

BIOLOGICAL SAFETY

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1.0 RESPONSIBILITIES

1.1 *Introduction*

The National Institutes of Health (NIH) and Centers of Disease Control and Prevention (CDC) defines 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' rDNA may be obtained from the Environmental Health and Safety Office.

1.2 Environmental Health and Safety Office Program

Environmental Health and Safety Office will:

- provide consultation and technical information for handling biological agents,
- review proposals and protocols for the use of hazardous biological agents, rDNA, and submit these to the Biological Safety Committee with recommendations,
- oversee the annual certification of biological safety cabinets by an outside contractor,
- present biological safety seminars upon request,
- review and approve purchases of biological safety cabinets and other safety-related equipment,
- survey laboratories for compliance with approved standards and policies of UTD, CDC and NIH,
- provide assistance or advice in the disinfection of facilities and equipment,
- assist in the development of safety plans and training programs.

1.3 Laboratory Management

Management will:

- observe the established guidelines and polices for biological safety
- ensure that laboratory personnel are trained in the hazards and safe handling procedures of biological agents,
- encourage employees to report any changes or suspected changes in their health status,
- advise Office of Environmental Health and Safety of any significant changes in the protocol for the use of hazardous biological agents.

1.4 Staff/Students

Staff/Students will:

- observe the established guidelines and policies for biological safety,
- 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,
- report all biological spills and accidents to their supervisor.

Responsibilities (continued)

1.5 Biological Safety Committee

The Biological Safety Committee will:

- establish general policies and standards for the safe use of biohazards at UTD,
- define categories of biohazardous agents to determine if specific policies are

- needed for their safe use,
- review Notification of Use applications and Registration Documents for research involving biological agents or recombinant DNA molecules for the proposed purpose,
- approve, recommend changes, or deny the intended use of the biological agent or recombinant DNA 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.

2.0 GENERAL GUIDELINES FOR HANDLING BIOLOGICAL AGENTS

2.1 *The Seven Basic Rules of Biosafety*

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

- Do not mouth pipette.
- Manipulate infectious fluids carefully to avoid spills and the production of aerosols and droplets.
- Restrict the use of needles and syringes to those procedure 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-resistance containers
- Use protective laboratory coat and gloves
- Wash hands following all laboratory activities, following contact with infectious materials.
- Decontaminate work surfaces before and after use, and immediately after spills.
- 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

Whenever handling biological material, wash your hands thoroughly:

- after working with any biohazards;
- after removing gloves, lab coat, and other contaminated protective clothing;
- before eating, drinking, smoking, or applying cosmetics;
- before leaving the laboratory area.

2.2 Personal Hygiene (continued)

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

Do not:

- eat
- drink
- smoke
- apply cosmetics in the work area

2.3 Clothing (Primary Barriers)

BSL 1

- recommended that lab coats or gowns be worn to prevent soiling of street clothes.
- gloves to be worn if skin on hands is broken or if a rash is present.
- protective eyewear if splashes are anticipated.
- use of contact lenses is not recommended; if contact lenses are used, laboratory eye protection or face protection must be worn.
- do not wear potentially contaminated protective clothing out of the laboratory area.

BSL 2

- 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.

BSL 3

- solid front or wrap around gowns are used when in the lab; re-usable 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.

Procedures for Protective Clothing Removal

1.	Remove booties from the back.
2.	Remove head covering from the peak.
3.	Wear gloves while untying gown.
4.	Remove gloves from uncontaminated side.
5.	Slip finger UNDER sleeve cuff to remove gown.

2.4 Handling Procedures

Always

- use mechanical pipetting devices and cotton-plugged pipettes.
- perform all procedures with a minimum of aerosol production.
- add a disinfectant to water baths used with infectious substances.
- use trunnion cups with screw caps 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.

2.5 Hand washing

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

Laboratory personnel should wash their hands:

- when coming on duty
- on leaving the laboratory for whatever reason
- when hands are obviously soiled
- before and after completion of a task in a biological safety cabinet, even if gloves are worn
- before contact around one's face or mouth
- upon completion of duty.

2.6 Protocol for Hand washing

A protocol for hand washing is as follows:

- Turn on faucets and wet hands with tepid water.
- Dispense nonantiseptic soap or antiseptic compound into a cupped hand.
- Spread soap or compound around both hands and between fingers. If needed, add a little more water to facilitate spread and lathering.
- Wash hands for about 10 seconds. Vigorously rub both sides of hands starting from a few inches above the wrist, extending downward between the fingers and around and under the fingernails.
- Rinse thoroughly under the tepid running water. Rinsing should start above the wrist area and proceed to the tips of the fingers. Note: if faucets are not knee- or foot-operated, do not turn off water (do not touch faucet handles) yet.
- Dry hands thoroughly with paper towels. If faucets are hand operated, turn them off now, using a dry paper towel to protect clean hands.

2.7 Syringes

Avoid using syringes and needles whenever possible. If a syringe is necessary:

- use the needle-locking type, or a disposable syringe-needle unit;
- place syringes into a pan of disinfectant without removing the needle;
- do not place syringes in pans containing pipettes or other glassware requiring sorting;
- do not recap needles;
- dispose of needles in leak proof, puncture resistant containers specifically designed for sharps disposal.

2.8 Controlling the Biohazard Area

- Keep laboratory doors closed while experiments are in progress.
- Keep laboratory doors locked when vacant.
- Limit access into 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.

2.9 Housekeeping

- Decontaminate work surfaces:
 - daily and
 - 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 UTD Policy for recycle and disposal of chemicals and biohazardous material.
- Keep books and journals only in clean areas of the laboratory.
- All equipment must be completely decontaminated prior to sending the equipment for routine maintenance or repair work.

2.10 Containment

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

Laboratory practice and techniques include the following:

- strict adherence to standard microbiological practices and procedures,
- be aware of any potential hazards and be trained and proficient in the practices and techniques associated with the materials being handled;
- use of appropriate safety equipment for the specific procedure;
- the laboratory supervisor must be trained in laboratory techniques, safety procedures, and hazards associated with handling potentially infectious agents.

Safety equipment

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

2.11 Biosafety Levels

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

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

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

unknown. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials.

Biosafety Level 3 - practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

Biosafety Level 4 - practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Additionally, agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and autoinoculation.

NOTE: UTD does not currently operate any BLS-4

2.12 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 Centers for Disease Control (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. (See UTD Policy)

3.0 GLOVE USE

3.1 Introduction

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

3.2 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 button, telephones or computers.
- 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,
- creating new hazards by decreasing dexterity.

3.3 Glove Materials and Types

The most common glove material used when handling biologicals are:

- latex
 - powdered
 - powder free
- polyvinyl chloride (PVC)
- nitrile

3.4 Glove Selection

When selecting gloves one should 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 gloves 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 powder less 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 against a biological hazard, they may not protect against a chemical or a mixture of chemicals.
- Higher temperatures than room temperature decrease break-through time of most materials.
- Thicker materials or multiple layers are usually better for combined biological and chemical use.

Glove Selection (continued)

- 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.

4.0 SHIPMENT OF HAZARDOUS AGENTS

4.1 Overview

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 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).

In agreement with the Civil Aviation Security Field Office and the Federal Aviation Administration, UTD has established a system to meet these requirements. No UTD employee will be permitted to ship chemicals without having completed certified DOT training. Since the definition of hazardous materials is very broad, any such shipments must be reviewed by a representative from the Environmental Health and Safety Office (EHS) prior to shipment. EHS staff and

Materials Management has successfully completed the training course in order to carry out these responsibilities. Please direct questions to EHS at extension 2113

5.0 TRANSPORTATION AND TRANSFER OF BIOLOGICAL AGENTS

5.1 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.

5.2 Transfer and transportation of biological agents

Please contact the Office of Environmental Health and Safety if you wish to transfer or ship biological agents and/or specimens to ensure proper regulations are being followed.

6.0 AEROSOLS

6.1 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.

6.2 Risks

Aerosol particles one to five microns in size presents 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.

6.3 Aerosol Production Aerosols may be produced in the use of:

- 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
- separator funnel

6.4 Minimizing Aerosol Production

To reduce/minimize aerosol production:

- perform activities that may produce aerosols in a biological safety cabinet or a chemical fume hood;
- 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,
- wrap with disinfectant-soaked gauze when:
 - removing the needle from the rubber stopper of a test tube or vial,
 - breaking the cap on an ampoule,

Minimizing Aerosol Production (continued)

- removing stoppers or plugs from tubes, or
- expelling air or surplus solution from a syringe; and
- perform centrifugation using balanced trunnion cups with disinfectant added between the tube and the cup.

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.

7.0 REPRODUCTIVE HAZARDS

Hazards that may affect the desire to procreate.

Hazard

Risk

Infectious Diseases

Fatigue, decreased libido, impotence

7.1 Known Human Teratogens

Cytomegalovirus (CMV)
 Herpes virus hominis I&II
 Parvo virus B-19 (Erthema Infectiosum)
 Diabetes
 Rubella Virus
 Syphilis
 Toxoplasmosis
 Phenylketonuria
 Venezulelan equine encephalitis virus
 Virilizing tumors
 Rheumatic disease

8.0 DISINFECTION AND STERILIZATION

8.1 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 - to destroy all nonspore 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).

8.0 DISINFECTION AND STERILIZATION

8.1 Definitions (continued)

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 organism or inhibiting their growth and multiplication.

8.2 General Procedures

Disinfect frequently all floors, cabinet tops, and equipment where biohazardous materials are used.

- Decontaminate all infectious materials and contaminated equipment prior to being 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 clearly marked holding containers such as "NON-INFECTIOUS" or "BIOHAZARDOUS TO BE AUTOCLAVED".

8.3 Choosing a Method

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

- the target organism (the biological agent) and
- the characteristic(s) of the materials or areas to be cleaned.

8.4 Selecting a Disinfectant

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

DESCRIPTIONS OF COMMONLY USED DISINFECTANTS

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 virus; 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 (50-500ppm) active against vegetative bacteria and most viruses; higher concentration (2500ppm) 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	

8.5 Sterilization Methods

Wet Heat (Steam)

This method requires approximately 15psi pressure with a chamber temperature of at least 250mF (121mC). 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). Monitor steam sterilization effectiveness with a biological indicator, *Bacillus stearothermophilus*.

Dry Heat

This is less effective than steam, and requires more time (two to four hours) and a higher temperature (320-338mF or 160-170mC). Monitor dry heat sterilization with a *Bacillus subtilis* biological indicator.

Ethylene Oxide Gas (EO)

Ethylene oxide sterilization is not available at UTD.

9.0 BIOLOGICAL SAFETY CABINETS

9.1 Overview

A biological safety cabinet 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.

9.0 BIOLOGICAL SAFETY CABINETS

9.1 Overview (continued)

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

9.2 Primary Containment: Biological Safety Cabinets

Biological Safety Cabinets (BSCs) are among the most effective and the most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Class I, II, III) have performance characteristics and applications which are described in this 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 (Biosafety Level 2 and 3 agents). Both Class I and 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 high-efficiency particulate air filtration (HEPA filtration) of the air flow down 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.

9.3 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. 1) 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 radiolabel led 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.

9.0 BIOLOGICAL SAFETY CABINETS

9.4 Class II (continued)

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 International, (2) Ann Arbor, Michigan. 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 two 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. 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 2b, 2c, and 2d, 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 radionuclide.

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 supersede 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.

9.5 Class III

The Class III Biological Safety Cabinet (Fig. 3) 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.

9.5 Class III

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 door 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.

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. 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.

References:

U.S. Department of Health and Human Services. Primary Containment of Biohazards: Selection, Installation and Use of Biological Safety Cabinets. (Washington: GPO, 1995)

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

9.6 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.

9.7 Table 1 - Comparison of Biological Safety Cabinets

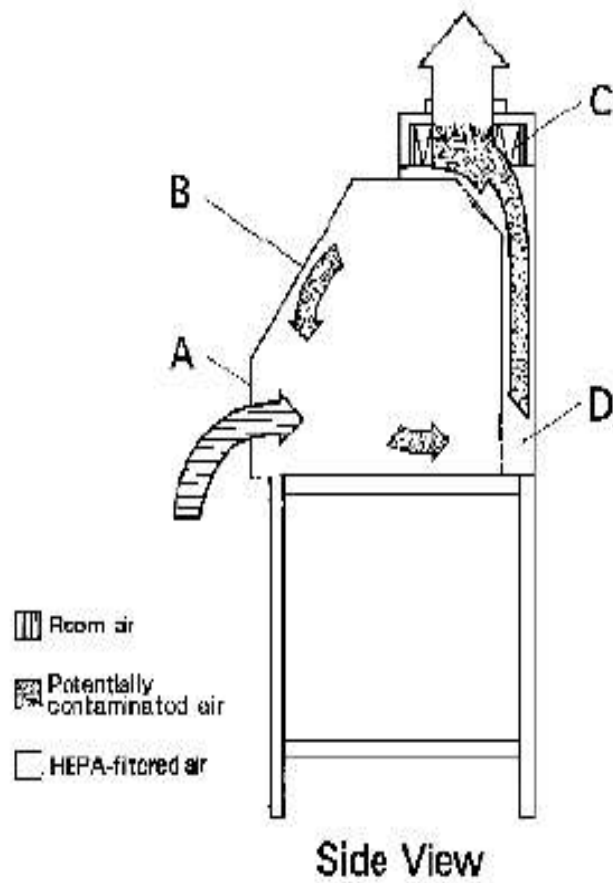
Type	Face velocity (lfpm)	Airflow Pattern	Radionuclide/ Toxic Chemicals	Biosafety Level (s)	Product Protection
Class I* open front	75	In at front; rear and top No through HEPA filter	No	2,3	
Class II Type A	75	70% recirculated through HEPA; exhaust through HEPA	No	2,3	Yes
Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Yes (Low levels/volatility)	2,3	Yes
Type B2	100	No recirculation; total exhaust via HEPA and hard ducted	yes	2,3	Yes
Type B3	100	Same as IIA, but plena under negative pressure to room and exhaust air is ducted	Yes	2,3	Yes
Class III	NA	Supply air inlets and exhaust through 2 HEPA filters	Yes	2,4	Yes

* 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/radionuclide.

9.8 Class I Biological Safety Cabinet

Figure 1.
Class I Biological Safety Cabinet.

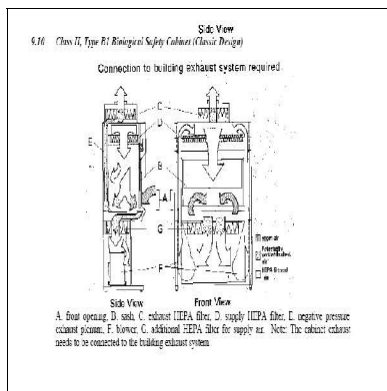
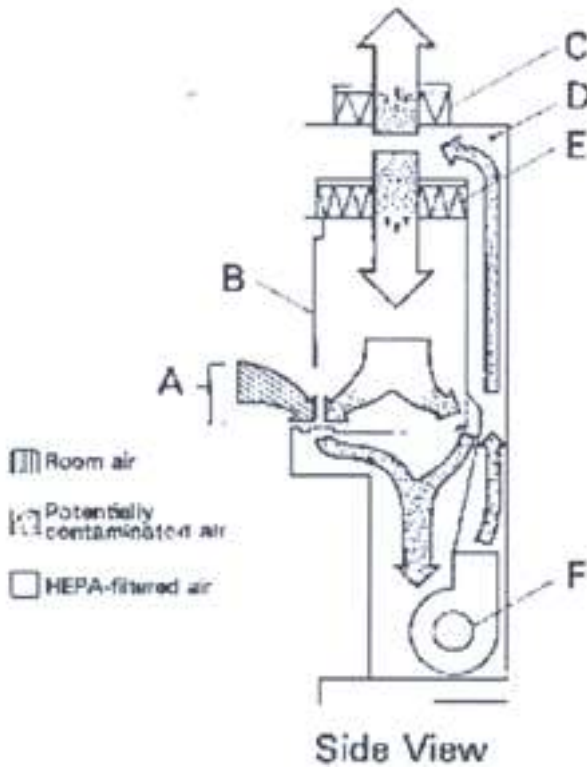
A. front opening, B. sash, C. exhaust HEPA filter, D. exhaust plenum



9.9 Class II, Type A Biological Safety Cabinet

Figure 2a
Class II, Type A Biological Safety Cabinet.

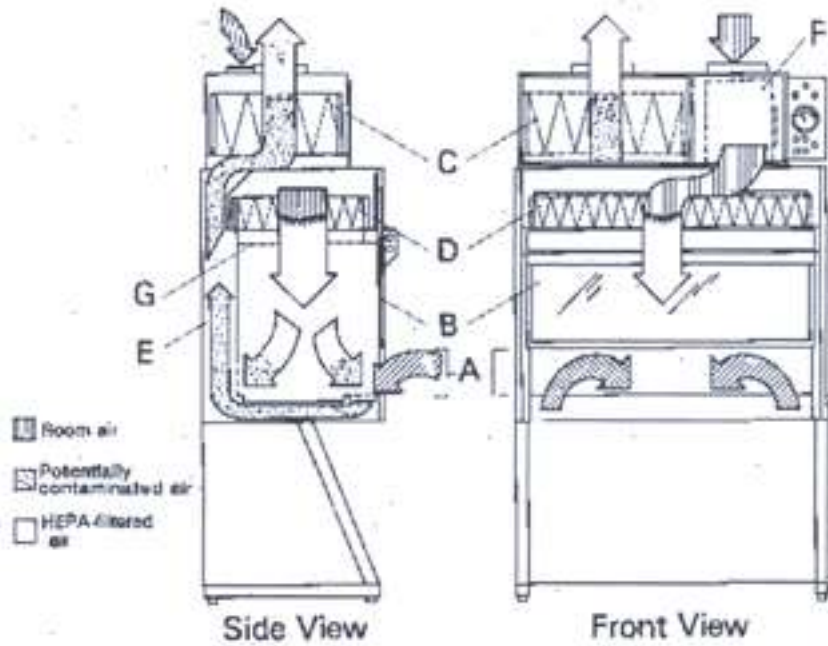
A, front opening, B, sash, C, exhaust HEPA filter, D, rear plenum, E, supply HEPA filter, F, blower



Class II Type B1 Biological Safety Cabinet

9.11 Class II, Type B2 Biological Safety Cabinet

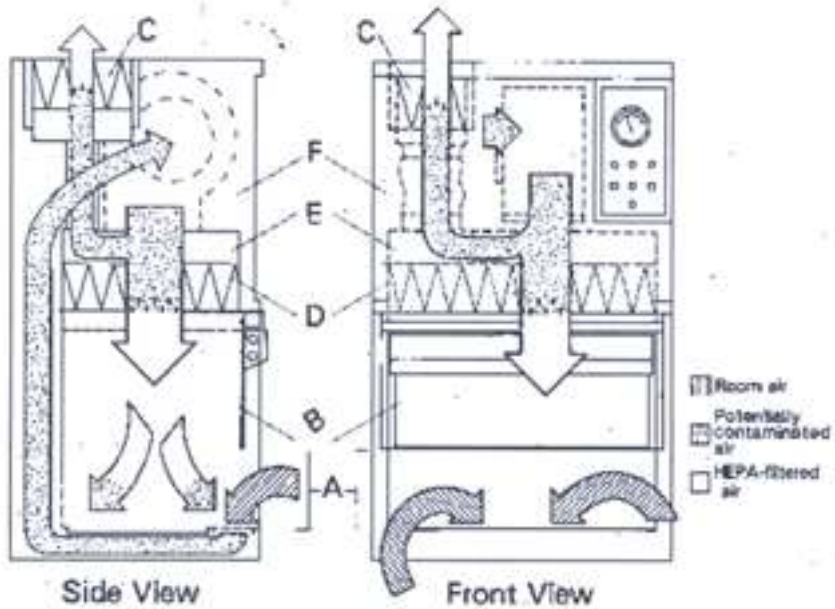
Connection to building exhaust system required.



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 needs to be connected in the building exhaust system.

9.12 Table Top Model of a class II, Type B3 Biological Safety Cabinet

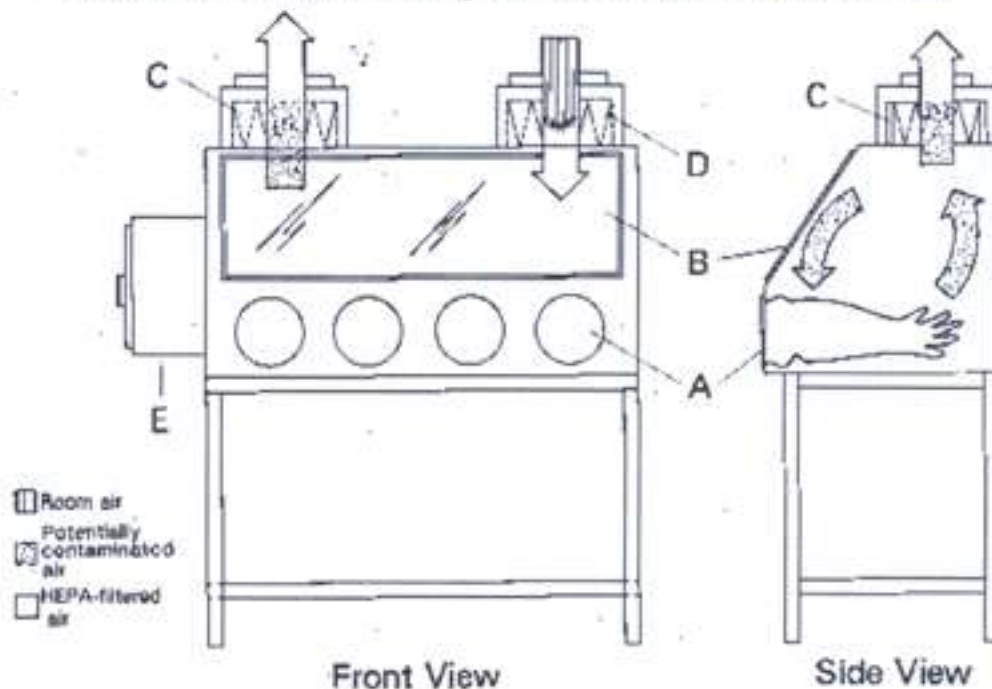
Connection to building exhaust system required.



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 needs to be connected to the building exhaust system.

9.13 Class III Biological Safety Cabinet

Connection to building exhaust system required.



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 installed which would be located beneath the work surface of the BSC with access system from above. The cabinet exhaust needs to be connected to the building exhaust system.

9.14 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

10.0 SAFETY RULES FOR USE OF CLASS I AND II CABINETS

10.1 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.

10.2 Biohazardous Spills in the Cabinet

In case a biohazardous spill occurs inside the cabinet:

- decontamination steps should be taken while the cabinet is operating to prevent the escape of contaminants.
- spray or wipe walls, work surface, and all apparatus that is affected with an appropriate disinfectant detergent. (Make sure to wear gloves while doing this.)
- 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 caviicide.

After a spill is decontaminated, the cabinet shall 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.

10.3 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.

11.0 PROCEDURES FOR CENTRIFUGATION

11.1 Low-speed Centrifugation

All low-speed centrifugation must be done in capped tubes in centrifuge safety cups or centrifuge rotors which 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:

- Each operator should be trained on proper operating procedures

11.0 PROCEDURES FOR CENTRIFUGATION

11.1 Low-speed Centrifugation (continued)

- 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 $\frac{3}{4}$ full. Always cap tubes before spinning.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins
- 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.
- Decontaminate safety carriers or rotors and centrifuge interior after each use.

11.2 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.

11.3 Centrifuge Spill

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

- hold breath,
- close the centrifuge lid,
- turn centrifuge off,
- immediately leave the laboratory,
- notify others to evacuate the lab,
- post laboratory door with a biohazard spill note,
- presume the aerosolized material is contaminated,
- treat the incident as a potential exposure.

PROCEDURES FOR CENTRIFUGATION (continued)

11.4 Decontamination Procedures

- Allow area to rest for 30 minutes prior to entry for decontamination
- Notify the supervisor or Principal Investigator and EHS ext. 6111.
- 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 manufacturers 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 Environmental Health and Safety Office ext. 2113 for information.

12.0 PROCEDURES FOR SPILLS OF BIOHAZARDOUS MATERIALS

12.1 Spills in the Laboratory

Spills in the laboratory, outside a biological safety cabinet or other physical containment device, need to be reported to the supervisor or Principal Investigator and documented. Reported and documented spills are any spills that may contain the presence of potentially infectious materials or the possibility of splashes and generation of aerosols or airborne particles.

12.2 In Case of a Spill

Immediate action needed in case of spill

- Stop work immediately.
- Notify others in Lab of spill.
- Avoid inhaling airborne materials.
- Leave the room immediately.
- Remove contaminated clothing, turn exposed area inward, place in red bag or autoclave bag as appropriate to biosafety level. Wash all exposed skin with antiseptic soap and water.
- Notify the supervisor or Principal Investigator and EHS ext. 2113 during normal work hours (8:00 a.m. - 5:00 p.m.) and UTD Police at ext. 2331 after normal working hours.

12.3 Decontamination and Clean-up Procedures

HOW TO CLEAN-UP AND DECONTAMINATE AFTER A SPILL

Major spill is any spilled volume over 10 ml.

- a) Label the room off-limits for at least 30 minutes. This allows droplets to settle and the ventilation system to purge the air.
- b) After 30 minutes don PPE (HEPA filtered respirator, gown, gloves (double glove) and shoe covers to enter the room).
- c) Contain the spill with absorbent towels or pads (do not use plastic backed pads). Slowly pour 10% chlorine bleach solution, Cavicide or any appropriate disinfectant around the perimeter of the spill working toward the center of the spill to inactivate infectious materials.

- d) Allow the disinfectant to stand for 30 minutes. Carefully remove the absorbent material and place in either a red bag or autoclave bag. Pick up any glass or other sharps with tongs and discard in a sharps container.
- e) Once all materials have been removed, decontaminate the area again with an appropriate disinfectant.
- f) If a mop is used, soak the mop in fresh disinfectant for 30 minutes before rinsing for reuse. Wash reusable gloves with the disinfectant.
- g) Remove PPE and wash hands thoroughly with soap and water.

Minor spills are a small volume of less than 10 ml or a volume that can be covered by paper towels.

- a) Take steps outlined in the "IMMEDIATE ACTION NEEDED IN CASE OF SPILL 12.2"
- b) Cover the spill with absorbent material
- c) Pour disinfectant around and onto the absorbent material. Allow to stand for 30 minutes contact time.
- d) After 30 minutes, carefully soak up the spill with absorbent material.
- e) Pick up any glass or sharps with tongs or tweezers and discard in a sharps container.
- f) Dispose of absorbent material in a red bag or autoclave bag as appropriate.
- g) Decontaminate the area again with an appropriate disinfectant.
- h) Remove PPE and wash hands thoroughly with soap and water.

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

Spills in Hallways

Procedure for spills in hallways:

- a) Restrict traffic through the area.
- b) Shut all room doors adjacent to the spill area
- c) Notify the supervisor or Principal Investigator and EHS ext. 6111 during normal work hours (8:00 a.m. - 5:00 p.m.) and CDAS ext. 2331 after normal working hours or on university holidays and ask for the Environmental Health and Safety Office "On-Call" person.
- d) Don PPE (HEPA filtered respirator, gown, gloves (double glove) and shoe covers.
- e) Cover the spill with absorbent material
- f) Pour disinfectant around and onto the absorbent material. Allow to stand for 30 minutes contact time.
- g) After 30 minutes, carefully soak up the spill with absorbent material.
- h) Pick up any glass or sharps with tongs or tweezers and discard in a sharps container.
- i) Decontaminate the area again with an appropriate disinfectant.
- j) Dispose of absorbent material in a red bag or autoclave bag as appropriate.

12.5 Re-occupancy of a Spill Area

Before reoccupying any area where a spill has occurred:

- the supervisor of the area or EHS 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.

13.0 RECOMBINANT DNA

13.1 Definition

Recombinant DNA 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, or
- molecules that result from the replication described above.

13.2 Research Approval Categories

rDNA research may be approved in one of two categories at UTD

- Experiments which require specific prior approval by the National Institutes of Health (NIH) and the UTD Biological Safety Committee.
- Experiments which require prior approval of only the UTD Biological Safety Committee.

See the Biological Safety Policy in this chapter for notification and approval procedures.

Note: The list of recombinant DNA molecules along with the reporting and containment requirements is revised periodically by the Director of the National Institutes of Health. Contact Environmental Health and Safety Office, for current guidelines for research involving recombinant DNA molecules at extension 2113.

14.0 NOTIFICATION OF USE FOR BIOLOGICAL AGENTS AND RECOMBINANT DNA

14.1 Introduction

A Notification of Use for Biological Agents and Recombinant DNA (NOU) form must be submitted to the UTMB Biological Safety 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.

14.1 Introduction (continued)

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 that an NOU 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.

14.2 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 outline in the OSHA Lab Standard (29) CFR 1910.1450 "Occupational Exposure to Hazardous Chemical in Laboratories".
 - Laboratories using select agents will maintain strict security in select agent storage as well as maintaining a locked laboratory when not occupied.

14.3 Select Agent List

Viruses:

Crimean-Congo haemorrhagic fever virus
Eastern Equine Encephalitis virus
Ebola viruses
Equine Morbillivirus
Lassa fever virus
Marburg virus
Rift Valley fever virus
South American Haemorrhagic 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.

Bacteria:

Bacillus anthracis
Brucella abortus, B. melitensis, B. suis
Burkholderia (Pseudomonas) mallei
Burkholderia (Pseudomonas) pseudomallei
Clostridium botulinum

14.3 Select Agent List (continued)

Bacteria: (continued)

Francisella tularensis
Yersinia pestis

Exemptions:

Vaccine strains as described in Title 9 CFR, Part 78.1 are exempt.

Rickettsiae:

Coxiella burnetii
Rickettsia prowazekii
Rickettsia rickettsii

Fungi:

Coccidioides immitis

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 in this list or their toxic subunits.

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 EHS extension x2113 for assistance prior to commencement of work with a select agent.

15.0 LABORATORY MOVES/TRANSFERS/CLOSURES

General 15.1

- 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, glass slides, etc.
- 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 HSS as required by law. All chemicals must be properly segregated and packaged.

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 and trash left in the lab.

Environmental Health and Safety personnel will inspect the lab with the individual responsible for the laboratory to insure that the lab has been cleared. If the lab has not been cleared properly, the Principal Investigator will be contacted for follow-up response.

15.0 LABORATORY MOVES/TRANSFERS/CLOSURES (continued)

15.2 Equipment

- Coordinate the decontamination of the Biological Safety Cabinet to be moved through EHS at least two weeks before the move. Once moved, they will need to be re-certified 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 previous section).
- 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 EHS 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.

15.3 Laboratory Equipment Decontamination Form (SEE NEXT PAGE)

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 the equipment 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. For questions regarding radioactive decontamination, call ext. 6111. For questions regarding chemical and biological decontamination, call ext. 6111.

The Laboratory Closure and Relocation Check List should be completed, signed by a faculty member and an Environmental Health and Safety representative and then posted on the door.

UTD LABORATORY EQUIPMENT DECONTAMINATION FORM

This form must be completed and attached to laboratory equipment that is to be salvaged, relocated or disposed of prior to removal from or abandonment of the laboratory.

To the best of my knowledge the following piece of equipment:

Manufacturer, Model#, Serial#

Inventory#

Has never been used with radioactive materials, hazardous chemicals or biological agents.

Signature

Date

Printed Name

Title

Department

has been used with the following materials:

Radioactive material(s)*

Radionuclide(s)

Hazardous Chemical(s)

Name High Risk Chemical(s)

Biological Agent(s)

Agent(s)

The above named equipment has been cleaned with _____, which is suitable

Describe process and agent used

for deactivating/removing the hazardous materials used with/in this equipment.

Date of decontamination

*Decontamination must be confirmed by wipe test.

Signature

Date

Printed Name

Title

Department

How to complete the form:

1. Notify the Environmental Health & Safety Office at ext 2369 of any moves regarding chemical fume hoods or biological safety cabinets. (Tissue Hoods)
2. Identify the equipment by providing the Manufacturer, Model #, Serial # and the UTD Inventory #.
3. Check the appropriate box indicating whether or not the equipment has been used with radioactive materials, hazardous chemicals or biological materials.
4. If the equipment was not used with hazardous chemicals or biological and or radioactive materials, sign and date the form. Attach the completed form to the equipment. The equipment is then ready for transport.
5. If the equipment was used with the hazardous materials, indicate which type of material by marking the appropriate box.
6. For usage with radioactive materials, list the radionuclide(s) used. The equipment must be wipe tested. The wipe tests will be done by personnel from the lab and the results posted with the equipment. If the equipment is free of contamination, document on the form the date the clearance was given and the name of the Safety personnel issuing the clearance. Sign and date the form and attach to the equipment.
7. For usage with hazardous chemicals, list the name of the chemical(s) and clean with the appropriate compound. Document the cleanup date and the cleaning compound used. Sign and date the form and attach to the equipment.
8. For usage with biological agents, list the agent(s) used. Clean with a suitable disinfectant, document date and disinfectant used. Sign and date the form and attach to the equipment.

16.0 RESPIRATORY PROTECTION PROGRAM

16.1 Overview

Respiratory protection was once only found 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 UTD, 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.
- Select respirators based on the hazards to which the worker is exposed.
- 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.
- Respirators shall be stored 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.

- Assigned persons to tasks requiring 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 (i.e., annually).
- Use approved or accepted respirators when they are available.

If you currently use a respirator or feel that a particular aspect of your job requires the use of one, please contact EHS ext 6114.

17.0 REPRODUCTIVE HAZARDS

17.1 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

17.0 REPRODUCTIVE HAZARDS

17.1 Introduction (continued)

The resultant effect of the above may be:

- spontaneous abortion
- infertility
- stillbirth
- malformed offspring

17.2 Teratogen

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, during the 3rd to 8th week of pregnancy the organs are developing, and the placenta, which acts as a barrier to many toxicants, is not completely formed until the 8th or 9th week.

17.3 Exposure Hazards

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' ova before birth. Exposures during the first trimester represent the greatest risk.

Male germ 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.

17.4 Know Human Teratogens

Cytomegalovirus (CMV)	Herpes Simplex virus
Parvo virus B-19 (Erythema Infectiosum)	Rubella Virus
Syphilis (Treponema Pallidum)	Toxoplasmosis
Varicella virus	Venezuelan equine encephalitis virus
Virilizing tumors	Rheumatic disease
Hepatitis B Virus	Human Immunodeficiency Virus

Note: See also Section 13.0

18.0 APPENDIX A: BIOLOGICAL SAFETY POLICY

18.1 Introduction

The Biological Safety Committee was established for the purpose of formulating and recommending to the governing body through the Vice President for Research a general policy for the safe use of biological agents at UTD. The Committee recognizes and supports the University's fundamental objectives of teaching, research and healthcare delivery. It also recognizes the University's obligation to pursue these objectives without compromising the health and safety of its students, staff and faculty and those members of the surrounding community. The Committee's goal, therefore, is not to be restrictive but to develop safety 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.

18.2 Biological Safety Committee Scope and Mission Statement

Scope

The scope of the Biological Safety Committee encompasses all UTD biological safety issues except those addressed by Healthcare Epidemiology. Healthcare Epidemiology addresses infection control issues specifically related to healthcare delivery for both patients and healthcare workers, including personnel in the clinical laboratories. Healthcare Epidemiology does not address infection control or biological safety issues in research laboratories unless consulted by the Biological Safety Committee.

Mission

The Mission of the Biological Safety Committee is to support the University's fundamental objectives of teaching, research and healthcare delivery by promoting the safe use and handling of biological agents and assuring that all

activities involving these agents are in compliance with the applicable guidelines, codes and regulations.

Responsibilities/Important Components of Biological Safety

- Assess all biosafety activities
- Develop institutional biological safety policies and procedures
- Maintain/review policies and procedures
- Identify problem areas
- Assure safety of students, employees, visitors, volunteers, patients, environment and community
- Review and assess grant projects for biological safety
- Monitor the handling of biological waste
- Report and advise university administration on biological safety issues
- Communicate to address:
 - Biological agents requiring biosafety review
 - Individual responsibilities with respect to biological safety
 - Biological issues prompted by public awareness/media coverage

APPENDIX A BIOLOGICAL SAFETY POLICY (continued)

Definition of a Biological Agent

Any agent, or component of an agent, that could cause harm to people, animals and/or the environment. The following are examples (not inclusive or comprehensive) of biological agents.

- rDNA molecules
- Organisms and viruses containing rDNA molecules
- Materials known or suspected to contain etiological agents
- Oncogenic viruses
- Human tissue and human products
- Organisms and viruses not containing rDNA

18.3 Definition of a Biological Agent

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, or
- human products (blood, tissue).

18.4 Review Classification

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 Biological Safety Committee.

The reclassification will be deemed necessary if the laboratory procedures will involve:

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

APPENDIX A BIOLOGICAL SAFETY POLICY (continued)

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

Recombinant DNA Research

The current procedures and requirements for recombinant DNA are specified in the current "NIH Guidelines for Research Involving DNA Molecules." UTD must comply with these guidelines if NIH funding is to be maintained. The Biological Safety 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.

18.5 Review Procedures

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 Environmental Health and Safety Office. This form should be submitted prior to, or simultaneously with, any proposal for funding of research.

Once this notification is received, Environmental Health and Safety staff will review the 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.

APPENDIX A BIOLOGICAL SAFETY POLICY

18.5 Review Procedures (continued)

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 Environmental Health and Safety Office for committee approval. This certification does not include rDNA or work with agents that require U.S. 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	<ol style="list-style-type: none"> 1. P.I. submits NOU to Health & Safety Services/Biological & Chemical Safety Program (HSS/B&C). 2. HSS/B&C 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 UTMB at the present time

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 Biological Safety Committee through the EHS Program. EHS will aid the Investigator in the interpretation of the NIH guidelines.

The Registration Document 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 a deliberate attempt will be made to obtain expression of a foreign gene and, if so, what protein will be produced;
- the containment conditions specified in the NIH guidelines.

The document must be dated and signed by the Investigator.

APPENDIX A BIOLOGICAL SAFETY POLICY (continued)

18.5 Review Procedures (continued)

No recombinant DNA experiments at UTD are exempt. All experiments must have the approval of the Committee before the researcher may begin.

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 (a copy of these recommendations is in this chapter). The Committee will cooperate with the Institutional Animal Care and Use Committee on all experiments involving both animals and biological agents.

18.6 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 Biological Safety Committee and should contain a suggestion for alternative classification and documentation in support of this suggestion. The Biological Safety Committee will then inform the Investigator of its decision.

Instructions for Page 1

A Notification of Use must be submitted to the UTD Biological Safety 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. Complete page 1 and Section I (pages 2 & 3).

Recombinant DNA: An NOU must be completed and submitted for ALL rDNA Class 1 and above. Complete page 1 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 question 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 the select biological agents and rDNA is on page 7. Refer to page 11 for listing of select biological agents and rDNA.

If you are working with human cells, it is a biological agent; if you are working with genes, it is rDNA.

Note: An NOU must be submitted whenever the biosafety level changes.

Typed document preferred. Handwritten document must be printed legibly and in black ink for copy clarity.

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SECTION I: Biological Agents

1. Name of organism(s) and/or human product _____

BSL 3

ABSL 3

11. Check the protective clothing or equipment used when handling this agent:

- | | |
|---|--|
| <input type="checkbox"/> Gloves/lab coat | <input type="checkbox"/> Safety Centrifuge/blender |
| <input type="checkbox"/> Cover gown/booties | <input type="checkbox"/> Chemical Fume Hood-Room location _____ |
| <input type="checkbox"/> Goggles | <input type="checkbox"/> Biological Safety Cabinet-Room location _____ |
| <input type="checkbox"/> Face Shield | <input type="checkbox"/> Respirator (Type) _____ |
| <input type="checkbox"/> Mask | <input type="checkbox"/> Other _____ |

12. Will an animal model be used? No Yes, if yes: what animal will be used and what is the route of administration? _____

12a. Does the infected animal present any human health risk after administration? Yes No

If yes, provide route of transmission and what precautions will be taken to prevent exposure:

13. How is biohazardous waste disposed? _____

14. What steps will be taken in the event of contamination or spill? _____

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Instructions for page 5

9. List ALL project personnel including the Principal Investigator, their experience and/or training in handling this or similar organisms (stating just the number of years is not sufficient to describe experience). Attach additional sheets if necessary (do not include curriculum vitas). Select agent users must provide detailed information regarding experience with the agent. If staff is to be trained by the P.I. include outline of training and proficiency testing.

Example of appropriate response:

Dr. Bob Agent has a Ph.D. in Molecular and Cellular Biology and Approximately 8 years of experience in recombinant DNA technology. His graduate work involved the cloning, sequencing, characterization and regulation of a gene encoding a penicillin-binding protein from

2. Description of Project: 2.

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Instructions for page 7

Source of DNA: Provide species.

Hosts & Vectors: Describe broadly e.g. plasmids, viral vectors, expression vectors, etc. Indicate the bacterial or eukaryotic host, unless not propagating in the laboratory.

Nature of Inserted Sequences: e.g. viral xgene, plant xgene, etc.

Protein(s) Produced: Protein(s) produced or potential for expression of proteins.

Example:

Source of DNA	Types of Vector	Nature of Insert	Protein Produced
Human	E. coli HMS 174DE3	Human cytb5	B5 reductase
Rat	Expression vector pET29a	Rat FP (OR262)	Rat FP reductase

Use additional sheet if necessary.

Description of Project: A brief description of the project to include purpose, objectives and methodology. This description is for the rDNA work. Add additional sheet if necessary. This description does not have to be filled in if already described in Section I, No. 2.

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Section III: Select Agents

1. Will select agent transfers (shipping and receiving) be conducted with institutions or agencies outside the UTD campus? No Yes
If yes, describe: _____

2. Will transfer of select agents be conducted within the UTD campus? No Yes
If yes, describe: _____

3. Name and phone number of the personnel responsible for laboratory security and emergency response.

	Primary	Secondary
Name	_____	_____
Work #	_____	_____
Pager#	_____	_____
Home #	_____	_____

4. Chemical Hygiene Plan. (Attach as Appendix A).

Instruction for page 9

1. EHS personnel will need to be present during packing of material for shipment and unpacking of material received. Transfers must be documented by use of the CDC.
2. Transfers of material within the UTD campus (from user to user) must be documented and EHS informed of the transfer.
3. Self-explanatory
4. Attach a Chemical Hygiene Plan (CHP) addressing ALL chemicals and the procedures involved with chemical use. A CHP contains general principles, responsibilities, laboratory facilities, basic rules, SOP's, chemical procurement/distributions/storage, monitoring, housekeeping, maintenance, inspections, medical program, PPE, records, signs/labels, spills, accidents, training, MSDS information, waste disposal and safety recommendations. Attach as appendix A.

Part 72 - Interstate Shipment of Etiologic Agents
Appendix A to Part 72 - Select Agents

Viruses

1. Crimean-Congo haemorrhagic fever virus
2. Eastern Equine Encephalitis virus
3. Ebola viruses
4. Equine Morbillivirus
5. Lass fever virus
6. Marburg virus
7. Rift Valley fever virus
8. South American Haemorrhagic fever viruses (Junin, Machupo, Sabia, Flexal, Guanarito)
9. Tick-borne encephalitis complex viruses
10. Variola major virus (Smallpox virus)
11. Venezuelan Equine Encephalitis virus
12. Viruses causing Hantavirus pulmonary syndrome
13. 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).

Bacteria

1. Bacillus anthracis
2. Brucella abortus, B. melitensis, B. suis
3. Burkholderia (Pseudomonas) mallei
4. Burkholderia (Pseudomonas) pseudomallei
5. Clostridium botulinum
6. Francisella tularensis
7. Yersinia pestis

Exemptions: vaccine strains as described in Title 9 CFR, Part 78.1 are exempt.

Rickettsiae

1. Coxiella burnetii

- 2. Rickettsia prowazekii
- 3. Rickettsia rickettsii

Fungi

Coccidioides immitis

Recombinant organisms/molecules

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

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.

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**Recommendation/Requirement
Immunization of UTD Personnel**

I request that the personnel listed below be provided immunization against the following biological agent(s) used in my lab:

Name Department Employee Number Job Title

As Principal Investigator, I understand that it is my responsibility to provide to the employee information regarding the above named biological agent and any known incidence of personnel exposure. The information will address at a minimum the following topics: benefits of immunization, symptomology of disease and documented incidents of laboratory exposures.

Department _____ P.I. Name (print) _____
 Date _____ P.I. Signature _____

Biological Safety Committee

The Biological Safety Committee has reviewed and approved a Notification of Use for the biological

agent: _____ at Biological Safety
 Level _____, Principal Investigator _____ on _____ .
(Date)

The Biological Safety Committee recommends requires that the above personnel be provided immunization against _____
(Name of biological agent)

 Chairperson (Signature)
 Biological Safety Committee

 Chairperson (Printed Name)

 Extension

 Date

For OEHS Use Only: NOU Number

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Instructions for page 12

List the name, department, employee number and job title of the person to receive the immunization, including the principal investigator.

Attach documentation of the information provided to the employee regarding benefits of immunization, decontamination, symptomology of disease and incidents of documented laboratory exposures.

Attach this form to the NOU packet for review by the Biological Safety Committee.

19.0 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, 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. Included are the more commonly encountered agents and 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, Biosafety Branch, Atlanta, Georgia 30333, Phone (404) 639-3883, FAX: (404) 639-2294
- National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496- 1357
- National Animal Disease Center, U.S, Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

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)

APPENDIX B-I - RISK GROUP 1 (RG1) AGENTS

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

Those agents not listed in Risk Groups (RGs) 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.

APPENDIX B-II. - RISK GROUP 2 (RG2) AGENTS

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

APPENDIX B-II-A. - RISK GROUP 2 (RG2) - BACTERIAL AGENTS INCLUDING CHLAMYDIA

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 Appendix B-III-A (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 Appendix B-III-A (RG3) including M. avium complex, M. asiaticum, M. bovis BCG vaccine strain, M. chelonae, 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. choleraesuis, 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

APPENDIX B-II-B - RISK GROUP 2 (RG2) - FUNGAL AGENTS

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

APPENDIX B-II-C - RISK GROUP 2 (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. granulosus*, *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. peruviana*,
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

APPENDIX B-II-D - RISK GROUP 2 (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

Calciwiruses

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

Herpes viruses - except Herpes virus simiae (Monkey B Virus)

(see appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents)

Cytomegalovirus

Epstein Barr virus

Herpes simplex types 1 and 2

Herpes zoster

Human herpes virus 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 Appendix B-III-D, Risk Group 3 (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)

APPENDIX B-II-D - RISK GROUP 2 (RG2) - VIRUSES (continued)

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)

APPENDIX B-III - RISK GROUP 3 (RG3) AGENTS

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

APPENDIX B-III-A - RISK GROUP 3 (RG3) BACTERIAL AGENTS INCLUDING RICKETTSIA

Bartonella

Brucella including B. abortus, B. canis, B. suis

Buckholderia (Pseudomonas) mallei, B. pseudomallei

Coxiella brunetii

Francisella tularensis

Mycobacterium bovis (except BDG strain, see Appendix B-II-A, Risk Group (RG2) - Bacterial Agents Including Chlamydia), 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

APPENDIX B-III-B - RISK GROUP 3 (RG3) - FUNGAL AGENTS

Coccidioides immitis (sporulating cultures; contaminated soil)

Histoplasma capsulatum, *H. capsulatum* var. *duboisii*

APPENDIX B-III-C - RISK GROUP 3 (RG3) PARASITIC AGENTS

None

APPENDIX B-III-D - RISK GROUP 3 (RG3) - VIRUSES AND PRIONS

Alphaviruses (Togaviruses) - Group A Arboviruses

Semliki Forest Virus

St. Louis encephalitis virus

Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))

Other viruses as listed in the reference source

Arenaviruses

Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

Hantaviruses including Hantaan virus

Rift Valley fever virus

APPENDIX B-III-D - RISK GROUP 3 (RG3) - VIRUSES AND PRIONS (continued)

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 (TME) 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

APPENDIX B-IV - 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.

APPENDIX B-IV - RISK GROUP 4 (RG4) - BACTERIAL AGENTS

None

APPENDIX B-IV - RISK GROUP 4 (RG4) - FUNGAL AGENTS

None

APPENDIX B-IV - RISK GROUP 4 (RG4) - PARASITIC AGENTS

None

APPENDIX B-IV - RISK GROUP 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
Herpes viruses (alpha)
 Herpes virus simiae (Herpes B or Monkey B virus)
Hemorrhagic fever agents and viruses as yet undefined

APPENDIX B-V - 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

Herpes viruses

- Herpes virus ateles
- Herpes virus 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

APPENDIX B-V-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.

APPENDIX B-V-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 U.S. Department of Agriculture regulations or administrative policies:

- African horse sickness
- African Swine fever virus
- Akabane virus
- Besnoitia besnoiti

APPENDIX B-V-2 - RESTRICTED ANIMAL PATHOGENS (continued)

- Borna disease virus
- Bovine spongiform encephalopathy
- Bovine infectious petechial fever agent
- Brucellosis melitensis

Camelpox virus
 Cochliomyia hominivorax (screw worm)
 Epizootic hemorrhagic 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 U. S. Department of Agriculture.

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

Low-Risk Oncogenic Viruses (continued)

Murine Leukemia
 Murine Sarcoma
 Polyoma
 Rat Leukemia
 Rat Mammary Tumor
 Rous Sarcoma
 Shope Fibroma
 Shope Papilloma

Moderate-Risk Oncogenic Viruses

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

APPENDIX B - FOOTNOTES AND REFERENCES

a) The original reference for this classification was the publication Classification of Etiologic Agents on the Basis of Hazard, 4th edition, July 1974, U.S. 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.

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

U. S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
Import-Export Products Staff
Room 756, Federal Building
6505 Blecrest Road
Hyattsville, MD 20782.
Telephone: (301) 436-7830 or (301) 436-8499
FAX: (301) 436-8226

c) 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).

d) National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses (October, 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75-790.

e) U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

f) Biosafety in Microbiological and Biomedical Laboratories, Centers for Disease Control and National Institutes of Health, 4th edition, May 1999.

20.0 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 Tables 1 of this section and Section IV. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1 (BSL-1)

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:

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 is 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 of the immediate laboratory are to be placed in a durable, leak proof container and closed for transport from the laboratory. 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 (see Appendix G).

20.0 APPENDIX C: LABORATORY BIOSAFETY LEVEL CRITERIA (continued)

Special Practices

None

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 is anticipated.

Laboratory Facilities (Secondary Barriers)

- Laboratories should have doors for access control.
- Each laboratory contains a sink for hand washing.
- 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.

Biosafety Level 2 (BSL-2)

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:

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.

Standard Microbiological Practices (continued)

- 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 of the immediate laboratory are placed in a durable, leak proof 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 (see Appendix G).

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.

Special Practices (continued)

- 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. Plastic ware 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.

Safety Equipment (Primary Barriers)

- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used 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.
 - ↳ 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.

Laboratory Facilities (Secondary Barriers)

- Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
- Consider locating new laboratories away from public areas.
- Each laboratory contains a sink for hand washing.
- 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.

Laboratory Facilities (Secondary Barriers) (continued)

- Install biological safety cabinets 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. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from 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 of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Biosafety Level 3 (BSL-3)

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 the inhalation route. 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 (i.e., 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, etc.), 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:

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 of the immediate laboratory are placed in a durable, leak proof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.
- An insect and rodent control program is in effect (see Appendix G).

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.

Special Practices (continued)

- 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. Plastic ware 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.

Special Practices (continued)

- ⇒ 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 (e.g., 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. 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 (see Appendix A).
- 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. 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 (see Appendix F). A clothes change room may be included in the passageway.

- Each laboratory room contains a sink for hand washing. The sink is hands-free or automatically operated and is located near the room exit door.

Laboratory Facilities (Secondary Barriers) (continued)

- The interior surfaces of walls, floors, and ceilings of areas where BSL-3 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 balances 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 (see Appendix A).

Laboratory Facilities (Secondary Barriers) (continued)

- 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.

TABLE 1. SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

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	BSL-1 practice plus: Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other Physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials. PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents. PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory

	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
4	Dangerous/exotic agents which pose high risk of lifethreatening disease, aerosol-transmitted lab infections; or related Shower on exit Dedicated supply and exhaust, vacuum, and decon systems supplied, positive pressure personnel suit agents with unknown risk of transmission	BSL3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon system Other requirements outlined in the text

21.0 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.

21.0 APPENDIX D: VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA (continued)

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) 1-4, 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 1-4, 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.

Animal Biosafety Level 1 (ABSL-1)

Animal Biosafety Level 1 (ABSL-1) 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.

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 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.
- 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.

Animal Biosafety Level 1 (ABSL-1) (continued)

- 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 (see Appendix G).

Special Practices

None.

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. (5)
- #### *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. (6) No recirculation of exhaust air should occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.
 - The facility has a hand washing sink.
 - Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180F.
 - Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

Animal Biosafety Level 2 (ABSL-2)

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. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.

VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA (continued)

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.(7)
 - 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.
 - Plastic ware 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 (see Appendix G).

VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA (continued)

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. 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 a material is unavoidable.
 - Personal protective equipment is used based on risk assessment determinations (see Section V). Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms that house nonhuman primates.(8)
 - 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. 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.

Facilities (Secondary Barriers) (continued)

- 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 180F.
- 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.

Animal Biosafety Level 3 (ABSL-3)

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. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

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.(9) 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.

Standard Practices (continued)

- 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 Special Practices #3 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.
 - ⇒ Plastic ware 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.

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.

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) is 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 BSL-3, all work should be done in a primary barrier; otherwise respirators should be worn by personnel in the room.

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-door 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 hand washing sink is provided in each animal room near the exit door. The sink trap is filled with an appropriate disinfectant after each use.

Facilities (Secondary Barriers) (continued)

- 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 (see Appendix A).

- Cages are washed in a cage washer. The mechanical cage washer has a final rinse temperature of at least 180F.
- 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 (i.e., 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.

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TABLE 1. SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR ACTIVITIES IN WHICH EXPERIMENTALLY OR NATURALLY INFECTED VERTEBRATE ANIMALS ARE USED

BS L	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy human adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	<ul style="list-style-type: none"> • Standard animal facility; • No recirculation of exhaust air; • Directional air flow recommended; • Hand washing sink recommended
2	Associated with human disease. Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	ABSL-1 practices plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • Sharps precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing 	ABSL-1 equipment plus: <ul style="list-style-type: none"> • Primary barriers containment equipment appropriate for animal species • PPES: laboratory coats, gloves, face and respiratory protection as needed 	ABSL-1 facility plus: <ul style="list-style-type: none"> • Autoclave available • Hand washing 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	<p>ABSL-2 practices plus:</p> <ul style="list-style-type: none"> Controlled access Decontamination of clothing before laundering Cages decontaminated before bedding removed Disinfectant foot bath as needed 	<p>ABSL-2 equipment plus:</p> <ul style="list-style-type: none"> 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 	<p>ABSL-2 facility plus:</p> <ul style="list-style-type: none"> Physical separation from access corridors Self-closing, double door access Sealed penetrations Sealed windows Autoclave available in facility

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
4	Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown risk of transmission	<p>ABSL-3 practices plus:</p> <ul style="list-style-type: none"> Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting All wastes are decontaminated before removal from the facility 	<p>ABSL-3 equipment plus:</p> <ul style="list-style-type: none"> Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air supplied positive pressure personnel suit) used for all procedures and activities 	<p>ABSL-3 facility plus:</p> <ul style="list-style-type: none"> Separate building or isolated zone Dedicated supply and exhaust, vacuum and decontamination systems Other requirements outlined in the text

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