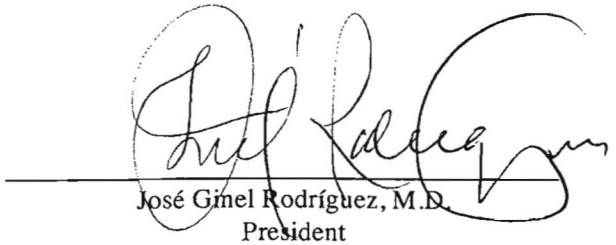
Universidad Central del Caribe
Biological Safety Manual



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Laboratory Practices and Techniques

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. Primary containment, refers to the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. Secondary containment, refers to the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. The purpose of containment is to reduce exposure of laboratory workers and other persons, and to prevent escape into the outside environment of potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for safely handling such material. The director or person in charge of the laboratory is responsible for providing or arranging for appropriate training of personnel.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory supervisor is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Each laboratory should develop or adopt a bio-safety or operations manual which identifies the hazards that will or may be encountered and which specifies practices and procedures designed to minimize or eliminate risks. Personnel shall be advised of special hazards and shall be required to read and follow the required practices and procedures. A scientist with training and knowledge in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct laboratory activities.

Laboratory personnel, safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

1. Engineering controls shall be examined and maintained or replaced on a regular schedule to ensure their effectiveness.

2. Employees shall wash their hands immediately or as soon as possible after removal of gloves or other personal protective equipment and after hand contact with blood or other potentially infectious materials.

3. All personal protective equipment shall be removed immediately upon leaving the work area or as soon as possible if overtly contaminated and placed in an appropriately designated area or container for storage, washing, decontamination or disposal.

4. Used needles and other sharps shall not be sheared, bent, broken, recapped, or resheathed by hand. Used needles shall not be removed from disposable syringes.

5. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a potential for occupational exposure.

6. Food and drink shall not be stored in refrigerators, freezers, or cabinets where blood or other potentially
infectious materials are stored or in other areas of possible contamination; they must be stored in labeled food storage refrigerators.

7. All procedures involving blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, and aerosolization of these substances, and shall comply with the Blood-Borne Pathogens Act.

Safety Equipment

Safety equipment includes biological safety cabinets and a variety of enclosed containers. The biological safety cabinet is the principal device used to provide containment of infectious aerosols generated by many laboratory procedures. Open fronted Class I and Class II biological safety cabinets are partial containment cabinets which offer significant levels of protection to laboratory personnel and the environment when used with good microbiological techniques. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

The safety centrifuge cup, as an example of an enclosed container, is designed to prevent aerosols from being released during centrifugation.

Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, and safety glasses. These personal protective devices are often used in combination with biological safety cabinets and other devices which contain the agents, animals, or materials being examined. In some situations in which it is impractical to work in biological safety cabinets, personal protective devices may form the primary barrier between personnel and the infectious materials. Examples of such activities include certain animal studies, animal necropsy, production activities, and activities relating to maintenance, service or support of the laboratory facility.

Personal Protective Equipment

When there is a potential for occupational exposure, the employer shall provide and assure that the employee uses appropriate personal protective equipment such as, but not limited to, gloves, gowns, fluid-proof aprons, laboratory coats, head and foot coverings, face shields or masks, eye protection, mouthpieces, resuscitation bags, pocket masks, or other ventilation devices.

1. The employer shall assure that appropriate personal protective equipment in the appropriate sizes is readily accessible at the work site or issued to employees. Hypoallergenic gloves shall be readily accessible to those employees who are allergic to the gloves normally provided.

2. The employer shall provide for the cleaning, laundering or disposal of personal protective equipment.

3. The employer shall repair or replace required personal protective equipment as needed to maintain its effectiveness.

4. Gloves shall be worn when the employee has the potential for the hands to have the direct skin contact with blood, other potentially infectious materials, mucous membranes, non-intact skin, and when handling items or surfaces soiled with blood or other potentially infectious material.

a. Disposable (single-use) gloves such as surgical or examination gloves shall be replaced as soon as possible when visibly soiled, torn, punctured or when their ability to function as a barrier is compromised. They shall not be washed or disinfected for re-use.

b. Utility gloves may be disinfected for re-use if the integrity of the glove is not compromised, however, they must be discarded if they are cracked, peeling, discolored, torn, punctured, or exhibit other signs of deterioration.

5. Masks and eye protection or chin-length face shields shall be worn whenever splashes, spray, spatter, droplets, or aerosols of blood or other potentially infectious materials may be generated and there is a potential for eye, nose, or mouth contamination.

6. Appropriate protective clothing shall be worn when the employee has potential for occupational exposure. The type and characteristics will depend upon the task and degree of exposure anticipated.

a. Gowns, lab coats, aprons or similar clothing shall be worn if there is a potential for soiling of clothes with blood or other potentially infectious materials.

b. Fluid resistant clothing, surgical caps or hoods shall be worn if there is a potential for splashing or spraying of blood or other potentially infectious materials.

c. Fluid-proof shoe covers shall be worn if there is a potential for shoes to become contaminated and/or soaked with blood or other potentially infectious materials.

D. Housekeeping

The work site shall be maintained in a clean and sanitary condition. All equipment, environmental enclosures and working surfaces shall be properly cleaned and disinfected after contact with blood or other potentially infectious materials.

1. Work surfaces shall be decontaminated with an appropriate disinfectant after completion of procedures; when surfaces are overtly contaminated; immediately after the spill of blood or other potentially infectious materials; and at the end of the work shift.

2. Protective coverings such as plastic wrap, aluminum foil, or imperviously-backed absorbent paper may be used to cover equipment and environmental surfaces. These coverings shall be removed and replaced at the end of the work shift or when they become overtly contaminated.

3. Equipment which may become contaminated with blood or other potentially infectious materials shall be checked routinely and prior to servicing or shipping and shall be decontaminated as necessary.

4. All bins, pails, cans, and similar receptacles intended for re-use which have a potential for becoming contaminated with blood or other potentially infectious materials shall be inspected, cleaned, and disinfected on a regularly scheduled basis and cleaned and disinfected immediately or as soon as possible upon visible contamination.

5. Broken glassware which may be contaminated shall not be picked up directly with the hands. It shall be cleaned up using mechanical means such as a brush and dust pan, tongs, cotton swabs or forceps.

6. Specimens of blood or other potentially infectious materials shall be placed in a closable, leak-proof container labeled or color-coded bag prior to being stored or transported. If outside contamination of the primary container is likely, then a second leak-proof container that is labeled or color-coded shall be placed over the outside of the first container and closed to prevent leakage during handling, storage, or transport. If puncture of the primary container is likely, it shall be placed in a leak-proof puncture-resistant secondary container.

7. Reusable items contaminated with blood or other potentially infectious materials shall be decontaminated prior to washing and/or reprocessing.

Infectious Waste Disposal
All infectious waste destined for disposal shall be placed in closable, leak-proof containers or bags that are color-coded or labeled.

1. If outside contamination of the container or bag is likely to occur then a second leak-proof container or bag which is closable and labeled or color-coded shall be placed over the outside of the first and closed to prevent leakage during handling, storage, and transport.

2. Disposal of all infectious waste shall be in accordance with procedures found in the "Biohazard/Biomedical Waste". Section

3. Immediately after use, sharps, i.e., broken glass, needles, pipettes, etc., shall be placed in closable, labeled or color-coded leak-proof, puncture resistant, disposable containers.

4. These containers shall be easily accessible to personnel and located in the area of use.

Biosafety Levels
Four biosafety levels are described which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazard posed by the infectious agents and for the laboratory function or activity.

1. Biosafety Level 1: Practices, safety equipment, and facilities are appropriate for facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. Bacillus subtilis, Naegleria gruberi, and infectious canine hepatitis virus are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, immunodeficient or immunosuppressed individuals. Vaccinia strains which have undergone multiple in-vivo passages should not be considered avirulent simply because they are vaccinia strains.

2. Biosafety Level 2: Practices, equipment, and facilities are applicable to clinical facilities in which work is done with the broad spectrum of endogenous 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 aerosols is low. Hepatitis B virus, the Salmonellae, and Toxoplasma spp. are representative of microorganism assignment to this containment level. Primary hazards to personnel working with these agents may include accidental autoinnoculation, ingestion, and skin or mucous membrane exposure to infectious materials. Procedures with high aerosol potential that may increase the risk of exposure to personnel, must be conducted in primary containment equipment or devices.

3. Biosafety Level 3: Practices, safety equipment, and facilities are applicable to facilities in which work is done with endogenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences. Autoinnoculation and ingestion also represent primary hazards to personnel working with these agents. Examples of such agents for which bio-safety Level 3 safeguards are generally recommended include Mycobacterium tuberculosis, St. Louis encephalitis virus and Coxiella burnetti.

4. Biosafety Level 4: Practices, safety equipment, and facilities are applicable to work with dangerous and exotic agents, which pose a high individual risk of life threatening disease. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel. Lassa Fever virus is representative of the microorganisms assigned to Level 4.

Work with known agents shall be conducted at the bio-safety level recommended by the Centers for Disease Control (CDC) or the National Institute of Health (NIH), unless specific information is available to suggest the virulence, pathogenicity, antibiotic resistance patterns, and the other factors are significantly altered to require more stringent or allow less stringent practices to be used.

Clinical laboratories, and especially those in health care facilities or disease diagnostic labs, receive clinical specimens with requests for clinical support services. Typically, clinical laboratories receive specimens without pertinent information such as patient history or clinical findings which may be suggestive of an infectious etiology. Furthermore, such specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputum samples submitted for "routine," acid fast, and fungal cultures).

It is the responsibility of the laboratory supervisor to establish standard procedures in the laboratory which realistically address the issue of ineffective hazard of clinical specimens. Except in extraordinary circumstances (e.g., suspected hemorrhagic fever) the initial processing of clinical specimens and identification of isolates can be and are safely conducted using a combination of practices, facilities, and safety equipment described as bio-safety level 2. Biological safety cabinets (Class I or II) should be used for the initial processing of clinical specimens when the nature of the test is requested or other information is suggestive that an agent readily transmissible by infectious aerosols is likely to be present. Class II biological safety cabinets are also used to protect the integrity of the specimens or cultures by preventing contamination from the laboratory environment.

Segregating clinical laboratory functions and limiting or restricting access to laboratory areas are the responsibility of the laboratory supervisor.

If needed, a specially designed suit area may be provided in the facility. Personnel who enter this area wear a one piece positive pressure suit that is ventilated by a life support system. The life support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from suit area is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area are sealed. A double door autoclave is provided for decontaminating waste materials to be removed from the suite area.

Here at UCC, the area of biological safety is under the guidance of the Biological Safety Committee (BSC) and the Biological Safety Officer. Research involving Biological Safety Levels III or IV shall contact the Biological Safety Committee for appropriate authorization, guidelines and inspections. For Biological Safety Levels I or II, researchers are strongly encouraged to work with the Committee for guidelines and inspections. All biological safety cabinets shall be certified and inspected by the Committee.

Procedures for Working with Human Blood or Other Potentially Infectious Material

1. Departments with employees who have occupational exposure to blood or other potentially infectious material must develop an Exposure Control Plan in compliance with the OSHA Bloodborne Pathogen Standard.

2. Universal precautions shall be observed at all times. Universal precautions apply to blood, any other body fluid containing visible blood, and other potentially infectious material.

a. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials.

b. Universal precautions do not apply to feces, nasal secretions, sputum, saliva, sweat, tears, urine, or vomitus unless they contain visible blood.

c. Other potentially infectious materials means the following human body fluids:

(1) semen,
(2) vaginal secretions,
(3) pericardial fluid,
(4) cerebrospinal fluid,
(5) synovial fluid,
(6) pleural fluid,
(7) peritoneal fluid,
(8) amniotic fluid,
(9) saliva in dental procedures,
(10) any body fluid that is visibly contaminated with blood,
(11) all body fluids in situations where it is difficult or impossible to differentiate between body fluids,
(12) any unfixed tissue or organ (other than intact skin) from a human, living or dead,
(13) human immunodeficiency virus (HIV)-containing cell or tissue cultures, organ cultures, and HIV or hepatitis B virus (HBV)-containing culture medium or other solutions, and
(14) blood, organs, or other tissues from experimental animals infected with HIV, HBV, or other diseases infectious to humans.

3. Employees must wash their hands immediately or as soon as feasible after removal of gloves or other personal protective equipment, and following contact with blood or other potentially infectious materials.

4. Contaminated needles or other contaminated sharps must not be recapped, sheared, bent, broken or resheathed by hand. Contaminated sharps must be placed in appropriate containers until properly reprocessed or disposed.
These containers shall be:

a. puncture resistant,

b. labeled or color-coded, and

c. leak-proof on the sides and bottom.

5. Specimens of blood or other potentially infectious materials should be placed in a labeled or color-coded container which prevents leakage during collection, storage, transport, or shipping. A secondary container must be used if the primary container is contaminated, punctured or leaking.

6. Equipment that has been in contact with blood or other potentially infected material must be examined and decontaminated by laboratory personnel as necessary prior to servicing or shipping.

7. If the equipment cannot be completely decontaminated, a readily observable label must be attached to the contaminated equipment and all parties who will be in contact with the equipment should be notified.

8. Gloves must be worn when there is potential for contact with blood, or other potentially infectious materials. Disposable (single use) gloves such as surgical or examination gloves must be replaced as soon as possible when visibly soiled, torn, punctured, or when their ability to function as a barrier is compromised.

9. Additional appropriate protective clothing should be selected and worn based upon the task and degree of exposure anticipated.

a. Gowns, laboratory coats, aprons or similar clothing should be worn if there is a potential for soiling of clothes with blood or other potentially infectious materials.

b. Fluid-resistant clothing should be worn if there is a potential for splashing or spraying of blood or other potentially infectious materials.

c. Surgical caps or hoods should be worn if there is a potential for splashing or spraying of blood or other potentially infectious materials.

d. Fluid-proof shoe covers should be worn if there is a potential for shoes to become contaminated and/or soaked with blood or other potentially infectious materials.

10. Work surfaces must be decontaminated with an appropriate disinfectant after completion of procedures; when surfaces are overtly contaminated; immediately after the spill of blood or other potentially infectious materials; and at the end of the work shift.

a. Appropriate germicidals include:

(1) EPA-registered "hospital disinfectant" chemical germicides that have a label claim for

tuberculocidal activity, and

(2) commercially available hard-surface germicides or solutions containing at least 500 parts per million free available chlorine (a 1:100 dilution of common household bleach - approximately ¼ cup of bleach per gallon of tap water).

b. For routine housekeeping or removal of soiling in the absence of visible blood contamination, EPA- registered "hospital disinfectants" (no label claim for tuberculocidal activity required) can be used.

c. Environmental surfaces such as floors, woodwork, or countertops that have become soiled, should be cleaned and disinfected using any cleaner or disinfectant agent that is intended for environmental use.

11. All bins, pails, cans, and similar receptacles intended for reuse that have a potential for becoming contaminated with blood or other potentially infectious materials should be inspected, cleaned, and disinfected on a regularly scheduled basis and cleaned and disinfected immediately or as soon as possible upon visible contamination.

12. Broken glassware that may be contaminated must not be picked up directly with the hands. It shall be cleaned up using mechanical means such as a brush and dustpan, a vacuum cleaner, tongs, cotton swabs or forceps.

13. Research and production facilities engaged in the culture, production, concentration, experimentation, and manipulation or HIV and HBV are to carry out their procedures at Biosafety Level 3. Written policies and procedures are to be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures are allowed to enter the work areas and animal rooms.

14. Employees working in HIV or HBV research laboratories and HIV or HBV production facilities are to receive the following initial training. This training must be documented.

a. Employees must demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the facility before being allowed to work with HIV or HBV.

b. Employees are to have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

c. A training program must be provided to employees who have no prior experience in handling human pathogens. Initial work activities must not include the handling of infectious agents. A progression of work activities is to be assigned as techniques are learned and proficiency is developed. Employees are to participate in work activities involving infectious agents only after proficiency has been demonstrated. Employees must watch a training video before starting work on a new technique.

EFFECTIVE USE OF BIOLOGICAL SAFETY CABINETS

Exposure to airborne microorganisms can result in infection of laboratory workers or contamination of research materials. Biomedical engineering and technology have provided safeguards, but these safeguards do not prevent mistakes or human errors. Danger to personnel and to the success of scientific investigation from carelessly or improperly used equipment cannot be overly emphasized.

The Laminar Flow Biological Safety Cabinet, designed to prevent escape of pathogens into the workers' environment and to bar contaminants from the research work zone, is a key element to safe, successful experimentation with biological materials. Escape of pathogens into the workers' area is prevented by an air barrier at the front opening and the cleaning action of the exhaust air filter. Inward flow of room air into the front air intake grill creates the air barrier. The amount of air drawn into the air intake grill and the amount of air exhausted through the exhaust filter are equal. The exhaust filter removes airborne biological contaminants which may be released in the cabinet. It does not remove chemical or radiological contaminants.

Contamination of the work area inside the cabinet is prevented by the cleaning action of the supply filters. Air flows through the cabinet work area in a downward direction at a uniform velocity. The air continues to be recirculated by the fan through the air flow plenum. Airborne biological contaminants are removed by the filters as the air is returned to the cabinet work area.

Certification and advance planning are of prime importance to safe operation. Only qualified personnel using approved test methods and equipment should provide performance certification at initial installation, after maintenance, and on an annual basis thereafter. Certification is also necessary after the cabinet has been moved and after filters have been replaced. Many cabinets have gauges to indicate pressure differential across the supply filters. If the filters must be replaced, the cabinet MUST be decontaminated first. This is the responsibility of the researcher to do or have done by a qualified contractor. Procedures must follow those outlined in the National Sanitation Foundation Standard Number 49. After decontamination, only qualified Site Support personnel should replace filters. Fan speed must also be readjusted by qualified maintenance technicians.

Deanship of Administration maintains a list of firms specializing in the decontamination and certification of biological safety cabinets. It is the responsibility of individual researchers and/or departments to ensure this process is accomplished at least annually. If your biological safety cabinet has not been certified, contact EHS for a list of names and phone numbers of certification agencies.

In a survey performed by a cabinet manufacturer, 65 of 100 cabinets failed to pass filtration system leak tests. The operators of these cabinets were unaware of the malfunction. Maximum safety and full use of the cabinet can be best achieved by adequate advanced planning. Ideally, advanced planning should follow a procedural check list to anticipate equipment, apparatus, media, order of events and the many other details necessary for the completion of the assignment.

When planning is completed, start-up procedures may be initiated. There are three start-up steps:

1. Turn on the lights,

2. Check the air intake and exhaust grill to make sure they are unobstructed

3. Turn on the fan

Allow the fan to operate a minimum of five minutes before manipulations are begun in the cabinet. In addition, the following points should be considered:

1. Some cabinets are equipped with ultraviolet light. These must be turned off during the day while laboratory personnel are occupying the room.

2. Hands and arms should be washed well with germicidal soap before and after work in the cabinet.

3. Technicians are encouraged to wear long-sleeve gowns with knit cuffs and rubber gloves. This minimizes the shedding of skin flora into the work area and protects the hands and arms from contamination by viable agents.

4. Interior surfaces of the work area should be disinfected by wiping them thoroughly with 70% alcohol.

5. The cabinets should not be overloaded. Everything needed for the complete procedure should be placed in the cabinet before starting so that nothing passes in or out through the air barrier until the procedure is completed.

6. Do not place anything over the front intake or rear exhaust grill in units having a solid work surface. As a general rule, keep equipment at least four inches inside the cabinet window and perform transfer of viable materials as deeply into the cabinet as possible.

7. After all materials have been placed in the cabinet, wait 2-3 minutes before beginning work. This will allow sufficient time for the cabinet air to purge airborne contamination from the work area.

8. Hold the activity in the room to a minimum. Unnecessary activity may create disruptive air currents. The ideal location for a cabinet is in a quiet end of the laboratory, removed from doorways, air conditioning and heating vents. Opening and closing laboratory doors can cause disruptive drafts that allow microorganisms to penetrate the air barrier.

9. Schedule uninterrupted work periods. The movement of objects including hands and arms causes turbulent air currents that disrupt the air barrier and allow escape and entrance of airborne contaminants.

10. Air turbulence caused by rotating laboratory equipment, such as a small clinical centrifuge, disrupt airflow within the cabinet and at the work opening. This is sufficient for contaminated air to escape to the laboratory environment. If a centrifuge must be used in the cabinet, do not perform other research activities in the cabinet while the centrifuge is operating.

11. Normal laboratory contamination control procedures and aseptic techniques are still necessary while working in the biological safety cabinet.

12. Equipment in direct contact with the biological agent should not be removed from the cabinet until enclosed or until the surface is decontaminated. Trays of discarded pipettes and glassware must be covered before removal from the cabinets.
13. If an accident occurs which spills or splatters the biological agent in the work area, all surfaces in the cabinet must be surface decontaminated before being removed.

14. Do not use a Bunsen Burner in a biological safety cabinet. The flame causes turbulence in the air stream and the heat generated may damage the HEPA filter. If a procedure requires the use of a flame, a burner with a pilot light should be used. It should be placed to the rear of the workspace where resulting air turbulence will have a minimal effect.

15. Do not mouth pipette.

16. Following completion of the work, the following steps must be performed:

a. Allow the cabinet to run 2-3 minutes with no activity. This will allow sufficient time for cabinet airflow to purge airborne contaminants from the work area;

b. Decontamination of the interior surfaces should be repeated after removal of all materials, cultures, apparatus, etc. A careful check of the work area should be made for spilled or splashed nutrients. They may support fungus growth and result in spore liberation that contaminates the protected work environment; and

c. Shut down by turning off the fan and lights. Use UV lights according to manufacturer's recommendations. Do not use the cabinet to store excess laboratory equipment.

BIOHAZARD WASTE

The following information regarding biohazard waste is being provided to eliminate any misunderstandings about the requirements for proper disposal of biohazard wastes. A full copy of the Puerto Rico Department of Health regulations is available from the University Biological Safety Officer and will be provided upon request. If you do not currently have satisfactory arrangements for the proper disposal of your biohazard wastes, please contact the University Biological Safety Officer for assistance.

A. Biohazard Wastes

are discarded materials "that are biological agents or conditions (as an infectious organism or unsecure laboratory condition) that constitutes a hazard to humans or their environment." This definition includes "any and all substances which contain materials to which organisms may cause injury or disease to humans or their environment, but which are not regulated as controlled industrial waste".

B. Infectious Wastes include the following categories:

- cultures and stocks of infectious agents and associated biologicals;

- human blood and blood products,

- pathological wastes,

- contaminated sharps,

- contaminated animal carcasses, body parts, and bedding,

- wastes from surgery, necropsy and other medical procedures,

- laboratory wastes,

- isolation wastes, unless determined to be non-infectious by the infection control committee at the health care facility,

- any other material and contaminated equipment which, in the determination of the facility infection control staff, presents a significant danger of infection because it is contaminated with, or may reasonably be expected to be contaminated with, etiologic agents.

C. Chemical Wastes:

These are subject to the requirements of biohazard waste regulations include wastes from the following categories:

- pharmaceutical wastes,

- laboratory reagents contaminated with infectious body fluids,

- all the disposable materials which have come into contact with cytotoxic/antineoplastic agents during the preparation, handling, and administration of such agents, and

- other chemicals that may be contaminated by infectious agents, as designated by experts at the point of generation of the waste.

D. Treated Biohazard Wastes:
Include all biohazard wastes that have been treated by one of the following methods and rendered harmless and biologically inert:
- incineration in an approved incinerator,

- steam sterilization at sufficient time and temperature to destroy infectious agents in the waste ("autoclaved"),

- chemical disinfection where contact time, concentration, and quantity of the chemical disinfectant are sufficient to destroy infectious agents in the waste, and

- any other method approved by the Oklahoma State Department of Health and is generally recognized as
effective.

E. Sharps:
Are used in animal or human patient care or treatment or in medical research, or industrial laboratories, including: hypodermic needles, syringes, (with or without the attached needle), pasteur pipettes, scalpel blades, suture needles, blood vials, needles with attached tubing, and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.

All sharps intended for disposal, whether contaminated or not, shall be enclosed in a sharps container. Recapping needles is dangerous and shall be avoided. Treat syringes as you would a controlled substance. It is recommended that all unwanted syringes be destroyed after disinfection but before disposal in the solid waste stream. Destroying an infectious sharp or syringe before disinfection could spread contamination. Special consideration should also be given to the disposal of contaminated pipettes.

Never clip or recap needles before putting them in the sharps container. The sharps container should be puncture-resistant, leak proof on the sides and bottom, and color-coded or labeled with the biohazard symbol. When selecting sharps containers, look for special safety features such as lids that lock tight for safe disposal, a container that can be sterilized by steam, gas, or chemicals, and a clear top that would allow inspection. If sharps containers are not specifically constructed to be autoclaved, the resulting mass of melted plastic is extremely hazardous due to the needles that often protrude.

F. Guidelines for Disposal

1. If any infectious waste is also a chemical waste, call the Chemical Safety Officer for assistance with disposal after disinfection. Antineoplastic/cytotoxic agents require special disposal.

2. Biomedical wastes that are also radioactive should be treated according to requirements for both biomedical and radioactive waste.

3. Prior to any treatment, all biomedical wastes, including those to be incinerated, should be enclosed in a
puncture-resistant, red biohazard bag that is color-coded or labeled with the biological hazard symbol.

4. Untreated biomedical waste is not to be disposed of in the municipal waste stream. All biomedical waste, including sharps and syringes, must be treated by incineration, steam sterilization, or chemical disinfection before disposal in the municipal waste stream.

5. After disinfection, but before disposal in the municipal waste stream, all treated biomedical wastes should be enclosed in an unmarked outer bag that is not red or labeled with the biohazard symbol. Any biomedical waste that has been treated as described above and packaged such that it is clearly evident that the waste has been effectively treated, is not subject to regulation as biomedical waste and may be collected, transported, and disposed of as municipal waste.

BIOHAZARD SPILLS

1. Spills on the Body

a. Remove contaminated clothing.
b. Vigorously wash exposed area with soap and water for one minute.
c. Obtain medical attention (if necessary).
d. Report the incident to the laboratory supervisor.

2. Biosafety Level 1 Organism Spill

a. Wear disposable gloves.
b. Soak paper towels in disinfectant and place over sill.
c. Place towels in a plastic bag for disposal.
d. Clean up spill area with fresh towels soaked in disinfectant.

3. Biosafety Level 2 Organism Spill

a. Alert people in immediate area of spill.
b. Put on protective equipment. This may include a laboratory coat with long sleeves, back-fastening gown or jumpsuit, disposable gloves, disposable shoe covers, safety goggles, mask or full-face shield.
c. Cover spill with paper towels or other absorbent materials.
d. Carefully pour a freshly prepared 1 to 10 dilution of household bleach around the edges of the spill and then into the spill. Avoid splashing.
e. Allow a 20-minute contact period.
f. After the spill has been absorbed, clean up the spill area with fresh towels soaked in disinfectant.
g. Place towels in a plastic bag and decontaminate in an autoclave.

B. Blood Spills

1. General Information

a. Universal precautions must be observed. Refer to the Campus Blood Borne Pathogens Plan or Departmental Exposure Control Plan for more information. Cleaning of blood spills should be limited to those persons who are trained for the task.
b. If an untrained person encounters a spill, he/she should limit access to the area and immediately call the person(s) assigned to this duty.
c. Only disposable towels should be used to avoid the difficulties involved in laundering.
d. If a spill involves broken glassware, the glass should **never** be picked up directly with the hands. It must be cleaned up using mechanical means, such as a brush and dustpan, tongs, or forceps.

2. Personal Protective Equipment

a. Persons who clean blood spills should wear disposable gloves of sufficient strength so they will not tear during cleaning activities. If the gloves develop holes, tears, or splits, remove them, wash hands immediately, and put on fresh gloves. Disposable gloves must never be washed or reused. Remove gloves one at a time by grasping the wrist opening and pulling toward the fingers so that the gloves come off as inside out. Double-bag gloves with other contaminated biomedical waste (such as towels).

b. If enough blood has been spilled to expect splashing during cleaning, additional protective equipment may be required

3. Disinfectants

Read and follow all manufacturer's handling instructions. All spills of blood and blood-contaminated fluids should be properly cleaned using any of these three disinfectants:

a. EPA-registered "hospital disinfectant" chemical germicides that have a label claim for tuberculocidal activity. These are chemical germicides that are approved for use as hospital disinfectants and are tuberculocidal when used at recommended dilutions.
b. Products registered by the Environmental Protection Agency as being effective against human immunodeficiency virus (HIV).
c. A solution of 5.25 percent sodium hypochlorite (household bleach) diluted between 1:10 and 1:100 with water (a 1:100 dilution of common household bleach yields 500 parts per million free available chlorine - approximately ¼ cup of bleach per gallon of tap water).

4. Cleaning Blood Spills on Hard Surfaces

To assure the effectiveness of any sterilization or disinfection process, surfaces must first be thoroughly cleaned of all visible blood or soil before a germicidal chemical is applied for disinfection.

a. Isolate the area, if possible.
b. Wear gloves and other protective apparel as needed.
c. Remove visible blood with disposable towels in a manner that will ensure against direct contact with the blood. For example, put towels over the spill to absorb the liquid.
d. Place contaminated towels in a plastic waste disposal bag.
e. The area should then be decontaminated with an appropriate germicide applied according to manufacturer's directions.
f. All contaminated towels and gloves should be double-bagged for disposal and labeled with the biohazard symbol.

5. Cleaning Blood Spills on Carpeting

Use only a registered germicide. Read and follow manufacturer's instructions. Do not use chlorine bleach solution on carpet.

a) Isolate the area--if possible.
b) Wear gloves and other appropriate apparel.
c) Procedures for small spills on carpets (smaller than a quarter) are as follows.
    i) Soak the spill with enough disinfectant to cover the spot.
    ii) Let dry at least overnight to ensure that the spot is disinfected.
    iii) Shampoo carpet, if needed, or use 3% hydrogen peroxide to remove discoloration.
d) Procedures for larger spills are as follows.
    i) Pour disinfectant on the spot and let stand at least 30 minutes to allow some disinfection to take place. Blot up excess liquid with disposable towels.
    ii) Soak the area with additional disinfectant. Allow to dry overnight. Shampoo carpet, if needed, or use 3% hydrogen peroxide to remove discoloration.
e) All contaminated towels and gloves should be double-bagged and labeled with the biohazard symbol.

C. Cytotoxic/Antineoplastic Spills

1. General Procedures

a. Spills and breakages of cytotoxic/antineoplastic drugs (CDs) should be cleaned up immediately by a properly trained person using the appropriate procedures.
b. Broken glass should be carefully removed.
c. A spill should be identified with a warning sign so that other persons in the area will not be contaminated.

2. Personnel Contamination

Overt contamination of gloves or gowns, or direct skin or eye contact should be treated as follows.

a. Immediately remove the gloves or gown.
b. Wash the affected skin area immediately with soap (not germicidal cleanser) and water. For eye exposure, immediately flood the affected eye with water or isotonic eyewash designated for the purpose for at least five minutes.
c. Obtain medical attention immediately.

3. Clean-up of Small Spills

Spills of less than 5 ml or 5 gm outside a hood should be cleaned immediately by personnel wearing gowns, double surgical latex gloves, and eye protection.

a. Liquids should be wiped with absorbent gauze pads, solids should be wiped with wet absorbent gauze.
b. The spill areas then should be cleaned (three times) using a detergent solution followed by clean water.
c. Any broken glass fragments should be placed in a small cardboard or plastic container and then into a CD disposal bag, along with the used absorbent pads and any non-cleanable contaminated items.
d. Reusable glassware or other contaminated items should be placed in a plastic bag and washed in a sink with detergent by a trained employee wearing double surgical latex gloves.

4. Clean-up of Large Spills

For spills of amounts larger than 5 ml or 5 gm, the spread should be limited by gently covering with absorbent sheets of spill-control pads or pillows or, if a powder is involved, with damp cloths or towels. Be sure not to generate aerosols. Access to the spill areas should be restricted.

a. Protective apparel should be used with the addition of a respirator when there is any danger of airborne powder or an aerosol being generated. The dispersal of CD particles into surrounding air and the possibility of inhalation is a serious matter and should be treated as such.
b. Chemical inactivators, with the exception of sodium thiosulfate, which can be used safely to inactivate nitrogen mustard, may produce hazardous by-products and should not be applied to the spilled drug.
c. All contaminated surfaces should be thoroughly cleaned with detergent solution and then wiped with clean water. All contaminated absorbents and other materials should be disposed of in the CD disposal bag.

5. Spills in Hoods

If the spill occurred in either a glove box, clean bench or biological safety cabinet, the HEPA filter (contained in the cabinet) is more than likely contaminated. Label the unit "Do Not Use--Contaminated With (name of substance)."

The HEPA filter and filter cabinet must be decontaminated and the filter changed and properly disposed of.

This procedure may require the services of an outside contractor trained in the use of specialized personal protective equipment.

6. Spill Kits

Spill kits, clearly labeled, should be kept in or near preparation and administrative areas. It is suggested that kits include a respirator, chemical splash goggles, two pairs of gloves, two sheets (12x12) of absorbent material, 250 ml and one liter spill control pillows and a small scoop to collect glass fragments. Absorbents should be suitable for incineration. Finally, the kit should contain two large CD waste-disposal bags.

INSTITUTIONAL BIOLOGICAL SAFETY (RECOMBINANT DNA RESEARCH)

It is the policy of this University that the PI is responsible for complying with the NIH "Guidelines for Research involving Recombinant DNA Molecules", regardless of the source of the funds supporting that research. There are three groups of experiments that probably encompass the majority of work being done on campus. If your work does not fall clearly into one of these groups, consult the NIH Guidelines, by clicking on "Recombinant DNA" at the website http://web.mac.com/uccresearch/UCCRESEARCH/Research_Committees.html

A. Experiments Requiring Prior Approval

The following experiments require prior approval from either the NIH, Recombinant DNA Advisory Committee (RAC), Food and Drug Administration, and/or the IBC:

a. Gene transfer experiments in humans;

b. Genes for toxins lethal for vertebrates;

c. Release of genetically engineered organisms to the environment;

d. Those using human or animal pathogens (biosafety level 2 and higher) as host-vector systems, including adenovirus vectors and murine retroviruses that infect human cells;

e. Cloning DNA from human or animal pathogens (biosafety level 2 and higher) into a non-pathogen host-vector system;

f. Cultures of more than 10 liters; and

g. Experiments involving whole plants or animals, including transgenic organisms.

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c), Minor Actions).

B. Responsibilities of the Institutional Biosafety Committee

1. Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the NIH Guidelines as specified in Section III, Experiments Covered by the NIH Guidelines, and approving those research projects that are found to conform with the NIH Guidelines. This review shall include: (i) independent assessment of the containment levels required by the NIH Guidelines for the proposed research; (ii) assessment of the facilities,

procedures, practices, and training and expertise of personnel involved in recombinant DNA research; and (iii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements required by the NIH Guidelines.

2. Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

3. Lowering containment levels for certain experiments as specified in Section III-D-2-a, Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host- Vector Systems.

4. Setting containment levels as specified in Sections III-D-4-b, Experiments Involving Whole Animals, and III-D-5, Experiments Involving Whole Plants.

5. Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the NIH Guidelines.

6. Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

7. Reporting any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/ORDA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892- 7010, (301) 496-9838.

8. The Institutional Biosafety Committee may not authorize initiation of experiments not explicitly covered by the NIH Guidelines until NIH (with the advice of the RAC when required) establishes the containment requirement.

9. Performing such other functions as may be delegated to the Institutional Biosafety Committee under Section IV-B-2, Institutional Biosafety Committee.

C. Responsibilities of the Principal Investigator (PI)

On behalf of the institution, the Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research.

1. Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections III-A, III-B, III-C, III-D, and III-E, Experiments Covered by the NIH Guidelines) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the NIH Guidelines.

2. Determine whether experiments are covered by Section III-E, Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation, and ensure that the appropriate procedures are followed.

3. Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to NIH/ORDA within thirty days. Reports are to be sent to the Institutional Biosafety Committee, Health and Safety Office, CB# 1650 and to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838.

For reporting serious adverse events for projects involving human gene therapy see Appendix M-VII-C below.

4. Report any new information bearing on the NIH Guidelines to the Institutional Biosafety Committee and to NIH/ORDA (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838).

5. Be adequately trained in good microbiological techniques.

6. Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination.

7. Comply with shipping requirements for recombinant DNA molecules.

D. Experiments Involving the Deliberate Transfer of Recombinant DNA or DNA or RNA Derived from Recombinant DNA into One or More Human Subjects.

Note: Consult the NIH website for the latest revisions in the Guidelines.

Research proposals involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer) will be considered through a review process involving both NIH/ORDA and Recombinant DNA Advisory Committee (RAC). Investigators shall submit relevant information on the proposed human gene transfer experiments to NIH/ORDA. Submission of human gene transfer protocols to NIH will be in the format described in Appendix M-I, Submission Requirements -- Human Gene Transfer Experiments. Submission to NIH/ORDA shall be for registration purposes and will ensure continued public access to relevant human gene transfer information in compliance with the NIH Guidelines. Investigational New Drug (IND) applications should be submitted to FDA in the format described in 21 CFR, Chapter I, Subchapter D, Part 312, Subpart B, Section 23, IND Content and Format.

Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects.

Recombinant DNA Advisory Committee (RAC) prefers that submission to NIH/Office of Recombinant DNA Activities (ORDA) in accordance with Appendix M-I, Submission Requirements --Human Gene Transfer Experiments, contain no proprietary data or trade secrets, enabling all aspects of the review to be open to the public. Following receipt by NIH/ORDA, relevant information shall be entered into the NIH human gene transfer database for registration purposes. Summary information pertaining to the human gene transfer protocol will be forwarded to RAC members. NIH/ORDA summary information shall include comparisons to previously registered protocols. Specific items of similarity to previous experiments include (but are not limited to): (i) gene delivery vehicle, (ii) functional gene, (iii) marker gene, (iv) packaging cell (if applicable), (v) disease application, (vi) route of administration, and (vii) patient selection criteria.

RAC members shall notify NIH/ORDA within 15 working days if the protocol has been determined to represent novel characteristics requiring further public discussion. Full RAC review of an individual human gene transfer experiment can be initiated by the NIH Director or recommended to the NIH Director by: (i) three or more RAC members, or (ii) other Federal agencies. An individual human gene transfer experiment that is recommended for full RAC review should represent novel characteristics deserving of public discussion. RAC recommendations on a specific human gene transfer experiment shall be forwarded to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate.