done in capped tubes in centrifuge safety cups or centrifuge rotors that provide a gasket for containment. Centrifuge safety cups must be used to be effective – if a tube breaks infectious material will stay in the bucket. Buckets can be removed and opened in the biosafety cabinet.

The following procedures should be followed during centrifugation:

- Ensure that each operator is trained in proper operating procedures.
- Keep a log book detailing operation records for centrifuges and rotors.
- Do not exceed safe rotor speed.
- Place a biohazard label on the centrifuge if used for infectious agents.
- Before centrifuging, inspect tubes for cracks and stress marks.
- Make sure the correct adapters are in place.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet.
- Wipe outside of tubes with disinfectant before placing in rotor.
- Wipe the exterior of safety carriers or rotors with disinfectant before removing from the biosafety cabinet.
- Never overfill centrifuge tubes as leakage invariably occurs when the tubes are filled to capacity. The maximum for general purpose centrifuge tubes/bottles is ¾ full. Always cap tubes before spinning.
- Stop the centrifuge immediately if an unusual noise or vibration is detected.
- If decontamination of a rotor or bucket is required, soak rotor in Cavicide or equivalent disinfectant, followed by a mild detergent, then water rinse.
- Always use sealed safety cups or sealed rotors with O-rings. A variety of safety tubes/bottles and safety centrifuge cups have been developed specifically for work with infectious agents.
- Sealed rotors or safety cups should be opened inside BSC after certifugation of infectious materials is complete.
- Decontaminate safety carriers or rotors and centrifuge interior after each use.

7.2 Microfuge use

Several models of microfuges are used in laboratories. Microfuges should be placed inside a biosafety cabinet. Microfuges with containment features may be used outside the biosafety cabinet, as long as the gasket in the rotor lid is in place and intact.

7.3 Centrifuge spill

If leak is outside the safety cup or sealed rotor when opening centrifuge:

1. Hold your breath.
2. Close the centrifuge lid.
3. Turn centrifuge off.

4. Immediately leave the laboratory.
5. Notify others to evacuate the lab.
6. Post laboratory door with a biohazard spill note.
7. Presume the aerosolized material is contaminated.
8. Treat the incident as a potential exposure.

7.4 Decontamination procedures

- Allow area to rest for 30 minutes prior to entry for decontamination.
- Notify the supervisor or Principal Investigator and EHSRM (at ext. 3690).
- Don appropriate personal protection equipment (cover gown, booties, gloves, eye protection, respiratory protection) before entering the laboratory.
- Use absorbent materials to cover spill areas before the addition of a disinfectant. Absorbent material reduces the potential of generating an additional aerosol due to the decontamination procedure itself.
- Decontaminate all exposed environmental surfaces before releasing the room for normal use.
- Remove rotor and place in a biological safety cabinet. To decontaminate the rotor, soak it in a disinfectant approved for use by the manufacturer's instruction and followed by mild detergent, then water rinse.
- All contaminated material will be disposed of according to procedures for the biosafety level of the laboratory.

Note: If unsure of procedure contact the Environmental Health and Safety Department (665-3690 or 882-5930) for more information.

8 Procedures for Spills of Hazardous Materials

8.1 Spills in the laboratory

Spills of biohazardous, corrosive, flammable, or caustic materials (collectively referred to as hazardous) in the laboratory that occur outside a biological safety cabinet, chemical fume hood, or other physical containment device need to be reported to the supervisor or principal investigator and documented. Biohazardous spills that must be reported and documented include any spills that may contain potentially infectious materials or the possibility of splashes and generation of aerosols or airborne particles.

8.2 Spills and response procedures

For any spills in laboratories or prep rooms, the instructor should follow these procedures:

8.2.1 Non-flammable liquid spills

For a spill involving 500 ml to four (4) liters of non-flammable liquid:

1. Move students out of affected area.
2. Contain spill with absorbent material from spill kit.
3. If chemical is corrosive, neutralize with appropriate agent.
4. Soak up material with absorbent from lab spill station.
5. Place contaminated materials in appropriate disposal bag.
6. Wash area with water.
7. Fill out an incident report.

For spills of more than four (4) liters of non-flammable liquids:

1. Contain spill with absorbent material from spill kit.
2. Evacuate the area.
3. Notify campus police and EHSRM of the spill and provide location information.
4. Neutralize corrosives, if possible.
5. Soak up material with absorbent from lab spill station
6. Place contaminated materials in appropriate disposal bag.
7. Wash area with water.
8. Fill out an incident report.

8.2.2 Flammable liquid spills

For a spill of 100 ml to one (1) liter of flammable chemicals:

1. Move students out of affected area.
2. Turn off ignition sources.
3. Open hood sash doors.
4. Contain spill with absorbent material from spill kit.
5. Notify campus police and EHSRM of the spill and provide location information.
6. Soak up material with absorbent from lab spill station.

7. Place contaminated materials in appropriate disposal bag.
8. Wash area with water.
9. Fill out an incident report.

For a spill of one (1) to four (4) liters of flammable chemicals:

1. Move students out of room.
2. Turn off ignition sources.
3. Open hood sash doors.
4. Contain spill with absorbent material from spill kit.
5. Leave room and notify campus police and EHSRM of spill and provide location information. After hours, notify campus police.
6. **Do not** re-enter the area until help is available (minimum of two people for clean-up).
7. If chemical vapor is a problem, do not enter without a respirator.
8. Soak up material with absorbent from lab spill station.
9. Place contaminated materials in appropriate disposal bag.
10. Wash area with water.
11. Fill out an incident report.

For spills of more than four (4) liters of flammable liquids:

1. Contain spill with absorbent material from spill kit.
2. Evacuate the laboratory.
3. Evacuate surrounding areas if there is a low flash point or if more than eight (8) liters of chemicals have spilled.
4. Turn off ignition sources
5. Open hood sash doors
6. Leave room and notify campus police and EHSRM of the spill and provide location information.
7. **If more than five (5) gallons of chemical are spilled and the chemical has a very low flash point, such as for alcohol, <u>notify campus police and advise them to evacuate the building and call the Fire Department.</u>**
8. Get out and stay out.
9. Do not re-enter until permission is granted by Fire Department personnel on the scene.
10. Do not enter without a respirator if air is saturated with vapor.
11. Fill out a incident report.

8.2.3 Extremely hazardous chemical spills

For a spill of any amount of chemicals such as cyanide or sulfide solution:

1. Evacuate all persons from all connecting laboratory areas.
2. Open hood sashes if possible, and make sure to close doors.
3. Contain spill with absorbent material from spill kit.
4. Notify campus police and EHSRM of spill and provide location information.
5. Do not re-enter.
6. Provide EHSRM with specific material information so that they can proceed with appropriate clean-up procedures.

7. Fill out an incident report.

Note: If the spilled biohazardous material is labeled/tagged with a radionuclide, refer to the Radiation Safety Manual.

8.3 Spills in hallways

Procedure for clearing hazardous spills in hallways:

1. Restrict traffic through the area.
2. Shut all room doors adjacent to the spill area.
3. Notify the supervisor or principal investigator, campus police at 882-4911, and EHSRM at 665-3690 or 882-5903. They will direct decontamination of the area as follows.
4. Put on personal protective equipment (HEPA filtered respirator, gown, gloves – double-glove if necessary – and shoe covers).
5. Cover the spill with absorbent material.
6. Pour disinfectant around and onto the absorbent material. Allow to stand for 30 minutes contact time.
7. After 30 minutes, carefully soak up the spill with absorbent material.
8. Pick up any glass or sharps with tongs or tweezers and discard in a sharps container.
9. Decontaminate the area again with an appropriate disinfectant.
10. Dispose of absorbent material in a red bag or autoclave bag as appropriate.
11. Fill out and submit spill report.

8.4 Reoccupancy of a spill area

Before reoccupying any area where a spill has occurred:

- An EHSRM representative must determine that the decontamination has been effective.
- Stringent decontamination measures must have been executed if the spilled agents were of a highly infectious nature.
- Follow-up steps such as surface swab sampling or medical surveillance may be necessary.

9 Biological Agents and Recombinant DNA

9.1 Introduction

A Notification of Use for Biological Agents and Recombinant DNA (NOU) form must be submitted to the UTRGV Institutional BioSafety Committee for review and approval when the project meets the following criteria:

- **Biological Agents**: An NOU must be completed and submitted for all Class 2 and above pathogens and all human products, human tissues, and human cell lines.
- **Recombinant DNA**: An NOU must be completed and submitted for all rDNA Class 1 and above. When genes are propagated in living cells, submission of an NOU for a biological agent and for the rDNA is required.
- **Select Agents**: select agent use requires an NOU to be completed for the biological agent and rDNA. select agent users must comply with regulations issued by the Centers for Disease Control and Prevention and Health and Human Services.

9.2 Recombinant DNA

9.2.1 Definition

Recombinant DNA (rDNA) molecules are either:

- molecules constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell
- Nucleic acid molecules that are chemically, or by other means, synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (synthetic nucleic acids)
- molecules that result from the replication described above

9.2.2 Research approval categories

rDNA research may be approved in one of two categories at UTRGV:

- Experiments that require specific prior approval by the National Institutes of Health (NIH) and the UTRGV IBC committee
- Experiments that require prior approval of only the UTRGV IBC committee

Note: The list of rDNA molecules, along with the reporting and containment requirements is revised periodically by the Director of the National Institutes of Health.

9.3 Select agent use

Specific steps for compliance are as follows:

- Laboratories handling select biological agents must meet requirements outlined in the CDC/HHS publication *Biosafety in Microbiological and Biomedical Laboratories*, current edition.

- Laboratories are subject to inspection by CDC or their designee for compliance with the regulation.
- Select agents must be destroyed or deactivated in the laboratory.
- Laboratories will comply with documentation and notification requirements when ordering or transferring agents.
- Laboratories will develop a Chemical Hygiene Plan and meet requirements outlined in the OSHA Lab Standard (29) CFR 1910.1450, Occupational Exposure to Hazardous Chemicals in Laboratories.
- Laboratories using select agents will maintain strict security in select agent storage and be kept locked when not occupied.

9.4 List of select agents

9.4.1 Viruses

- Crimean-Congo hemorrhagic fever virus
- eastern equine encephalitis virus
- Ebola viruses
- equine morbillivirus
- Lassa fever virus
- Marburg virus
- Rift Valley fever virus
- South American hemorrhagic fever viruses (Junin, Machupo, Sabia, Flexal, Guanarito)
- tick-borne encephalitis complex viruses
- variola major virus (smallpox virus)
- Venezuelan equine encephalitis virus
- viruses causing hantavirus pulmonary syndrome
- yellow fever virus

Exemptions:

- vaccine strains of viral agents:
    - Junin virus strain candid #1
    - Rift Valley fever virus strain MP-12
    - Venezuelan equine encephalitis virus strain TC-83
    - yellow fever virus strain 17-D

9.4.2 Bacteria

- *Bacillus anthracis*
- *Brucella abortus, B. melitensis, B. suis*
- *Burkholderia (Pseudomonas) mallei*
- *Burkholderia (Pseudomonas) pseudomallei*
- *Clostridium botulinum*
- *Francisella tularensis*
- *Yersinia pestis*

Exemptions:

- vaccine strains as described in Title 9 CFR, Part 78.1

9.4.3 Rickettsiae

- *Coxiella burnetii*
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*

9.4.4 Fungi

- *Coccidioides immitis*

9.4.5 Recombinant organisms/molecules

- Genetically modified microorganisms or genetic elements from organisms listed above shown to produce or encode for a factor associated with a disease.
- Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins listed here or their toxic subunits.

9.4.6 Other restrictions

The deliberate transfer of a drug resistance trait to microorganisms listed that are not known to acquire the trait naturally is prohibited by NIH Guideline for Research Involving Recombinant DNA Molecules, if such acquisition could compromise the use of the drug to control these disease agents in humans or veterinary medicine.

Contact EHSRM for assistance prior to commencement of work with a select agent.

10 Laboratory Moves, Transfers, or Closures

10.1 General

Before moving, transferring or closing a lab, everything needs to be cleaned. Leave nothing behind; the lab must be empty when the move is complete. Decontaminate all bench tops and other work surfaces. The Principal Investigator of the lab area will be held responsible for any materials, equipment, or trash left behind. EHSRM personnel will inspect the lab with the individual responsible for the laboratory to ensure that the lab has been cleared. If the lab has not been cleared properly, the Principal Investigator will be contacted for follow-up response.

- Sharps must be properly disposed of in puncture-resistant, leak-proof containers to prevent puncture wounds or accidents. Sharps include needles, glass Pasteur pipettes, capillary tubes, and glass slides.
- Do not block access to safety equipment or routes of escape from labs with moving boxes or equipment.
- Supervision of the packing will be conducted by EHSRM as required by law. All chemicals must be properly segregated and packaged.

10.2 Laboratory equipment decontamination form

All equipment that is to be relocated, disposed of, or salvaged must be tagged with the completed Laboratory Equipment Decontamination Form. This form will indicate to the individuals moving or receiving the equipment whether or not it has been used with hazardous chemical, biological, and/or radioactive materials. If the equipment was used with these types of materials, decontamination procedures must be performed by the laboratory prior to removal from or abandonment of the laboratory. This will ensure that people coming in contact with the equipment do not get exposed.

If the equipment is being abandoned or sent to inventory, remove all markings denoting its use with hazardous materials before tagging the equipment with the Laboratory Equipment Decontamination Form.

Note: Inventory and Physical Plant will not accept any equipment without the Laboratory Decontamination Form attached. Radiation decontamination will be referred to EHSRM.

The Laboratory Closure and Relocation Checklist should be completed, signed by a faculty member and EHSRM representative.

10.3 Equipment

- Coordinate the decontamination of any biological safety cabinets to be moved through EHSRM at least two weeks before the move. The cabinets will need to be re-certified at the new location before use.
- Decontaminate all used equipment prior to packing or moving.

- Chemical residues need to be removed from all work surfaces and equipment. Lab bench tops must be cleared and cleaned with appropriate detergent or disinfectant depending on laboratory procedures.
- Tag all equipment that is to be relocated, disposed of, or salvaged with the completed Laboratory Equipment Decontamination Form (see section 12.2).
- Freezers may be moved with contents inside. Once the freezer is put back in service, allow the contents to refreeze before checking for broken and spilled material. Contact EHSRM for additional information on securely packing contents of freezers and refrigerators.
- Wipe test all equipment if radioactive materials were used. Include a diagram of the areas wiped. Wipe tests should be initiated no later than the day prior to the move.

11 Respiratory Protection Program

11.1 Overview

Respiratory protection was once found only in the industrial setting. Current trends in safety and health have brought the respirator into not only the lighter industrial settings but the home as well. The use of respirators is even mandated by government agencies in healthcare areas for protection against such airborne diseases as tuberculosis.

If you are potentially exposed to airborne hazardous substances at UTRGV, your job duties should be evaluated. Following such an evaluation, the need for respiratory protection will be determined. Hazards can often be engineered out or procedures changed to minimize the hazard making respirator use unnecessary. In fact, using a respirator is the least preferable method of controlling exposures.

The General Industry Safety and Health Regulations lists minimal acceptable requirements for a respiratory protection program. The State of Texas has adopted these regulations by reference. Basically, these requirements are as follows:

- Establish written standard operating procedures governing the selection and use of the program.
- Select respirators based on the hazards to which the worker is exposed. Use approved or accepted respirators when they are available.
- Instruct and train each user in proper use and limitations of their respirator.
- Establish a maintenance schedule. Those respirators used by more than one worker must be thoroughly cleaned, disinfected and inspected after each use. Worn or deteriorated parts must be replaced. Emergency response equipment must be included in these schedules.
- Store respirators in a convenient, clean, and sanitary location.
- Conduct appropriate surveillance of work area conditions, degree of employee exposure, regular inspections, and evaluations to determine the continued effectiveness of the program.

Persons assigned to tasks requiring the use of respirators must be physically able to perform the work and use the equipment. The physician shall determine what health and physical conditions are pertinent. The respirator user's medical status should be reviewed periodically (annually).

If you currently use a respirator or feel that a particular aspect of your job requires the use of one, please contact the Environmental Health and Safety Department.

12 Reproductive Hazards

12.1 Introduction

Reproductive hazards may cause alterations in the genetic make-up of a cell, response to hormones, or metabolic pathways. Such hazards may affect both male and female reproductive systems. A reproductive hazard may

- inhibit implantation of a fertilized egg
- block fertilization
- cause death or abnormal development of an embryo

The above may lead to

- spontaneous abortion
- infertility
- stillbirth
- malformed offspring

12.2 Teratogens

A teratogen is an agent that causes congenitally malformed offspring. It may affect the mother directly through interference of transplacental exchange of nutrients, or by actually crossing the placental barrier and directly affecting the developing fetus. Teratogenic effects are normally not hereditary, but may result from mutagenic damage to germ cells, embryonic cells, or other toxic effects. They cause permanent alterations in the form or function of offspring by acting at specific times during development and timing is as critical as exposure during certain periods and results in specific adverse effects. For example, from the third to the eighth week of pregnancy the organs are developing, and the placenta, which acts as a barrier to many toxicants, is not completely formed until the eighth or ninth week.

12.3 Exposure hazards

12.3.1 Female

Females have a lifetime supply of eggs at birth, so any mutations to these eggs will be permanent. Furthermore, agents acting upon the female fetus at the time of egg formation could change the genetic structure of the fetus's ova before birth. Exposures during the first trimester represent the greatest risk.

12.3.2 Male

Male sperm cells are continually replenished, making damage to sperm cells temporary, affecting only those present at the time of exposure or damage. Still, exposure to agents may result in mutations in sperm that are transmittable to offspring. Miscarriages and birth defects may also be attributable to male exposure to agents.

12.4 Known human teratogens

- cytomegalovirus (CMV)
- diabetes
- hepatitis B virus
- herpes simplex virus
- herpes virus hominis I & II
- human immunodificiency virus (HIV)
- parvovirus B-19 (erthyma infectiosum)
- phenylketonuria
- rheumatic disease
- rubella virus
- syphilis (*Treponema pallidum*)
- toxoplasmosis
- varicella virus
- Venezulelan equine encephalitis virus
- virilizing tumors

13 Clinical Laboratories

13.1 Introduction

Clinical laboratory environment differ from those of a research or teaching laboratory. Clinical laboratories routinely work with unknown specimens and speciments that have the potential to be infected with multiple pathogens. Clinical diagnostic laboratory personnel may not know what infectious agent or other hazards exist in the specimen they handle and process. Most public and animal health clinical laboratories use Biosafety Level (BSL-2) facility, engineering, and biosafety practices. All UTRGV clinical laboratories follow BSL-2 practices, equipment, and facility design.

13.2 Clinical Laboratory Risk Assessment

Risk assessment generates information that guides the selection of appropriate microbiological practices, safety equipment, and facility safeguards that can reduce Laboratory associated infections (LAIs). The intergration of the risk assessment process into daily laboratory operations results in the ongoing identification and prioritization of risk and the establishment of risk mitigation protocols tailored to specific situations which promotes a positive culture of safety.

The clinical laboratory director is responsible for the overall operation and administration of the laboratory. As stated in the Clinical Laboratory Improvement Amendments (CLIA) regulations, the laboratory director must:

1 Ensure that testing systems developed and used for each of the tests performed in the laboratory provide quality laboratory services for all aspects of test performace, and

2 Ensure that the physical plant and environmental conditions of the laboratory are appropriate for the testing performed and provide a safe environment in which employees are protected from physical, chemical, and biological hazards.

The responsibility for ensuring the safe and secure handling of hazardous materials in a clinical laboratory should be shared. A multidisciplinary team should be responsible for the laboratories risk assessment which takes into consideration the knowledge and expertise of the laboratory, infection prevention, and safety professionals. Risk assessments should be documented and routinely evaluated, particularly when new instruments, tests, staff, or processes have been added to the laboratory environment. Also, risk assessments should be reevaluated when unanticipated or unusual events, near-misses, incidents, or accidents occur. The assessment team should determine what hazards may exist and the risks associated with those hazards. Clinical laboratories should consider what procedures or activities will be performed, where the work will be performed, who will perform the work, and what undesirable events could occur.

It is also essential to evaluate the potential routes of transmission of the suspected infectious agent (i.e., inhalation of aerosols, ingestion, percutaneous inoculation from sharps or non-intact skin, and direct mucous membrane contact from splashes or droplets). In general, blood and body fluids are not normally an inhalation risk, but there is a risk of percutaneous, mucous membrane contact, ingestion, or non-intact skin exposure in clinical laboratories. Protecting portals of entry (i.e., eyes, nose, mouth, and non-intact skin) can reduce initial exposure to hazards, subsequent transmission of infectious agents, and potential LAIs.

Clinical laboratories should consider a wide range of potential hazards when conducting a risk assessment. Examples of hazards unique to the clinical laboratory that should also be considered are listed below:

- Hazards associated with unknown specimens;
- Hazards associated with point-of-care (POC) and/or bedside testing; and
- Hazards associated with inadequate mitigation capabilities.

13.3 Clinical Laboratory Mitigation Measures

Standard precautions‖ refers to the concept that all patients and all laboratory specimens should be handled as if they are infectious and capable of transmitting disease. Standard precautions consist of the major features of universal precautions and body substance isolation and apply to all patients and their specimens regardless of their diagnosis or presumed infection status.

Standard precautions were issued by the CDC in 1996 and are based on the fact that all blood, body fluids, secretions, and mucous membranes can contain infectious agents. Standard precautions not only include the major components of blood and body fluid precautions and universal precautions but also provide guidelines for preventing infections in inclinical settings. Standard precautions include guidelines for patient isolation; PPE; protection of HCWs when in the presence of patients with contact-, droplet-, and airborne-transmissible diseases; hand hygiene; and manipulation of soiled

clothing and equipment. Therefore, standard precautions are more comprehensive than are either blood and body fluid or universal precautions.

Universal precautions were issued by the CDC in 1987 and extended the 1983 recommendations to all patients regardless of their bloodborne infectious status. Universal precautions consisted of guidelines to prevent transmission of HIV , HBV , HCV , and other bloodborne pathogens (from blood, body fluids, semen, vaginal fluids, pleural fluids, and tissues) when providing health care or first aid. They emphasized that blood and body fluids of all patients should be considered as potentially infectious for these pathogens and included guidelines for using PPE and for preventing injuries from needles, scalpels, and other sharp objects. Universal precautions did not apply to the following unless they were contaminated with blood: saliva, feces, nasal secretions, sputum, sweat, urine, tears, and vomitus.

New employees in the laboratory require specific education on basic principles and on their job- and procedure-specific application. In addition, seasoned employees require periodic refresher training to reinforce their knowledge and understanding of changes that occur as new infectious disease challenges evolve.

13.4 Components of Standard Precautions

Standard laboratory precautions should be used when handling or coming in contact with the following.

- All potential infectious materials from patients including all body fluids, secretions, excretions (except sweat), and tissue specimens regardless of whether they contain visible blood.
- Equipment, work surfaces, and materials that may have come in contact with potentially infectious materials.
- Reagents and QC, proficiency testing,and calibrator materials regardless of origin or documented absence of specific pathogens.

The risks of a laboratory worker‘s contact with potentially infectious materials should be eliminated or minimized through the use of engineering controls (eg, BSCs, PPE) and administrative controls (eg, no-hands procedures in handling contaminated sharps) which are covered in more detail above in accordance with BSL-2 containment information. Because these approaches to control have limitations, standard laboratory precautions remain an integral part of the strategy to interrupt disease transmission in laboratories.

Standard laboratory precautions eliminate the need for using specific biohazard warning labels (other than the OSHA-required biohazard label) on specimens obtained from patients infected with HBV, HIV, or other pathogens including antibiotic-resistant organisms. Warning labels used to indicate the (suspected or actual) infectious nature of specific specimens should NOT be used. The use of warning labels contradicts the principle that all specimens should be treated as infectious and capable of transmitting disease.

13.5 Clinical Safe Work Practices

Exposure control should be practiced in all phases of the laboratory workflow (preexamination, examination, and postexamination) that involve human specimens including procurement, transportation, accessioning, preparation, analysis, storage, and disposal.

Bloodborne pathogen exposures are estimated to occur between 600 000 and 800 000 times annually. Data suggest that at an average hospital, approximately 30 needlestick injuries per 100 beds per year occur to a variety of HCWs, with most being preventable.Seventy-five percent of all incidents are associated with disposable syringes and winged infusion sets and could be prevented by using safer equipment (engineering controls).

Whether collected at the bedside or in a dedicated blood collection site in the laboratory or office, all blood specimens should be regarded as potentially infectious (universal precautions). Care should be taken to prevent blood spills or splashes on environmental surfaces, the patient, or the laboratory worker. Plastic-backed, absorbent paper may be used to cover environmental surfaces and should be discarded after use. Special diligence should be exercised to avoid accidental needlesticks. For complete protection, gloves and laboratory coats/gowns should be worn when collecting blood specimens. Gloves should be the appropriate size and be of a material that does not permit blood or OPIM to pass through to or reach the employee‘s skin. Phlebotomists should practice standard precautions at all times and, when collecting blood from a patient on special precautions (eg, airborne, droplet, or contact precautions), should follow the directions posted on the isolation sign. In addition, only necessary phlebotomy supplies should be taken into the patient‘s room. The manufacturer‘s instructions for the use of blood collection devices and the institutional guidelines for the collection of blood specimens should be followed. Phlebotomists should be trained on appropriate spill response when human blood is involved. Any resulting environmental contamination should be decontaminated as soon as possible.

In addition to the BSL-2 safe work practices detailed above, clinical laboratory personnel should receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (eg, HBV vaccine, *N. meningitidis* vaccine, or tuberculosis [TB] skin testing). If possible, occupationale/employee health services hould be consulted when assessing requirements for immunizations. The immunization program should be reviewed annually to determine appropriate coverage of vaccine-preventable exposures and to assess employee eligibility for vaccination. A system of documented acceptance or declination of vaccination should be maintained. Additional medical surveillance may also be required (eg, TB skin testing or interferon-gamma release assay).

Appendix A: Biological Safety Policy

A.1 Introduction

All work regarding the use of BSL-2 agenst and rDNA mandate committee approval

The Biological Committee was established for the purpose of formulating and recommending to the governing body through the Dean of Health Sciences, a general policy for the safe use of biological agents at UTRGV. The committee recognizes and supports the University's fundamental objectives of teaching, research, and development. It also recognizes the University's obligation to pursue these objectives without compromising the health and safety of its students, staff, and faculty and members of the surrounding community. The committee's goal, therefore, is not to be restrictive but to develop policies and procedures that will promote the safe use and handling of biological agents while allowing necessary research to proceed.

The committee has used the *Guidelines for Research Involving Recombinant DNA Molecules*, August 1999, NIH revision and *Biosafety in Microbiological and Biomedical Laboratories*, May 1999, CDC/NIH, as a basis for this policy. The classification of etiologic agents and oncogenic viruses and the levels of containment of this policy will be updated to reflect changes in the CSC, NIH, and NCI guidelines.

A.2 Biological, Chemical, and Radiation Safety Committee

A.2.1 Scope, mission, and responsibilities

The scope of the IBC Committee encompasses all UTRGV biological/chemical safety issues including personnel in the clinical laboratories.
The mission of the IBC Committee is to support the University's fundamental objectives of teaching, research and development by promoting the safe use and handling of biological and chemical agents and assuring that all activities involving these agents are in compliance with the applicable guidelines, codes and regulations.

Responsibilities and important components of IBC Committee:

- Assess all biosafety activities.
- Establish policies that support university environmental health and safety programs and standards developed by the Environmental Health and Safety Department,
- Maintain and review policies.
- Identify problem areas.
- Assure safety of students, employees, visitors, environment, and community.
- Review and assess grant projects for biological safety.
- Report and advise university administration on biological safety issues.
- Use of radioactive materials and the safe use of chemicals.
- Communicate to address:

- biological agents requiring biosafety review
- individual responsibilities with respect to biological safety
- biological issues prompted by public awareness/media coverage

A.3 Definition of a biological agent

A biological agent is any agent, or component of an agent, that could cause harm to people, animals, and/or the environment. For the purpose of this policy, a biological agent is considered to be any of the following:

- recombinant DNA molecules
- organisms and viruses containing recombinant DNA molecules
- materials known or suspected to contain etiological agents
- oncogenic viruses
- human products (blood, tissue)

A.4 Review classification

A.4.1 Etiologic agents and oncogenic viruses

For the purpose of establishing review policies, etiologic agents and oncogenic viruses shall be classified into low-, moderate- and high-risk groups. Those agents in the high-risk group require stringent controls for their containment because they are extremely hazardous to laboratory personnel or could cause widespread disease if released into the environment. Adequate safety for agents in the low-risk groups is ensured through standard microbiological practices and basic laboratory facilities.

The moderate-risk group includes biological agents that present a significant risk to the laboratory workers and the surrounding personnel and require the use of a containment facility. Each group corresponds to a specific biosafety level. Each biosafety level consists of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the risk group.

The following classifications and biosafety levels shall be used as guidelines. The group classification corresponds to varying levels of review necessary to handle the biological agent. Any specific agent may be placed in a higher group classification by the IBC Committee. The reclassification will be deemed necessary if the laboratory procedures will involve any of the following:

- large quantities or highly concentrated preparations of infectious agents
- manipulations that are likely to produce a large volume or aerosol
- manipulations that are otherwise intrinsically considered hazardous by the committee

Group	**Biosafety level**	**Biological agents**
Low risk	2	Class 1 etiologic agents Class 2 etiologic agents Low-risk oncogenic viruses

Moderate risk	3	Class 3 etiologic agents Moderate-risk oncogenic viruses
High risk	4	Class 4 etiologic agents

Table A1: Group biosafety level biological agents

A.4.2 Recombinant DNA research

The current procedures and requirements for recombinant DNA are specified in the current NIH Guidelines for Research Involving DNA Molecules. UTRGV must comply with these guidelines if NIH funding is to be maintained. The IBC committee shall act as the Institutional Biosafety Committee as defined in those guidelines. Any update to the NIH guidelines shall be considered as a revision to this Biological Safety Policy.

Due to the complexity of the NIH guidelines, only a brief overview of the classification and review procedures will be given in this policy. The laboratory facilities and procedures for each experiment involving specific agents are specified in the guidelines. As with the review classification for etiologic agents and oncogenic viruses, the group classifications correspond to varying levels of review by the committee. All persons proposing to use recombinant DNA must file a registration document.

A.5 Review procedures

A.5.1 Etiologic agents and oncogenic viruses

All persons currently conducting or proposing to conduct research involving biological agents, as defined by this policy, shall submit a properly completed Notification for Use of Biological Agents and Recombinant DNA Form (NOU) to the EHSRM department or the Instutional Bilogical Safety Committee. The form should be submitted prior to, or simultaneously with, any proposal for funding of research.

Once this notification is received, EHSRM department staff will review the templates, facilities, equipment, and general procedures with the Principal Investigator. The committee will then certify the laboratory at a specific Biosafety Level. The specific requirements that must be met for each biosafety level are included in this chapter.

After an Investigator obtains this certification, the agent corresponding to that level or below may be used in that laboratory. Research involving any additional biological agent requires a Notification of Use Form to be submitted to EHSRM and the IBC for committee approval. This certification does not include rDNA or work with agents that require US Department of Agriculture approval before importation, possession, or use (referred to as Class 5). These agents must be addressed on a case-by-case basis.

Any research involving agents in the high-risk group classification shall require prior approval by the committee.

The following table summarizes the review procedures specified on the previous page.

Group	Biosafety level	Review procedure
Low and moderate risk	2 and 3	1. PI submits NOU to EHS/IBC program. 2. EHS/IBC reviews facilities and procedures for compliance with appropriate biosafety level corresponding to the classification of the biological agent. 3. Committee certifies facility for work at a specific biosafety level. 4. Research on any agent at that biosafety level or below requires submission of a NOU.
High risk	4	Due to the highly specialized equipment and containment facilities required, no Biosafety Level 4 activity may be approved at UTRGV/TSC at the present time.

Table A2: Summary of review procedures

A.5.2 Recombinant DNA experiments

All persons proposing to conduct recombinant DNA research must submit a Notification for Use of Biological Agents and Recombinant DNA to the IBC committee through the EHSRM department and will aid the Investigator in the interpretation of the NIH guidelines.

The NOU must contain a description of

- the source(s) of DNA
- the nature of the inserted DNA sequences
- the hosts and vector to be used
- whether any rDNA sequences will be employed in the expression of a protein
- the containment conditions specified in the NIH guidelines

The document must be dated and signed by the Investigator. No recombinant DNA experiments at UTRGV are exempt. All projects must meet the approval of the committee before the researcher may begin.

A.5.3 Experiments involving animal and biological agents

Any experiment involving both animals and biological agents will be classified and reviewed at the appropriate level. The Vertebrate Animal Biosafety Level recommendations as given in the latest edition of *Biosafety in Microbiological and Biosafety Laboratories* will be used as a guide for the Investigator. The committee will cooperate with the Institutional Animal Care and Use Committee on all experiments involving both animals and biological agents.

A.6 Appeals

Any Investigator who believes that a biological agent has been improperly classified may request a reconsideration of the classification. The request should be submitted to the chairman of the IBC committee and should contain a suggestion for alternative classification and documentation in support of this suggestion. The IBC committee will then inform the Investigator of its decision.

A.7 NOU form and instructions

The following is an example of an NOU form and instructions for its completion:**Notification of Use for Biological Agents and Recombinant DNA**

A.7.1 General instructions for NOU submission

A Notification of Use must be submitted to the UTRGV IBC committee for review and approval when the project meets the following criteria:

- **Biological Agents:** An NOU must be completed and submitted for all Class 2 and above pathogens and all human products, human tissues, and human cell lines.
- **Recombinant DNA:** An NOU must be completed and submitted for ALL recombinant DNA (rDNA) Class 1 and above. Complete the cover page and Sections I and II. When genes are propagated in living cells, submission of an NOU for a biological agent (Section I) and for the rDNA (Section II) is required. When genes are not propagated for rDNA work, Section I, questions 1 and 2 would be not applicable, but Sections I questions 3 through 15 as well as Section II would need to be addressed.
- **select agents:** select agent use requires that an NOU be completed for the biological agent and rDNA (Sections I and II) as well as meeting the additional requirements outlined in Section III, select agents. The list of select biological agents and rDNA can be found in section 12.3 of this manual.

An NOU must be submitted whenever the biosafety level changes. The following outlines the notification and approvalprocedures for submitting protcols to the IBC.

Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) is a federally mandated university committee responsible for reviewing research activities utilizing recombinant DNA (rDNA) and biohazardous materials to ensure that UTRGV principal investigators and lab personnel are adequately trained and utilize best practices when employing biological agents in research.

All research by UTRGV investigators that involves the following biological agents, regardless of funding source, must be reviewed and approved by the Institutional Biological Safety (IBC) prior to initiation of work:

- **Recombinant DNA** is defined in the NIH Guidelines as; (1) nucleic acid molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, and (2) DNA molecules that result from the replication of these molecules.
- **Hazardous Biological Agents (HBA's)** these include infectious organisms classified as BSL-2 or above agents by the NIH (CDC) such as pathogenic bacteria, protozoans, fungi, viruses, etc., including attenuated lab & vaccine strains. Human and primate biological products (cells, fluids, etc.), applicable primary and immortal cell lines, organotypic and tissue cultures involved in research that require universal precautions are also included.

What is the IBC?

The IBC reports to the National Institutes of Health Office of Biotechnology Activities (NIH-OBA), serves in an advisory and consultative capacity to UTRGV's President and Senior Vice President for Research, Innovation and Economic Development, works with the Environmental, Health, Safety and Risk Management Office in matters pertaining to biological hazards. UTRGV's IBC reviews, approves/disapproves or forwards applications for rDNA work to the NIH, advises on the safe handling, transport, shipment, storage, and disposal of potentially hazardous biological agents; may review plans for areas designated to be constructed or remodeled for biohazardous work, monitors adherence to best practices for research with biohazardous agents and facilities designed for use with such agents.

Who is on the IBC?

The Institutional Biosafety Committee (IBC) is comprised of faculty and staff members from each UTRGV school that conduct research with rDNA and/or infectious agents, a member from the EH&S, a representative from the campus health clinic, a compliance office representative and two community members. Faculty IBC members must have expertise in the use of rDNA and hazardous biological agents while IBC community members represent community interests in protecting the environment and the local population.

UTRGV's IBC Membership:

- **Daniele Provenzano**, Ph.D., Professor Dept. Biology; Bacterial Genetics - Chair
- **Julie Mustard**, Ph.D., Associate Professor, Biology – Voting Member – Vice Chair
- **Megan Keniry**, Ph.D., Associate Professor, Biology – Voting Member
- **Hyeongjun Kim**, Ph.D., Assistant Professor Dept. Physics & Astronomy – Voting Member
- **Dae Kim**, Ph.D., Associate Professor, Molecular Science – Voting Member
- **Lynne Depeault**, Community representative – Voting Member
- **Laura Decanini**, Community representative – Voting Member

- **Cordelia Rasa,** Director of Animal Care Programs and BSL-3 facility Ex Officio, Non-Voting Member
- **Richard Costello**, Dr.PH., EHSRM Director – Ex Officio, Non-Voting Member
- **Glorimar Colon**, Interim Director of Research Compliance – Ex Officio, Non-Voting Member
- **Amy Mutore**, Senior Research Compliance Specialist – Ex Officio, Non-Voting Member

When and where does the IBC meet?

UTRGV's IBC meeting schedule for the 2017-2018 academic year:

- **June 24, 2022**; 1:30-3:30 pm; via Zoom
- **April 8, 2022**; 1:30-3:30 pm; via Zoom
- **February 25, 2022**; 1:30-3:30 pm; via Zoom
- **December 3, 2021**; 1:30-3:30pm; via Zoom
- **October 15, 2021**; 1:30-3:30pm; via Zoom

How do I register my research involving rDNA and Hazardous Biological Agents?

Filled IBC and HBA registration forms should be submitted either through campus mail or by email to Lynne Depeault, Senior Research Compliance Specialist, Research Compliance Office Biomedical Research Building (BRHP) 2.210 or electronically to Amy.Mutore@utrgv.edu. A preliminary review of the application will be conducted and then sent to the committee for review. The IBC typically meets five times a year and as needed. Registration documents should be submitted three weeks prior to the meeting dates.

IBC Forms

Forms and Submission Information

- IBC: Protocol Registration Form - rDNA
- IBC: Protocol Registration Form – HBA
- IBC: Annual Renewal Form - rDNA
- IBC: Annual Renewal Form - HBA
- IBC: New/Temporary Personnel Form - IBC/HBA
- Laboratory Biosafety Checklist

Contacts

Dr. Daniele Provenzano
Chair - Institutional Biological Safety Committee
Professor, Bacterial Genetics
BRHP 1.125
956-882-5045
Daniele.Provenzano@utrgv.edu

Dr. Richard Costello
Director - Environmental, Health Safety and Risk Management
Dept. 956-665-3690
Office 956-665-2903
Richard.Costello@utrgv.edu

Amy Mutore
Senior Research Compliance Specialist
956-665-2889
Amy.Mutore@utrgv.edu

Appendix B: Classification of Organisms on the Basis of Hazard

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic, mutated, and recombined; and non-pathogenic species and strains are considered. Non-infectious life-cycle stages of parasites are excluded.

The appendix reflects the current state of knowledge and should be considered a resource document. The more commonly encountered agents are included, but the list is not meant to be all inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories*. Further guidance on agents listed in this section may be obtained through:

- Centers for Disease Control and Prevention
  https://www.cdc.gov/about/lab-safety/index.html
- National Institutes of Health, Division of Occupational Health and Safety
  https://ors.od.nih.gov/sr/dohs/safety/laboratory/BioSafety/Pages/bio_chem_safety.aspx
- National Animal Disease Center, US Department of Agriculture
  https://www.ars.usda.gov/midwest-area/ames/nadc/

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Table B1: Basis for the classification of biohazardous agents by risk group (RG)

B.1 Risk Group 1 (RG1) agents

RG1 agents are not associated with disease in healthy adult humans. Example of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis, Escherichia Coli*-K12, and adeno-associated virus types 1 through 4.

Those agents not listed in Risk Groups 2,3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

B.2 Risk Group 2 (RG2) agents

RG2 agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.

B.2.1 RG2 bacterial agents

Acinetobacter baumannii (formerly *Acinetobacter calcoaceticus*)
Actinobacillus
Actinomyces pyogenes (formerly *Corynebacterium pyogenes*)
Aeromonas hydrophila
Amycolata autotrophica
Arizona hinshawii-all serotypes
Bacillus anthracis
Bartonella henselae, B. quintana, B. vinsonii
Bordetella including B. pertussis
Borrelia recurrentis, B. burgdorferi
Burkholderia (formerly *Pseudomonas* species) except those listed in section B.3.1 (RG3)
Campylobacter coli, C. fetus, C. jejuni
Chlamydia psittaci, C. trachomatis, C. pneumoniae
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix rhusiopathiae
Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive, and strains bearing K1 antigen, including *E. coli* O157-H7
Haemophilus ducreyi, H. influenzae
Helicobacter pylori
Klebsiella - all species except *K. oxytoca* (RG1)
Legionella including *L. pneumophila*
Leptospira interrogans - all serotypes
Listeria
Moraxella
Mycobacterium (except those listed in section B.3.1 (RG3) including *M. avium complex, M.asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae,M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai,M. ulcerans, M. xenopi Mycoplasma,* except *M. mycoides* and *M. agalactiae*, which are restricted animal pathogens.
Neisseria gonorrhoea, N. meningitidis
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
Rhodococcus equi

Salmonella including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C
S. typhi, S. typhimurium
Shigella including S. boydii, S. dysenteriae type 1, *S. flexneri, S. sonnei*
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus including *S. pneumoniae, S. pyogenes*
Treponema carateum, T. pallidum, and *T. carateum*
Vibrio cholerae, V. parahemolyticus, V. vulnificus
Yersinia enterocolitica

B.2.2 RG2 fungal agents

Blastomyces dermatitidis
Cladosporium bantianum, C. (Xylohypha) trichoides
Cryptococcus neoformans
Dactylaria galopava (Ochroconis gallopavum)
Epidermophyton
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Microsporum
Paracoccidioides brasiliensis
Penicillium marneffei
Sporothrix schenckii
Trichophyton

B.2.3 RG2 parasitic agents

Ancylostoma human hookworms including *A. duodenale. A. ceylanicum*
Ascaris including *Ascaris lumbricoides suum*
Babesia including *B. divergens, B. microti*
Brugia filaria worms including *B. malayi, B. timori*
Coccidia
Cryptosporidium including *C. parvum*
Cysticercus cellulosae (hydatid cyst, larva of *T. solium*)
Echinococcus including *E. granulosis, E. multilocularis, E. vogeli*
Entamoeba histolytica
Enterobius
Fasciola including *F. gigantica, F. hepatica*
Giardia including *G. lamblia*
Heterophyes
Hymenolepis including *H. diminuta, H. nana*
Isospora
Leishmania including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
Loa loa filaria worms
Microsporidium

Naegleria fowleri
Necator human hookworms including *N. americanus*
Onchoerca filaria worms including *O. volvulus*
Plasmodium including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
Sarcocystis including *S. sui hominis*
Schistosoma including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
Strongyloides including *S. stercoralis*
Taenia solium
Toxocara including *T. canis*
Toxoplasma including *T. gondii*
Trichinella spiralis
Trypanosoma including *T. Brucei brucei, T. brucei gambiense, T. Brucei rhodesiense, T. cruzi*
Wuchereria bancrofti filaria worms

B.2.4 RG2 viruses

Adenoviruses - human, all types
Alphaviruses (Togaviruses) – Group A Arboviruses

- Eastern equine encephalomyelitis virus
- Venezuelan equine encephalomyelitis virus
- Western equine encephalomyelitis virus

Arenaviruses

- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source

Bunyaviruses

- Bunyamwera virus
- Rift Valley fever virus vaccine strain MP-12
- Other viruses as listed in the reference source

Calciviruses
Coronaviruses
Flaviviruses (Togaviruses) – Group B Arboviruses

- Dengue virus serotypes 1,2,3, and 4
- Yellow fever virus vaccine strain 17D
- Other viruses as listed in the reference source

Hepatitis A, B, C, D, and E viruses
Herpesviruses – except Herpesvirus simiae (Monkey B Virus)
(see section B.4.4, RG4 viral agents)

- Cytomegalovirus
- Epstein Barr virus

- *Herpes simplex* types 1 and 2

Herpes zoster

- Human herpesvirus types 6 and 7

Orthomyxoviruses

- Influenza viruses types A, B, and C
- Other tick-borne orthomyxoviruses as listed in the reference source

Papovaviruses

- All human papilloma viruses

Paramyxoviruses

- Newcastle disease virus
- Measles virus
- Mumps virus
- Parainfluenza viruses types 1, 2, 3, and 4
- Respiratory syncytial virus

Parvoviruses

- Human parvovirus (B19)

Pecornaviruses

- Coxsackie viruses types A and B
- Echoviruses – all types
- Polioviruses – all types, wild and attenuated
- Rhinoviruses – all types

Poxviruses – all types except monkeypox virus (see section B.3.4, RG3 viruses and prions) and restricted poxviruses including alastrim, smallpox, and whitepox
Reoviruses – all types including coltivirus, human rotavirus, and Orbivirus (Colorado tick fever virus)
Rhabdoviruses

- Rabies virus – all strains
- Vesicular stomatitis virus – laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)

- Rubivirus (rubella)

B.3 Risk Group 3 (RG3) agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

B.3.1 RG3 bacterial agents

Bartonella

Brucella including *B. abortus, B. canis, B. suis*
Buckholderia (Pseudomonas) mallei, B. pseudomallei
Coxiella brunetii
Francisella tularensis
Mycobacterium bovis (except BDG strain, see section B.2.1, RG2 bacterial agents), *M. tuberculosis*
Pasteurella multocida type B – "buffalo" and other virulent strains
Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R. siberica. R.
tsutsugamushi, R. typhi (R. mooseri)
Yersinia pestis

B.3.2 RG3 fungal agents

Coccidioides immitis (sporulating cultures; contaminated soil)
Histoplasma capsulatum, H. capsulatum var. *duboisii*

B.3.3 RG3 parasitic agents

None

B.3.4 RG3 viruses and prions

Alphaviruses (Togaviruses) – Group A Arboviruses

- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine train TC-83, see section B.2.4)
- Other viruses as listed in the reference source

Arenaviruses

- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Flaviviruses (Togaviruses) – Group B Arboviruses

- Japanese encephalitis virus
- Yellow fever virus
- Other viruses as listed in the reference source

Poxviruses
Monkeypox virus
Prions

- Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents)

Retroviruses

- Human immunodeficiency virus (HIV) types 1 and 2
- Human T-cell lymphotropic virus (HTLV) types 1 and 2
- Simian immunodeficiency virus (SIV)

Rhabdoviruses

- Vesicular stomatitis virus

B.4 Risk Group 4 (RG4) agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

B.4.1 RG4 bacterial agents

None.

B.4.2 RG4 fungal agents

None.

B.4.3 RG4 parasitic agents

None.

B.4.4 RG4 viral agents

Arenaviruses (Togaviruses) – Group A Arboviruses

- Guanarito virus
- Lassa virus
- Junin virus
- Machupo virus

Bunyaviruses (Nairovirus)

- Crimean-Congo hemorrhagic fever virus

Filoviruses

- Ebola virus
- Marburg virus

Flaviruses (Togaviruses) – Group B Arboviruses

- Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis,
- Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, Russian spring-summer encephalitis viruses

Herpesviruses (alpha)

- Herpesvirus simiae (Herpes B or Monkey B virus)

Hemorrhagic fever agents and viruses as yet undefined

B.5 Animal viral etiologic agents in common use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cell, e.g. amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses

- Herpesvirus ateles
- Herpesvirus saimiri
- Marek's disease virus
- Murine cytomegalovirus

Papovaviruses

- Bovine papilloma virus
- Polyoma virus
- Shope papilloma virus
- Simian virus 40 (SV40)

Retroviruses

- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

B.5.1 Murine retroviral vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retroviruses can be maintained, handled, and administered, under BL1 containment.

B.5.2 Restricted animal pathogens

Nonindigenous pathogens of domestic livestock and poultry may require special laboratory design, operation, and containment features not generally addressed in this document. The importation, possession, or use of the following agents is prohibited or

restricted by law or by US Department of Agriculture regulations or administrative policies:
African horse sickness
African swine fever virus
Akabane virus
Besnoitia besnoiti
Borna disease virus
Bovine spongiform encephalopathy
Bovine infectious petechial fever agent
Brucellosis melitensis
Camelpox virus
Cochliomyla hominivorax (screw worm)
Ephermerl fever virus
Foot and mouth disease virus
Fowl plague virus (lethal avian influenza)
Hog cholera virus
Histoplasma (Zymonema) farciminosum
Louping ill virus
Lumpy skin disease virus
Mycoplasma agalactiae (contagious agalactia of sheep)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Nairobi sheep disease virus (Ganjam virus)
Newcastle disease virus (velogenic strains)
Peste des petits ruminants (pest of small ruminants)
Pseudomonas ruminantium (heartwater)
Rift Valley fever virus
Rhinderpest virus
Sheep and goat pox
Swine vesicular disease virus
Teschen disease virus
Theileria annulata
Theileria bovis
Theileria hirei
Theileria lawrencei
Trypanosoma evansi
Trypanosoma vivax (Nagana)
Vesicular exanthema virus
Viral hemorrhagic disease of rabbits
Wesselsbron disease virus

The importation, possession, use or interstate shipment of animal pathogens other than those listed above may also be subject to regulations of the US Department of Agriculture.

B.5.3 Low-risk oncogenic viruses

AD7-SV40
Adenovirus
Avian leukosis
Bovine leukemia
Bovine papilloma
CELO
Dog sarcoma
Guinea pig herpes
Hamster leukemia
HTLV I/II
Lucke (Frog)
Marek's
Mason-Pfizer monkey virus
Mouse mammary tumor
Murine leukemia
Murine sarcoma
Polyoma
Rat leukemia
Rat mammary tumor
Rous sarcoma
Shope fibroma
Shope papilloma
SV-40

B.5.4 Moderate-risk oncogenic viruses

Ad2-SV40
EBV
FeLV
FeSV
GaLV
HV Ateles
HV Saimiri
SSV-1, Yaba

B.6 Notes

The original reference for this classification was the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th edition, July 1974, US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purpose of these guidelines, this list has been revised by the NIH.

A USDA permit, required for import and interstate transport of pathogens, may be obtained from:

US Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
National Import Export Service (NEIS)

4700 River Road, Unit 2
Riverdale, MD 20737

Telephone: 301-851-3300
APIE@usda.gov

All activities, including storage of variola and whitepox, are restricted to the single national facility, World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta.

Appendix C: Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table C1 at the end of this appendix and Table D1 at the end of the next. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

C.1 Biosafety Level 1 (BSL1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 1.

C.1.1 Standard microbiological practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label. Materials to be decontaminated outside of the immediate laboratory are packaged

in accordance with applicable local, state, and federal regulations before removal from the facility.

- A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.
- An insect and rodent control program is in effect (Esparza Pest Control).

C.1.2 Special practices

None.

C.1.3 Safety equipment (primary barriers)

- Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
- It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
- Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.
- Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials are anticipated.

C.1.4 Laboratory facilities (secondary barriers)

- Laboratories have doors for access control.
- Each laboratory contains a sink for handwashing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- If the laboratory has windows that open to the exterior, they are fitted with fly screens. UTRGV has no laboratory windows that open to the outside.

C.2 Biosafety Level 2 (BSL2)

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2.

C.2.1 Standard microbiological practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
- An insect and rodent control program is in effect. (Esparza Pest Control)

C.2.2 Special practices

- Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
- The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g. immunization) may enter the laboratory.
- A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
- Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g. hepatitis B vaccine or TB skin testing).

- When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
- Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
    - Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
    - Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Nondisposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    - Syringes which re-sheathe the needle, needle-less systems, and other safety devices are used when appropriate.
    - Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.
- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance

or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

- Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- Animals not involved in the work being performed are not permitted in the lab.
- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used at the following times:
    - Whenever procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
    - When high concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
- Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g. cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
- Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

C.2.3 Laboratory facilities (secondary barriers)

- Lockable doors are provided for facilities that house restricted agents (as defined in 42 CFR 72.6).
- Where possible, new laboratories are located away from public areas.
- Each laboratory contains a sink for handwashing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- Biological safety cabinets are installed in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
- An eyewash station is readily available.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

C.3 Biosafety Level 3 (BSL3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (e.g. double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g. diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing), may be achieved in a Biosafety Level 2 facility, providing 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended standard microbiological practices, special practices, and safety equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment, and facilities apply to agents assigned to Biosafety Level 3:

C.3.1 Standard microbiological practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
- Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Infectious waste from BSL3 laboratories should be decontaminated before removal for off-site disposal.
- An insect and rodent control program is in effect.

C.3.2 Special practices

- Laboratory doors are kept closed when experiments are in progress.
- The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.
- The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g. immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
- When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special

requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

- Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g. hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.
- Baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.
- A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.
- The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
    - Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
    - Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Nondisposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    - Syringes which re-sheathe the needle, needle-less systems, and other safe devices are used when appropriate.
    - Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.

- All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.
- Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
    - Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.
    - Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.
- Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- All potentially contaminated waste materials (gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
- Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
- Animals and plants not related to the work being conducted are not permitted in the laboratory.

C.3.3 Safety equipment (primary barriers)

- Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.
- Gloves must be worn when handling infectious materials, infected animals, and contaminated equipment.
- Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
- All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs , etc., are conducted in a Class II or Class III biological safety cabinet.
- When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g. respirators, face shields) and physical containment devices (e.g. centrifuge safety cups or sealed rotors) are used.

- Respiratory and face protection are used when in rooms containing infected animals.

C.3.4 Laboratory facilities (secondary barriers)

- The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable. A clothes change/anteroom may be included in the passageway.
- Each laboratory room contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the room exit door.
- The interior surfaces of walls, floors, and ceilings of areas where BSL3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of covered floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- All windows in the laboratory are closed and sealed.
- A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e. autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
- Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.
- A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from “clean” areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to

prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

- HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g. an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.
- Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
- Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).
- An eyewash station is readily available inside the laboratory.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.
- Additional environmental protection (e.g. personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

C.4 Biosafety Level 4 (BSL4)

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents. Access to the laboratory is strictly controlled by the laboratory director. The facility is either in a separate building or in a controlled area within a

building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted.

Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices, equipment, and facilities apply to agents assigned to Biosafety Level 4:

C.4.1 Standard microbiological practices

- Access to the laboratory is limited by the laboratory director when experiments are in progress.
- Policies for safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All waste is decontaminated before disposal by an approved method such as autoclaving.
- An insect and rodent control program is in effect.

C.4.2 Special practices

- Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women, are not allowed in the laboratory or animal rooms. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazard control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook, signed by all personnel, indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.
- When infectious materials or infected animals are present in the laboratory or animal rooms, hazard warning signs, incorporating the universal biohazard symbol, are posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any

special requirements for entering the area (e.g. the need for immunizations or respirators).

- The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 4, all personnel demonstrate a high proficiency in standard microbiological practices and techniques, and in the special practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in these unique safe microbiological practices and techniques.
- Laboratory personnel receive available immunizations for the agents handed or potentially present in the laboratory.
- Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibodies to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.
- A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.
- Personnel enter and leave the laboratory only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the laboratory. Personnel use the airlocks to enter or leave the laboratory only in an emergency.
- Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the laboratory.
- When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.
- Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use.
- After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

- Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Nondisposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Syringes that re-sheathe the needle, needle-less systems, and other safety devices are used when appropriate.
- Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass must be decontaminated before disposal, according to any local, state, or federal regulations.

- Biological materials to be removed from the Class III cabinet or from the Biosafety Level 4 laboratory in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.
- No materials, except biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the laboratory. Equipment or material that might be damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.
- Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Equipment is decontaminated before it is sent for repair or maintenance.
- Spills of infectious materials are contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with concentrated infectious material. A spill procedure is developed and posted within the laboratory.
- A system is established for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting surveillance system is the availability of a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

- Materials not related to the experiment being conducted (e.g. plants, animals, and clothing) are not permitted in the facility.

C.4.3 Safety equipment (primary barriers)

All procedures within the facility are conducted in the Class III biological safety cabinet or in Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.

C.4.4 Laboratory facility (secondary barriers)

There are two models for Biosafety Level 4 laboratories: (A) the cabinet laboratory where all handling of the agent is performed in a Class III biological safety cabinet, and (B) the suit laboratory where personnel wear a protective suit. Biosafety Level 4 laboratories may be based on either model or a combination of both models in the same facility. If a combination is used, each type must meet all the requirements identified for that type.

(A) Cabinet laboratory

- The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through a minimum of two doors prior to entering the room(s) containing the Class III biological safety cabinet (cabinet room). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the cabinet room. A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom for decontamination is provided at the containment barrier for passage of those materials, supplies, or equipment that are not brought into the cabinet room through the change room.
- Daily inspections of all containment parameters (e.g. directional airflow) and life support systems are completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.
- Walls, floors, and ceilings of the cabinet room and inner change room are constructed to form a sealed internal shell which facilitates fumigation and is resistant to entry and exit of animals and insects. Floors are integrally sealed and coved. The internal surfaces of this shell are resistant to liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Openings around doors into the cabinet room and inner change room are minimized and are capable of being sealed to facilitate decontamination. Any drains in the cabinet room floor are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters and protection against vermin.
- Bench tops have seamless or sealed surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is of simple open construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used

in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

- A hands-free or automatically operated handwashing sink is provided near the door of the cabinet room(s) and the outer and inner change rooms.
- If there is a central vacuum system, it does not serve areas outside the cabinet room. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the cabinet room are protected by devices that prevent backflow.
- If water fountains are provided, they are operated automatically or by foot and are located in the facility corridors outside the laboratory. The water service to the fountain is isolated from the distribution system supplying water to the laboratory areas and is equipped with a backflow preventer.
- Access doors to the laboratory are self-closing and lockable.
- Any windows are breakage-resistant and sealed.
- Double-door autoclaves are provided for decontaminating materials passing out of both the Class III biological safety cabinet(s) and the cabinet room(s). Autoclaves that open outside of the containment barrier must be sealed to the wall of the containment barrier. The autoclave doors are automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.
- Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s).
- Liquid effluents from the dirty-side inner change room (including toilets) and cabinet room sinks, floor drains (if used), autoclave chambers, and other sources within the cabinet room are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and clean-side toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.
- A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate any system malfunction. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the cabinet room is provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and the HVAC control system is designed to prevent sustained positive pressurization of the laboratory. The Class III cabinet should be directly connected to the exhaust system. If the Class III cabinet is connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinet.
- The supply air to and exhaust air from the cabinet room, inner change room, and anteroom pass through HEPA filter(s). The air is discharged away from occupied

spaces and air intakes. The HEPA filter(s) are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for *in-situ* decontamination of the filter prior to removal, or removal of the filter in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.

- The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.
- Appropriate communication systems are provided between the laboratory and the outside (e.g. voice, fax, computer).

(B) Suit laboratory

- The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through the changing and decontamination areas prior to entering the room(s) where work is done with BSL4 agents (suit area). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the suit area. A specially designed suit area is maintained in the facility to provide personnel protection equivalent to that provided by Class III biological safety cabinets. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system protected by HEPA filtration. The life support system includes redundant breathing-air compressors, alarms, and emergency backup breathing-air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. An automatically starting emergency power source is provided at a minimum for the exhaust system, life support systems, alarms, lighting, entry and exit controls, and BSCs. The air pressure within the suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area, chemical shower, and airlocks, are sealed.
- A daily inspection of all containment parameters (e.g. directional airflow, chemical showers) and life support systems is completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.
- A double-doored autoclave is provided at the containment barrier for decontaminating waste materials to be removed from the suit area. The autoclave door, which opens to the area external to the suit area, is sealed to the outer wall of the suit area and is automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle. A dunk tank, fumigation chamber, or ventilated airlock for decontamination is provided for passage of

materials, supplies, or equipment that are not brought into the suit area through the change room. These devices can also be used for the safe removal of materials, supplies, or equipment from the laboratory that cannot be decontaminated in the autoclave.

- Walls, floors, and ceilings of the suit area are constructed to form a sealed internal shell, which facilitates fumigation and is animal and insect prohibitive. The internal surfaces of this shell are resistant to liquids and chemicals, facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floor of the suit area contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters.
- Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area.
- Bench tops have seamless surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is of simple open construction capable of supporting anticipated loading and uses. Non-porous materials are preferable. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- A hands-free or automatically operated handwashing sink is provided in the suit area(s); handwashing sinks in the outer and inner change rooms should be considered based on the risk assessment.
- If there is a central vacuum system, it does not serve areas outside the suit area. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the suit area are protected by devices that prevent backflow.
- Access doors to the laboratory are self-closing and lockable. Inner and outer doors to the chemical shower and inner and outer doors to airlocks are interlocked to prevent both doors from being opened simultaneously.
- Any windows are breakage-resistant and are sealed.
- Liquid effluents from sinks, floor drains (if used), autoclave chambers, and other sources within the containment barrier are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.
- A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. Redundant supply fans are recommended. Redundant exhaust fans are required. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. An appropriate visual pressure

monitoring device that indicates and confirms the pressure differential of the suit area must be provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and an HVAC control system is installed to prevent positive pressurization of the laboratory.

- The supply air to the suit area, decontamination shower, and decontamination airlock is protected by passage through a HEPA filter. The general room exhaust air from the suit area, decontamination shower and decontamination airlock is treated by a passage through two HEPA filters in series prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA filters are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for *in-situ* decontamination of the filter prior to removal. Alternatively, the filter can be removed in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.
- The positioning of the supply and exhaust points should be such that dead air space in the suit room is minimized.
- The treated exhaust air from Class II biological safety cabinets, located in a facility where workers wear a positive pressure suit, may be discharged into the room environment or to the outside through the facility air exhaust system. If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system.
- The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.
- Appropriate communication systems should be provided between the laboratory and the outside.

BSL	**Agents**	**Practices**	**Safety equipment (primary barriers)**	**Facilities (secondary barriers)**
1	Not known to consistently cause disease in healthy adults	Standard microbiological practices	None required	Open bench-top sink required
2	Associated with human disease. Hazard: percutaneous injury, ingestion, mucous membrane exposure	BLS1 practice plus: - limited access - biohazard warning signs - "sharps" precautions - biosafety manual defining any needed	Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulation of agents that cause splashes or aerosols of infectious materials PPEs: lab coats,	BSL1 plus: - autoclave available

		waste decontamination or medical surveillance policies	gloves; face protection as needed	
3	Indigenous or exotic agents with potential for aerosol transmission: disease may have serious or lethal consequences	BSL2 practice plus: - controlled access - decontamination of all waste - decontamination of lab clothing before laundering - baseline serum	Primary barriers: Class I or II BSCs or other physical containment devices used for all open manipulation of agents Protective lab clothing, gloves; respiratory protection as needed	BSL2 plus: - physical separation from access corridors - self-closing double-door access - exhausted air not recirculated - negative airflow into laboratory
4	Dangerous or exotic agents which pose high risk of life-threatening disease; aerosol-transmitted lab infections, or related agents with unknown risk of transmission	BSL3 practice plus: - clothing change before entering - shower on exit - all material decontaminated on exit from facility	Primary barriers: all procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body air-supplied positive-pressure personnel suit	BSL3 plus: - separate building or isolated zone - dedicated supply and exhaust, vacuum, and decon systems - other requirements outlined in the text

Table C1. Summary of recommended biosafety levels for infectious agents

Appendix D: Vertebrate Animal Biosafety Level Criteria

If experimental animals are used, institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, and care. Laboratory animal facilities are simply a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

However, it is best to remember that the animal room can present some unique problems. In the microbiological laboratory, hazardous conditions are caused by personnel or by the equipment being used. In the animal room, the activities of the animals themselves can present new hazards. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic disease.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g. *Guide for the Care and Use of Laboratory Animals* and *Laboratory Animal Welfare Regulations*) and that appropriate species have been selected for animal experiments. In addition, the organization should have an occupational health and safety plan. The recent publication of the Institute of Medicine, *Occupational Health and Safety in the Care of Research Animals* is most helpful in this regard.

Ideally, facilities for laboratory animals used in studies of infectious or noninfectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross-contamination should be considered in the plans. A “clean/dirty hall” layout may be useful to minimize this risk.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals infected with agents that cause, or may cause, human infection. These four combinations, designated Animal Biosafety Levels (ABSL) 1 to 4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to Biosafety Levels 1 to 4, respectively.

Investigators inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in the standards for commonly used laboratory animals. *Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates*, prepared by the Subcommittee on Arbovirus Laboratory Safety (SALS) of the American Committee on Arthropod-Borne

Viruses, serves as a useful reference in the design and operation of facilities using arthropods.

D.1 Animal Biosafety Level 1 (ABSL1)

Animal Biosafety Level 1 (ABSL1) is suitable for work involving well-characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

D.1.1 Standard practices

- The animal facility director establishes policies, procedures, and protocols for emergency situations. Each project is subject to pre-approval by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC). Any special practices are approved at this time.
- Only those persons required for program or support purposes are authorized to enter the facility. Before entering, persons are advised of the potential biohazards and are instructed on the appropriate safeguards.
- An appropriate medical surveillance program is in place.
- A safety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done in only designated areas and are not permitted in animal or procedure rooms.
- All procedures are carefully performed to minimize the creation of aerosols or splatters.
- Work surfaces are decontaminated after use or after any spill of viable materials.
- All wastes from the animal room (including animal tissues, carcasses, and contaminated bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended.
- Policies for the safe handling of sharps are instituted.
- Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g. the need for immunizations and respirators).
- An insect and rodent control program is in effect.

D.1.2 Special practices

None.

D.1.3 Safety equipment (primary barriers)

- The wearing of laboratory coats, gowns, and/or uniforms in the facility is recommended. Laboratory coats remain in the animal room. Gowns and uniforms are not worn outside the facility.
- Persons having contact with non-human primates should assess their risk of mucous membrane exposure and wear appropriate eye and face protection.

D.1.4 Facilities (secondary barriers)

- The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
- External facility doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
- Windows are not recommended. Any windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens.
- If floor drains are provided, the traps are always filled with water and/or an appropriate disinfectant.
- Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*, latest edition. No recirculation of exhaust air should occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.
- The facility has a handwashing sink.
- Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

D.2 Animal Biosafety Level 2 (ABSL2)

Animal Biosafety Level 2 involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL1.

D.2.1 Standard practices

- Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional

Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC).

- Access to the animal room is limited to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g. hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented.
- A biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.
- All procedures are carefully performed to minimize the creation of aerosols or splatters.
- Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
- All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. The outer surface of the containers is disinfected prior to moving the material. Autoclaving of the contents prior to incineration is recommended.
- Policies for the safe handling of sharps are instituted.
- Needles and syringes or other sharp instruments are restricted for use in the animal facility only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
- Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
- Plasticware should be substituted for glassware whenever possible.
- Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements (e.g. the need for immunizations and respirators) for entering the animal room.
- An insect and rodent control program is in effect.

D.2.2 Special practices

- Animal care laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. Records of all training provided are maintained. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal facility unless special procedures can eliminate the extra risk.
- Only animals used for the experiment(s) are allowed in the room.
- All equipment must be appropriately decontaminated prior to removal from the room.
- Spills and accidents which result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

D.2.3 Safety equipment (primary barriers)

- Gowns, uniforms, or laboratory coats are worn while in the animal room. The laboratory coat is removed and left in the animal room. Gowns, uniforms, and laboratory coats are removed before leaving the animal facility. Gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.
- Personal protective equipment is used based on risk assessment determinations. Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms that house nonhuman primates.
- Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g. respirators, face shields) are used whenever conducting procedures with a high potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals.
- When needed, animals are housed in primary biosafety containment equipment appropriate for the animal species. Filter-top cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.

D.2.4 Facilities (secondary barriers)

- The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
- Access to the facility is limited by secure locked doors. External doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.

- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
- Any windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens.
- If floor drains are provided, the traps are always filled with an appropriate disinfectant.
- Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals*, latest edition. The direction of airflow in the animal facility is inward; animal rooms should maintain negative pressure compared to adjoining hallways.
- Cages are washed manually or in an appropriate cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
- An autoclave is available in the animal facility to decontaminate infectious waste.
- A hand washing sink is in the animal room where infected animals are housed, as well as elsewhere in the facility.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

D.3 Animal Biosafety Level 3 (ABSL3)

Animal Biosafety Level 3 involves practices suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease. ABSL3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL2.

D.3.1 Standard practices

- Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
- The laboratory or animal facility director limits access to the animal room to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g. hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented. In general, persons who may be at increased risk of acquiring infection, or for whom infection might have serious consequences, are not allowed in the animal facility unless special procedures can eliminate the extra risk. Assessment should be made by the occupational health physician.

- A biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done only in designated areas and are not permitted in animal or procedure rooms.
- All procedures are carefully performed to minimize the creation of aerosols or splatters.
- Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
- All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse animal tissues) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended. The outer surface of the containers is disinfected prior to moving the material (see item 3 of section D.3.2 below).
- Policies for the safe handling of sharps are instituted:
    - Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
    - Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
    - Plasticware should be substituted for glassware whenever possible.
- Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g. the need for immunizations and respirators).
- All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s).
- Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. As necessary, personnel receive updates and/or additional training on procedural or policy changes. Records of all training provided are maintained.
- An insect and rodent control program is in effect.

D.3.2 Special practices

- Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. Equipment must be decontaminated

according to any local, state, or federal regulations before being packaged for transport or removal from the facility for repair or maintenance.

- A spill procedure is developed and posted. Only personnel properly trained and equipped to work with infectious materials are to clean up spills. Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- All wastes from the animal room must be autoclaved prior to incineration or other appropriate terminal treatment.
- Materials not related to the experiment (e.g. plants, animals) are not permitted in the animal room.

D.3.4 Safety equipment (primary barriers)

- Uniforms or scrub suits are worn by personnel entering the animal room. Wrap-around or solid-front gowns should be worn over this clothing. Front-button laboratory coats are unsuitable. The gown must be removed and left in the animal room. Before leaving the animal facility, scrub suits and uniforms are removed and appropriately contained and decontaminated prior to laundering or disposal.
- Personal protective equipment used is based on risk assessment determinations.
    - Personal protective equipment is used for all activities involving manipulations of infectious material or infected animals.
    - Personnel wear gloves when handling infected animals.
    - Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.
    - Appropriate face/eye and respiratory protection (e.g. respirators and face shields) are worn by all personnel entering animal rooms.
    - Boots, shoe covers, or other protective footwear, and disinfectant foot baths are available and used where indicated.
- The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems, such as open cages placed in inward flow ventilated enclosures (e.g. laminar flow cabinets), solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems.
- Biological safety cabinets and other physical containment devices are used whenever conducting procedures with a potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals. At BSL3, all work should be done in a primary barrier; otherwise respirators should be worn by personnel in the room.

D.3.5 Facilities (secondary barriers)

- The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.

- Access to the facility is limited by a self-closing and self-locking door. This exterior entry door may be controlled by a key lock, card key, or proximity reader. Entry into the animal room is via a double-door entry which includes a change room and shower(s). An additional double-door access (air-lock) or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility, respectively. Doors to animal rooms open inward and are self-closing. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
- The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant. Penetrations in floors, walls, and ceiling surfaces are sealed and openings around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
- A hands-free or automatically operated handwashing sink is provided in each animal room near the exit door. The sink trap is filled with an appropriate disinfectant after each use.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
- Windows are not recommended. Any windows must be resistant to breakage and must be sealed.
- If floor drains are provided, they are always filled with an appropriate disinfectant.
- Ventilation should be provided in accordance with criteria from the *Guide for Care and Use of Laboratory Animals*, latest edition. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, and specific agent manipulations and use conditions.
- The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the animal room entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the animal spaces. Audible alarms should be considered to notify personnel of HVAC system failure.
- HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the animal room if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g. an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used, they should be directly connected to the exhaust system. If the Class III cabinets are connected to

the supply system, it is done in a manner that prevents positive pressurization of the cabinets.

- Cages are washed in a cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F.
- An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious waste before moving it to other areas of the facility.
- If vacuum service (central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- The completed Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
- Additional environmental protection (e.g. personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state, or local regulations.

D.4 Animal Biosafety Level 4 (ABSL4)

Animal Biosafety Level 4 involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening disease, aerosol transmission, or related agents with unknown risk of transmission. ABSL4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL3. Procedures must be developed locally to address specific operations of the Class III cabinet line or the suit laboratory.

D.4.1 Standard practices

- Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
- The laboratory or animal facility director limits access to the animal room to the fewest individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- A medical surveillance program must be instituted for all persons entering an ABSL4 facility. This program must include appropriate immunizations, serum collection, and availability of post-exposure counseling and potential prophylaxis. In general, persons who may be at increased risk of acquiring infection, or for

whom infection might have serious consequences, are not allowed in the animal facility unless special procedures can eliminate the extra risk. Assessment should be made by the occupational health physician.

- A site-specific biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done only in designated areas and are not permitted in animal or procedure rooms.
- All procedures are carefully performed to minimize the creation of aerosols or splatters.
- Equipment and work surfaces in the room are routinely decontaminated with an appropriate disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
- A spill procedure is developed and posted. Only personnel properly trained and equipped to work with infectious materials are to clean up spills. Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- All wastes (including animal tissues, carcasses, and contaminated bedding), other materials for disposal, and clothing to be laundered, are sterilized in a double-door autoclave located in the secondary barrier wall of the facility. Disposable wastes are incinerated.
- Policies for the safe handling of sharps are instituted.
    - Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
    - Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
    - Plasticware should be substituted for glassware whenever possible.
- A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g. the need for immunizations and respirators).
- Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. Records are maintained on all training provided.
- Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with infectious materials, and especially after spills, splashes, or other contamination

by infectious materials. Equipment must be decontaminated according to any local, state, or federal regulations before removal from the facility for repair or maintenance.

- Personnel assigned to work with infected animals should work in pairs. Based on the risk assessment, use of squeeze cages, working only with anesthetized animals, or other appropriate procedures to reduce possible worker exposure must be instituted.
- Materials not related to the experiment (e.g., plants, animals) are not permitted in the facility.

D.4.2 Special practices

- Additional measures are effected to control access (e.g., 24-hour guard and check in/out system). Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel should not enter or leave the facility through the air locks, except in an emergency.
- In a Class III cabinet operation, personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by personnel entering the facility. When exiting, personnel remove laboratory clothing in the inner change room before entering the shower area. Soiled clothing is sterilized in an autoclave.
- In an ABSL4 suit operation, a complete clothing change is required. A personal shower is required following removal of the decontaminated suit. Soiled lab clothing is autoclaved before laundering.
- Supplies and materials are introduced into the facility via a double-door autoclave or fumigation chamber. After the outer door is secure, personnel inside the facility open the inner door to retrieve the materials. The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a sterilization cycle or the fumigation chamber has been decontaminated.
- A system is established for the reporting of accidents, incidents, exposures, and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such a reporting/surveillance system is the availability of a facility for the quarantine, isolation, and medical care of persons with potential or known laboratory associated illnesses.
- The serum samples collected are analyzed at intervals. The results are communicated to the participants.

D.4.3 Safety equipment (primary barriers)

- Laboratory animals infected with Biosafety Level 4 agents must be housed within a Class III biological safety cabinet in a BSL4 cabinet laboratory. In a BSL4 suit laboratory, all personnel are required to wear one-piece positive pressure suits ventilated with a life support system. Infected animals should be housed in a partial containment system (such as open cages placed in ventilated enclosures,

solid wall and bottom cages covered with filter bonnets and opened in laminar flow hoods, or other equivalent primary containment systems).

- The use of disposable material that does not require cleaning, including animal caging, should be considered. Disposable materials must be autoclaved on exit from the facility and then incinerated.

D.4.4 Facilities (secondary barriers)

BSL4 animal areas may be included as an integral part of BSL4 cabinet laboratories or suit laboratories as described in section C.4.4 (B). The facility requirements described in the BSL4 laboratory section should be utilized in conjunction with the caging described in the equipment section above.

BSL	**Agents**	**Practices**	**Safety equipment (primary barriers)**	**Facilities (secondary barriers)**
1	Not known to consistently cause disease in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility No recirculation of exhaust air Directional air flow recommended Handwashing sink recommended
2	Associated with human disease. Hazard: percutaneous injury, ingestion, mucous membrane exposure	ABLS1 practice plus: - limited access - biohazard warning signs - sharps precautions - biosafety manual - decontamination of all infectious wastes and of animal cages prior to washing	ABSL1 equipment plus: - primary barriers containment equipment appropriate for animal species - PPEs: lab coats, gloves; face and respiratory protection as needed	ABSL1 plus: - autoclave available - handwashing sink available in the animal room - mechanical cage washer used
3	Indigenous or exotic agents with potential for aerosol transmission: disease may have serious health effects	ABSL2 practice plus: - controlled access - decontamination of clothing before laundering - cages decontaminated before bedding removed - disinfectant foot bath as needed	ABSL2 equipment plus: - containment equipment for housing animals and cage dumping activities - Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols - PPEs: appropriate respiratory protection	BSL2 facility plus: - physical separation from access corridors - self-closing double-door access - sealed penetrations - sealed windows - autoclave available in facility

4	Dangerous or exotic agents which pose high risk of life-threatening disease; aerosol-transmitted lab infections, or related agents with unknown risk of transmission	ABSL3 practice plus: - entrance through change room where personal clothing is removed and lab clothing put on - shower on exit - all wastes are decontaminated before removal from facility	ABSL3 equipment plus: - maximum containment equipment (i.e. Class III BSCs or partial containment in combination with full-body air-supplied positive-pressure personnel suit) used for all procedures and activities	ABSL3 facility plus: - separate building or isolated zone - dedicated supply and exhaust, vacuum, and decontamination systems - other requirements outlined in the text

Table D1: Summary of recommended biosafety levels for activities in which experimentally or naturally infected vertebrate animals are used

Appendix E: Relevant publications

Benenson, Abram S, ed. *Control of Communicable Diseases Manual*, 17th ed. Washington DC: American Public Health Association, 2000.

Bodily, HL, "Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections" *General Administration of the Laboratory* Eds. HL Bodily, EL Updyke, JO Mason. New York: American Public Health Association, 1970. 11-28.

Centers for Disease Control and Prevention 1998. *Fatal Cercopithecine herpesvirus 1 (B Virus) Infection Following a Mucocutaneous Exposure and Interim Recommendations for Worker Protection*. MMWR 47(49); 1073-6,1083.

Chatigny, MA, "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures" *Advances in Applied Microbiology* Ed. WW Umbreit. New York: Academic Press, 1961. 3:131-192.

Collins, CH *Laboratory-acquired Infections* London: Butterworths, 1983.

Collins, CH, EG Hartley and R Pilsworth *The Prevention of Laboratory Acquired Infection* Public Health Laboratory Service, Monograph Series No. 6. 1974.

Committee on Hazardous Biological Substances in the Laboratory, Board on Chemical Sciences and Technology, Commission on Physical Sciences, Mathematics, and Resources, and National Research Council *Biosafety in the Laboratory* Washington DC: National Academy Press, 1989.

Darlow, HM "Methods in Microbiology" *Safety in the Microbiological Laboratory* Eds. JR Norris and DW Robbins. New York: Academic Press, Inc., 1969. 169-204.

Fleming, Diane O, John H Richardson, Jerry J Tulis, Donald Vesley, eds. *Laboratory Safety: Principles and Practices* 2nd ed. Washington DC: American Society for Microbiology, 1995.

Furr, A Keith, ed. *CRC Handbook of Laboratory Safety*. 4th ed. Boca Raton: Chemical Rubber Company, 1995.

Garvin, Michael L *Infectious Waste Management: A Practical Guide* Boca Raton: Lewis Publishers, 1995.

Hellman, A, MN Oxman, and R Pollack, eds. *Biohazards in Biological Research* Cold Spring Harbor: Cold Spring Harbor Laboratory, 1973.

Institute for Laboratory Animal Research, *Occupational Health and Safety in the Care of Research Animals* Washington, DC: National Academy Press, 1996.

Institute for Laboratory Animal Resources Commission on Life Sciences National Research Council *Guide for the Care and Use of Laboratory Animals* Washington DC: National Academy Press, 1996.

Liberman, Daniel F and Judith G Gordon, eds. *Biohazards Management Handbook* New York: Marcel Dekker, 1989.

National Sanitation Foundation Standard 49.. *Class II (Laminar Flow) Biohazard Cabinetry*. Ann Arbor, Michigan, 1983.

Reinhardt, Peter A and Judit G Gordon. *Infectious and Medical Waste Management* Chelsea: Lewis Publishers, 1991.

Richmond, Jonathon Y, ed. *Anthology of Biosafety: I. Perspectives on Laboratory Design* Mundelein: American Biological Safety Association, 1999.

Richmond, Jonathon Y, ed. *Designing a Modern Microbiological/Biomedical Laboratory* Washington DC: American Public Health Association, 1997.

Subcommittee on Arbovirus Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates. 1980. *Am J Trop Med Hyg* 29(6):1359-1381.

United States Department of Agriculture *Laboratory Animal Welfare Regulations* - 9 CFR, Subchapter A, Parts 1, 2, and 3.

United States Department of Health. *Laboratory Safety at the Center for Disease Control*. Education and Welfare Publication No. CDC 75-8118. Washington: GPO, September 1974.

United States Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. *Classification of Etiologic Agents on the Basis of Hazard*. 4th ed. Washington: GPO, July 1974.

United States Department of Health, Education and Welfare. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses.* Publication No. (NIH) 75-790. Washington: GPO, October 1974.

United States Department of Health, Education and Welfare, Public Health Service, and National Institutes of Health. *National Institutes of Health Biohazards Safety Guide*. Stock No. 1740-00383. Washington: GPO, 1974.

United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. HHS Publication No. (CDC) 21-1112. Washington: GPO, 2007.

United States Department of Health and Human Services. *Primary Containment of Biohazards: Selection, Installation and Use of Biological Safety Cabinets* Washington: GPO, 1995.

United States Federal Register. Vol. 47, No. 167, Part IV. *Guidelines for Research Involving Recombinant DNA Molecules* Washington: GPO, 1996.

United States Office of the Federal Register National Archives and Records Administration. Commerce and Foreign Trade. Title 15 *Code of Federal Regulations* Parts 300–799. Washington: GPO, 1998.

Yale University, *BioSafety Manual* 1996.