UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MEDICAS
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS

OFICINA DEL RECTOR
OFFICE OF THE CHANCELLOR

COMITE DE BIOSEGURIDAD
BIOSAFETY COMMITTEE

September 1, 2016

To whom it may concern

The Institutional Biosafety Committee hereby certify that the following documents are under review:

- Biomedical Waste Management Plan
- Chemical Hygiene Plan
- Biosafety Manual

The revision of these documents is also performed to comply with the changes in regulations of the local and states agencies. However, this documents will be effective until the new changes are incorporated in the manual. All these manuals are available at the Medical Sciences Campus web page (www.rcm.upr.edu) under the research section and in the Regulatory Committees

If there is any question or require further clarification, please contact me at 787-758-2525 extension 5509 or 5510, or at my email address at joseph.bloom@upr.edu

Sincerely,

Joseph Bloom, Ph.D.
Chairperson
Institutional Biosafety Committee (IBC)

“This manual is under review in order to comply with the changes in regulations of the local and states agencies. However, this document will be effective until the new changes are incorporated in the manual”.

UNIVERSITY OF PUERTO RICO
MEDICAL SCIENCE CAMPUS

Biosafety Manual

José R. Carlo, MD, FAAN
Chancellor

2007

PREFACE

The University of Puerto Rico (UPR) Biosafety Manual (BSM) outlines the policies and procedures concerning the procurement and use of biological infectious materials at the Medical Science Campus.

The intent of the Medical Science Campus Safety Committee is to facilitate the conduct of all work with biological infectious materials while observing Federal Regulations designed to eliminate needless exposures to infectious materials and needless contamination of the working environment.

The ultimate responsibility for the safe handling and use of biological infectious materials is in the hands of the individual user and this manual is intended to give him or her guidance based on the accumulated past experience in the field.

José R. Carlo, MD, FAAN
Chancellor

Julio A. Lavergne, Ph.D.
Biosafety Committee Chairperson

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BioSafety Committee Members:

Date: September 1, 2004

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Expiration Date: January 1, 2013

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CHAPTER 1, General

1.1 Purpose and Scope

This manual describes policies and procedures for use of infectious material at the UPRMSC. The provisions of this manual are applicable to all users. The Chancellor, the BioSafety Committee and the BioSafety Office have approved this manual for implementation. Every attempt has been made to include sufficient information and details to enable safe use of infectious material by simply following its provisions. It is impossible, however, to anticipate all possible circumstances and situations. In addition, Federal Regulations may change from time-to-time. Therefore, readers are advised to contact the BioSafety Officer with any questions or concerns.

CHAPTER 2, BioSafety Management

2.1 Management

It is the responsibility of Chancellor to assure that the BioSafety Program is properly funded, staffed and to oversee the Program. It is the responsibility of the BioSafety Officer and BioSafety Committee to notify the management if the BioSafety Program is deficient in any area. The Chancellor is ultimately responsible for the program.

2.2 BioSafety Committee

The Institutional BioSafety Committee (IBC) establishes policies for the safe use of infectious material at the UPRMSC in order to assure that infectious exposures and contamination to workers, students, and the public are kept to a minimum.

One specific responsibility is to evaluate all applications from new users or to re-approve users of infectious material and on any other matters concerning biosafety. The Committee has the authority to curtail or prohibit the use of infectious material and recommend to the chancellor the freezing of external funding by anyone who is: (a) misusing infectious material; (b) violating the terms of any infectious material license, registration, or regulation; or (c) otherwise creating unsafe conditions.

Individuals who plan to use infectious materials must submit proposals and appropriate forms to the IBC. Any use of infectious material which is judge to be used in a hazardous manner to personnel, patients, or the environment, will not be approved. The applicant must receive approval prior to ordering, receipt and usage of any infectious material. The Chairperson of the Committee and BioSafety Officer may give temporary approval to a qualified applicant for grant application(s) only until the Committee gives it's approval during its next Committee meeting.

The Committee reviews and revises biological health and safety aspects of requests for use of infectious material in the context of the investigator's proposal.

In addition, the Committee will review and revises the BioSafety Protection Program as needed. The Committee meets as often as is necessary to conduct its business, usually once a month. Members may also be contacted and polled at any time concerning specific issues. A quorum must consist of at least one-half of the membership, which must include a person from Management, BioSafety Chairperson, faculty members with expertise in chemicals, recombinant DNA, microbiology, clinical research, a representative of the Animal Care Committee, the BioSafety Officer, and the Compliance Officer. The BioSafety Office will maintain the approved minutes of these meetings.

2.3 BioSafety Officer

The BioSafety Officer (BSO) has the responsibility to ensure that the UPRMSC complies with the conditions of it's BioSafety Program and other Federal Regulations designed to eliminate needless

exposures to infectious material and needless contamination to the working environment. The BioSafety Office derives authority from the UPRMSC Chancellor and is responsible to the IBC regarding implementation of infectious protection measures and control within the Medical Campus.

Responsibilities of the Biosafety Officer include:

A. Implementing and maintaining infectious protection program. This requires periodic inspections to ensure that laboratory standards are rigorously followed.
B. Reporting to the IBC and the institution any significant problems, violations to the NIH guidelines, and any significant research-related accidents or illnesses of which the BSO becomes aware unless the BSO determines that a report has already been filled by the Principal Investigator.
C. Controlling the purchase, receipt, distribution, use, and proper disposal of infectious materials used under approved protocols.
D. Providing additional training to personnel related to laboratory security, infectious protection procedures, blood borne pathogens, use of chemicals, and recombinant DNA.
E. Develop emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving chemicals, biological agents, and recombinant DNA

F. Provide professional support to the Principal Investigator regarding use, disposal of infectious materials, and other research safety procedures.
G. Follow the recommendations listed Centers for Disease Control and Prevention and the National Institutes of Health.

Responsibilities of the Biosafety Office include:

A. Maintaining copies of the BSC minutes, records from all the MSC investigators and correspondence necessary to insure compliance with government regulations.

B. Review appropriate person protective equipment, including devices and biological safety cabinets to insure compliance.

C. Conducting routine surveys of areas approved for infectious material usage.

D. Supervising and/or review biological emergencies and special operations.

E. Ensure proper disposal of infectious waste using sound practices.

2.4 Principal Investigators (see Appendix A for application)

The Principal Investigator (PI) is the individual in whose name and on whose qualifications permit the use of infectious material. These qualifications are:

a. A doctoral degree, in the biological sciences or in a related field.

b. At least 40 hours of classroom training and three years working experience-using infectious materials. The three years may be shortened by the BSC if the committee feels the applicant has received enough training to properly handle infectious material, understand the characteristics of infectious material, and the biological hazards of exposure to infectious material appropriate to the type and forms of material to be used.

c. If an applicant has prior approval from another university to use infectious materials, a letter is needed from that facility stating the infectious material(s) and if possibly the number of years that the applicant was authorized to use the materials at that facility. This letter will be used to document training and experience of the applicant.

The PI is responsible for:

a. Possessing a copy of the BioSafety Manual (BSM) and having a thorough understanding of its contents.

b. Assuring that the individual users under his supervision are familiar with the contents of the BSM.

c. Complying with all Federal Regulations for the safe use and handling of infectious material as described in the BSM.

d. Instruction/training of personnel under his supervision in the safe use and handling of infectious materials. The training document must be forwarded to the BSO prior to handling infectious materials. (See Appendix I) Training must include the practices and techniques to ensure safety and procedures for dealing with accidents.

e. Instruct personnel under his supervision to attend BioSafety seminars for continuing education.

f. Direction of all personnel under his supervision to comply with all recommendation, which are designed to reduce their risk to exposure and contamination.

g. Adequately planning of an experiment or procedure to assure that proper safety precautions are taken.

h. Prior notification to the BSO regarding changes in protocol, techniques, and changes in physical location that might lead to increased personnel exposure or increased contamination in the laboratory or increased release of infectious materials to the environment.

i. Limiting the use/access of infectious materials to only those individuals authorized under that principal investigator's permit. The PI is also responsible for notifying the BSO of changes in personnel.

j. Maintaining current inventory records including receipt, use, and disposal of infectious materials.

k. Needles, syringe, or other sharp instruments should be restricted and used only when there is no alternative. Only use needle-locking syringes or disposable syringe units. Do not allow recapping of the needle.

l. Prohibit eating, drinking, smoking, and applying cosmetics in laboratories that infectious materials are stored or used.

m. Assure that laboratory coats, gloves, gowns, shoe covers, boots, respirators, face shields, safety glasses or goggles and protective clothing are worn.

n. Assure that the room(s), work areas, storage areas (hoods & refrigerators), and trash containers are properly labeled.

o. Post biological warning signs so all rooms using infectious materials.

p. Reporting spills or accidents involving infectious material immediately to the BioSafety Office and submit an Incident Report within two weeks on an appropriate form obtained from the BSM.

q. If the PI is absent for an extended time, a qualified individual must be responsible for any research that is continued in their absence. This does not relieve the PI of his/her responsibility for the regulatory requirements.

r. The design of all facilities involving the use, handling, or storage of infectious materials shall be reviewed by the IBC to assure maintenance of adequate environmental protection. New proposed procedures and techniques will be likewise reviewed.

s. Must comply with all conditions of the UPRMSC BioSafety Manual and Federal Regulations.

t. Review and if necessary update the "Yearly Inventory Form" to renew the authorization to use of radioactive materials.

u. Maintain records of laboratory personnel to ensure that laboratory personnel receive appropriate immunization or tests for the agents handled or potentially present in the laboratory.

v. When appropriate, must have access to medical surveillance monitoring. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

w. When appropriate, consider the agents(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored.

x. Each project is subject to pre-approval by the IBC and if applicable the Institutional Animal Care and Use Committee (IACUC).

y. Responsible for assessing risks to determine Biosafety level for the work that is required.

z. Make available to all laboratory staff the protocols, MSDS, etc. that describe the potential biohazards and the precautions to be taken.

aa. Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics if applicable).

2.6 Active/Inactive Status

An authorized user can apply for authorization to use infectious materials. Being placed on the active status will allow that individual to order, posses, and use biohazardous/infectious material as outlined by the biosafety manual.

An authorized user can request to be listed "Inactive" instead of terminating the use of infectious materials. The Inactive Status is used to keep your authorization for grants or for future use but eliminates the need of the record keeping, posting of the rooms, training, etc. However, upon requesting to become "Active", the authorized individual is require to comply with all the regulations prior to the order/receipt/use of infectious materials.

2.7 Termination

I. TERMINATION OF USE OF INFECTIOUS MATERIALS

The procedures listed here are primarily intended to assure the appropriate disposition of infectious materials when an application is discontinued. It is the authorized user's responsibility to initiate appropriate action to satisfy the procedures listed here prior to the time of departure.

Termination of Infectious Materials Application: Whenever an approved use of material is to be discontinued, the person responsible for its use must:

a. Notify the BioSafety Office of his/her intention to discontinue the use of infectious materials.

b. Inventory all Infectious materials on hand including all unused material and material considered waste.

c. Verify that all areas are free of infectious contamination.

The BioSafety Office upon receipt of such a notice will:

a. Conduct a survey of all laboratories in which the infectious materials have been used or stored.

b. Ensure that all infectious materials have been disposed or transfer to another authorized user.

Termination of Employment: Authorized user who are leaving the employment of the UPRMSC must:

a. Notify the BioSafety Office at least two weeks prior to their departure.

b. Inventory all biohazardous/infectious materials on hand, including all unused material and material considered waste.

c. Verify that all areas are free of infectious contamination.

The BioSafety Officer upon receipt of such a notice will:

a. Conduct a survey of all laboratories in which the infectious materials have been used or stored.

b. Assist the authorized user in making the final disposition of all infectious materials on hand.

Leave of Absence: An authorized user, who takes a leave of absence greater than two months must notify the BSO at least two weeks prior to his/her departure.

CHAPTER 3, Infectious Materials

3.1 General

In order to use biohazardous/infectious materials at the UPRMSC, all applicants must fill out an "Application For Possession and Use of Infectious Materials". (See Principal Investigators 2.4)

The BioSafety Committee (IBC) will review the applicant's proposal, qualifications and any recommendations made by the Safety Office. If satisfied that the proper precautions are to be taken, they will approve the request, binding the users to all statements represented and to this manual. If the Committee considers additional recommendations appropriate, a written condition shall be added to the applicant's authorization. This is required to evaluate for all potential hazards.

Modifications to approved authorizations shall be submitted to the BSC for approval. Authorized users will be notified by the BioSafety Office on the outcome of the Committee review of the application and/or amendment request.

The BioSafety Officer may withdraw approvals at any time if safety violations occur or use of infectious material is found not to be in compliance with conditions of the approved use, this manual, other published safety policies, or Federal Regulations. See Policy on Enforcement Actions for Items of Noncompliance (Appendix B).

Housekeeping

Because supervision of general or shared facilities is usually limited or lacking, responsibility for the condition of the room and its equipment is the responsibility of the principal investigator. The exception is the floor care, which custodians do periodically. Laboratory personnel should keep the rooms well organized and clean. Custodial personnel should not clean floors without the approval from the principal investigator. The principal investigator must arrange times that both is convenient for the laboratory staff and not hazardous to the custodians. All chemicals, biological, and radioactive materials must be secured from being accidentally disturbed or relocated by the custodians.

- Do not order more supplies than are actually needed. Do not use the floor as a storage area for supplies.
- Laboratory personnel are responsible for cleaning up spills of infectious materials from counters and floors and picking up razor blades, pipette tips, and other sharps from the floor before allowing the custodians to sweep, mop, or refinish the floors.
- If items need to be moved, the laboratory personnel must move the items to allow the custodians to perform their duties.

Maintenance personnel

Maintenance personnel should only enter laboratories when laboratory staff is present. All possibly hazards must be removed from the area before any particular work is performed.

Contact the Safety Office if items contain a chemical, biological, or radiation hazard prior to the start of work.

3.2 Caution Signs

a. The following required signs and notices could be obtained from the Safety Office.

"Caution - Biohazardous Material" - This sign is to be posted in each area where biohazardous materials are used or stored.

Labels Required for:

Storage Containers

Refrigerators/Freezers where infectious materials are stored

Fume hoods where materials are used or stored

Waste/Trash containers for infectious material

Work areas where biohazardous materials are used, stored or handled.

3.5 Use of Infectious/Biohazardous Materials

General

Prior to the use of infectious materials, the BioSafety Office may perform an initial safety inspection. This inspection will identify any equipment or facility problems, which must be resolved prior to approval for its use.

The BioSafety Office will conduct periodic safety inspections. These inspections of each facility will identify any unsafe practices and immediate action will be taken to assure correction. In extreme cases, use of infectious material within the laboratory will be suspended.

The BioSafety Office must approve all rooms that use infectious materials before infectious materials are brought into the laboratory. Also prior to leaving the UPRMSC or changing rooms a close out survey must be performed to ensure that the lab is not contaminated. The BioSafety Office will confirm that the Principal Investigator performed the close out survey.

Procedures For Minimizing Exposure and Contamination

a. All biohazardous/infectious materials are to be confined within zones with adequate boundaries and facilities suitable to prevent dispersal or exposure beyond the zone. These zones are confined to work areas within authorized rooms.

b. Personnel will use protective clothing, laboratory coats, shoe covers, gloves, etc., whenever handling biohazardous material or whenever infectious hazards exist.

c. SMOKING, DRINKING, EATING OR APPLICATION OF COSMETICS IN LABORATORIES OR WORK ROOMS IN WHICH BIOHAZARDOUS/INFECTIOUS MATERIALS ARE STORED OR USED IS PROHIBITED.

d. Pipetting by mouth is prohibited.

e. Food product containers shall not be used to hold infectious/biohazardous materials.

f. Storage of food or beverages in refrigerators, freezers, cold rooms, or laboratories marked for and/or containing biohazardous materials is prohibited. Any food or beverages found will be considered contaminated and have to be disposed of as infective waste.

g. Personal items such as purses, combs, cosmetics, etc., shall not be stored where biohazardous/infectious materials are used.

h. Telephones, papers, calculators, etc., shall not be handled if there is a possibility of contaminating them.

i. All biohazardous/infectious materials and samples shall be conspicuously labeled to include the infectious content. (see Caution Signs)

j. Unused biohazardous/infectious material and samples shall be returned to a proper storage areas when not in use. Please consult the BioSafety Office if there are questions concerning appropriate storage containers.

k. Discharging infectious material into the sanitary sewer that is prohibited.

l. Disposal of infectious material in ordinary trash is prohibited.

m. Dispose of sharp objects (syringe needles, razor blades, scalpel blades, broken glass, etc) only within puncture proof containers.

n. Do not mix items that are contaminated with uncontaminated items. Follow the Biohazard Waste Policy, (see Appendix C).

o. A catch pan of unbreakable material shall be placed under any vessel or equipment, which may leak, burst or spill a infectious material. The area of the workbench where biohazardous/infectious

liquids are used should be covered with absorbent material. The absorbent material should be changed frequently; e.g., following completion of a procedure or series of procedures.

p. Before beginning any new procedure, develop a detailed plan for carrying out the various steps. If the procedure involves hazardous risk levels, i.e. aerosol or airborne contaminates, rehearse the procedure without infectious materials before undertaking the actual experiment.

q. Sources of biohazardous/infectious materials must be secured against unauthorized removal from the place of storage. (see 3.7 Security of Areas Using Biohazardous/Infectious Material)

r. Each individual using biohazardous/infectious materials is responsible for being familiar with this BioSafety Manual and BioSafety Policies published by the BioSafety Committee or BioSafety Office. Also a copy of CDC is available for review in the BioSafety Office.

3.7 Security of Areas Using Biohazardous/Infectious Material

As required by the Federal Regulations, all areas within a laboratory in which biohazardous materials are used or stored will be conspicuously labeled with a proper sign (see Caution Signs). All areas of the department in which biohazardous materials (BL3&BL4) are stored will be locked at all times other than when under direct supervision of laboratory personnel or in direct use during an experiment.

3.8 Procurement, Receiving Biohazardous/Infectious Materials, Inventory Control, Storage and Transfer of Material

a. Procurement and Inventory Control -

All orders for biohazardous/infectious materials to be purchased through the UPRMSC shall not be processed until approved by the BioSafety Office. Different forms may be used for approval; this will be done on a case-by-case procurement. All requests should include the following: Principal Investigator's Name, Biohazardous/Infectious Material, Amount of material, and Vendor. If orders do not have all of the information listed, it will cause a delay upon approval of the material.

All orders must be delivered directly to the laboratory after approval from the BioSafety Office.

b. Receiving Biohazardous/Infectious Materials -

1. All packages destined for University must be approved by the BioSafety Office. The BioSafety Office will provide the form "Biohazardous/Infectious Material Receipt and Disposal Record". This form must be kept for receipt/disposal of the material. The internal check will be done by an authorized individual (from the laboratory) that has been properly trained by the authorized

user. The procedures in Appendix E will be followed. All documentation will be kept on the Biohazardous/Infectious Material Receipt and Disposal Form. (See Appendix E)

2. Always open Biohazardous/Infectious Material behind protective bearers inside a hood. Contents may be under pressure (especially packages on dry ice). Wear protective clothing such as gloves and a lab coat when opening packages. If gloves are a tight fit use two pairs of gloves. It is well known that gloves have small pores and when stretched the pores will open up and let infectious materials contaminate the user's hands.

3. In the event that packages are delivered after hours, the following procedures will be followed:

   a. The Authorized User will be responsibly for arranging receipt for the package.

   b. If the package is visibly damaged or leaking: the receiving person shall immediately notify the BSO or a member of the BioSafety staff and attempt to detain the carrier until it can be determined that neither the driver nor the vehicle is contaminated. If not, the person accepting the delivery shall obtain the name of the driver, a delivery company or other identifying information required.

   c. Isolate the damaged package from further handling.

   d. Keep all personnel away from the immediate vicinity of the package and under no circumstances shall anyone, other than BioSafety personnel, attempt to open the package.

c. Inventory Control

1. The BioSafety Staff (BS) will compare requisitions with database prior to the authorizing the purchase of infectious material. Any package that was not approved by the BS will not be purchased. In the event that a free sample or material is received from another university, the laboratory will notify the BS within the next business working day. The material cannot be used until the BS has been notified and authorization has been given authorization to use the material. The BS will then add the material to the database and will provide an inventory sheet for the material. If it has been determined that a package was received and the BS was not informed, the authorized individual will have their authorization suspended. The BioSafety Officer will make this determination.

2. When the biohazardous/infectious material arrives, it is the responsibility of the laboratory to perform all necessary survey(s) to the package. If for any reason the laboratory uses the material before the survey(s) has been preformed, the authorized use will be suspended.

3. The BioSafety Office provides inventory sheets to users so that the final disposition (e.g., waste disposal) of the infectious material can be reported. Users must return the inventory sheet once the material is transferred or disposed

4. The BioSafety Office maintains a database of all biohazardous/infectious material delivered to and possessed by the users. The database is referred to as the "Inventory Sheet" and each Principal Investigator will receive a copy once each year to check inventory and renew their authorization to use biohazardous/infectious materials. This database is used to assure that investigators do not exceed their limits.

5. Users may maintain their own records of use and disposal, if the laboratory transfers the final information to the inventory sheet.

d. Storage

1. Upon receipt of the package, all material must be stored in authorized rooms. (see Security of areas using biohazardous/infectious material)

c. Transfer of Biohazardous/Infectious Material -

1. Individuals must obtain the permission from the BioSafety Office to receive and/or transfer material to or from any commercial or non-commercial source. Additionally, any material that is either donated or received free-of-charge (e.g., received on a trial basis or free samples, or samples from other research facilities) must be approved prior to receipt.

2. When transferring material use the following as a guide:

   a. Use a strong, tight inner container with a secure cap or seal.

   b. Surround this inner container with enough absorbent material to contain at least twice the volume of any liquid within the container.

   c. Place the container and absorbent material into a DOT approved box. If you do not have a box, the BioSafety Office may have one. Do not seal up the box.

   d. Bring the box to the BioSafety Office with the following information:

      1. Name and address of sender
      2. Name and address of recipient
      3. A copy of the recipient license
      4. Infectious material, amount, and any special handling instructions
      5. A Federal Express account number for billing

3. The BioSafety Office will fill out the "Biohazardous/Infectious Material Shipment Form" (see Appendix J) and mail the package to the recipient.

4. Users must record any transfers on the Biohazardous/Infectious Material Inventory Form.

3.9 Biohazardous/Infectious Waste Disposal (see Appendix C)

The UPRMSC will dispose all biohazardous/infectious material. The material will be neutralized by either chemical, autoclaving, or by incineration.

CHAPTER 4, Principles of Biosafety

4.1 General

Microbiological laboratories are special and require unique work environments that may pose identifiable infectious disease risks to persons in or near them. Infections have been contracted in the laboratory throughout the history of microbiology. These infections, in some cases, have caused several fatalities. The infections that have occurred in the United States concluded that poor techniques in the handling of infectious materials attributed to employees becoming infected. The spread of contamination was through "handling of cultures or specimens, inhalation of dust or aerosols, pipetting by mouth, and the use of needles and syringes."

The BioSafety Manual (BSM) underlying principles, which seek to ensure safe practices, procedures and facilities, are applicable to the control of infectious contamination. The prudence use of the Biosafety Level 1-4 practices, procedures, and facilities described for manipulations of etiologic agents in the laboratory setting and animal facilities. Strict adherence to these guidelines does contribute to a healthier and safer work environment for laboratorians, their co-workers, and the surrounding community. To further reduce the potential for laboratory-associated infections, the guidelines presented here should be considered minimal guidance for contamination. They must be customized foe each individual laboratory and can be used in conjunction with other available scientific information.

4.2 Biological Safety Cabinets

Safety equipment (primary barriers) includes biological safety cabinets (BSCs) and other engineering controls designed to remove or minimize exposure to hazardous biological materials. The BSC is the principal device used to provide containment of the infectious splashes or aerosols generated by many microbiological procedures. The main three types are:

Class 1	Low protection to personnel and materials being manipulated inside the BSC	Opened front
Class 2	Moderate protection to personnel and materials being manipulated inside the BSC	Opened front
Class 3	Highest protection to personnel and materials being manipulated inside the BSC	Gas tight

Another type of a biological safety cabinet for non-work manipulation could be considered the centrifuge. To minimize aerosols from being released during centrifugation, centrifuge cups must be used with infectious agents that can be transmitted through the aerosol route of exposure.

Biosafety Level 1 (BSL-1)
BSL-1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and minimal potential hazard to laboratory personnel and the environment. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used.

The following standard and special practices, safety equipment and facilities apply to agents assigned to BSL-1:

A. Standard Microbiological Practices
   1. Access to the laboratory is limited or restricted.
   2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
   3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the laboratory.
   4. Mouth pipetting is prohibited.
   5. Sharps containers must be available in the laboratory.
   6. All procedures are performed carefully to minimize splashes or aerosols. This may include centrifuging, centrifuge safety cups, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers, inoculating animals intranasally, and infected tissues from animals or embryonate eggs.
   7. Work areas/surfaces are decontaminated at least once per day and after any spill of viable materials.
   8. All cultures, stocks, and other regulated waste are autoclaved before disposal or placed in a durable, leakproof container and closed for transport.
   9. A biohazard sign is placed at the entrance of the laboratory.

Animal Biosafety Level 1 (ABSL-1)

ABSL-1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and minimal potential hazard to laboratory personnel and the environment. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used.

The following additional standard and special practices must be added to BSL-1 inside the animal facility:

A. Additional Standards Microbiological Practices
   1. Only those persons required for the program or support purposes are authorized to enter the facility.
   2. Doors to animal rooms open inward, are self-closing, and are kept closed.
   3. Windows must be resistant to breakage, sealed or fitted with screens.
   4. If floor drains are provided, traps are always filed with water and/or appropriate disinfectant.
   5. Rooms are kept under negative pressure.
   6. Cages are washed before reuse.
   7. 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.
   8. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.

9. A biohazard sign must be posted on the entrance. In addition, the infectious agents in use, the name(s) and telephone numbers of contact personnel, and special requirements foe entering the room are posted.
10. The wearing of laboratory coats, gowns, and/or uniforms in the facility are required. Laboratory coats, gowns, and/or uniforms are not worn outside the facility.
11. Persons having contact with non-human primates must wear appropriate eye and face protection.

Plants Biosafety Level 1 (PBL-1)

1. Access to the green house shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.
2. Prior to entering the green house, personnel shall be required to read and follow instructions on PBL-1 greenhouse practices and procedures.
3. A record shall be kept of experiments currently in progress in the greenhouse facility.
4. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
5. A program shall be implemented to control undesired species (e.g., weeds, rodents, or arthropods pests and pathogens).
6. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the green house, precautions shall be taken to minimize escape from the greenhouse facility.
7. Experiments involving other organisms that require a containment level lower than PBL-1 may be conducted in the greenhouse concurrently provide that all work is conducted in accordance with PBL-1 greenhouse practices.

Biosafety Level 2 (BSL-2)

BLS-2 is similar to BSL-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; (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.

The following standard and special practices, safety equipment and facilities apply to agents assigned to BSL-2:

A. Standard Microbiological Practices
    1. Work is confined to Class I biological safety cabinets.
    2. Access to the laboratory is controlled. Lockable doors are required.
    3. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

4. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the laboratory.
5. Mouth pipetting is prohibited.
6. All procedures are performed carefully to minimize splashes or aerosols. This may include centrifuging, centrifuge safety cups, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers, inoculating animals intranasally, and infected tissues from animals or embryonate eggs.
7. Work areas/surfaces are decontaminated at least once per day and after any spill of viable materials.
8. All cultures, stocks, and other regulated waste are autoclaved before disposal or placed in a durable, leakproof container and closed for transport.
9. A biohazard sign is placed at the entrance of the laboratory.
10. Agents posted and the door.
11. Required immunizations are posted on the door.
12. Laboratory coats, gowns or uniforms are worn to prevent contamination to street clothes. Clothing cannot be taken home by personnel to be cleaned. Clothing is decontaminated before being laundered.
13. Gloves must be worn. Always-double glove.
14. Protective eyewear must be worn.
15. All windows that can be opened are fitted with screens or permanently sealed.
16. Laboratory must have a sink for hand washing.
17. Laboratory must have bench tops impervious to water and resistant to laboratory chemicals.
18. Sharps containers must be available in the laboratory.
19. Needles, syringe, or other sharp instruments should be restricted and used only when there is no alternative. Only use needle-locking syringes or disposable syringe units. Do not allow recapping of the needle.
20. Plastic-ware should be substituted for glassware whenever possible.
21. Spills and accidents that result in overt exposure to infectious material are immediately reported to the PI and BioSafety Officer and followed-up with medical evaluation, surveillance and treatment.
22. Animals not involved in the work being performed are not permitted in the laboratory.

Animal Biosafety Level 2 (ABSL-2)

ABSL-2 is suitable for work 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.

The following additional standard and special practices must be added to BSL-2 inside the animal facility:

A. Additional Standards Microbiological Practices
    1. Only those persons required for the program or support purposes are authorized to enter the facility. Personnel who must enter the room for the program or service purposes are advised of the potential hazard.

2. Doors to animal rooms open inward, are self-closing, and are kept closed.
3. Windows must be resistant to breakage, sealed or fitted with screens.
4. If floor drains are provided, traps are always filed with water and/or appropriate disinfectant.
5. Rooms are kept under negative pressure.
6. Cages are washed before reuse.
7. 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. The outer surface is disinfected prior to moving the material. All material is autoclaved prior to incineration.
8. An autoclave is available in the animal facility to decontaminate infectious waste.
9. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
10. A biohazard sign must be posted on the entrance. In addition, the infectious agents in use, the name(s) and telephone numbers of contact personnel, and special requirements (e.g., the need for immunizations and respirators) for entering the room are posted.
11. The wearing of laboratory coats, gowns, and/or uniforms in the facility are required. Laboratory coats, gowns, and/or uniforms are not worn outside the facility.
12. Persons having contact with non-human primates must wear appropriate eye and face protection.
13. When needed, animals are housed in primary Biosafety containment equipment appropriate for the animal species. Filter cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.

Plants Biosafety Level 2 (PBL-2)

1. Access to the green house shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.
2. Prior to entering the green house, personnel shall be required to read and follow instructions on PBL-2 greenhouse practices and procedures.
3. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
4. A record shall be kept of experimental plants currently in progress in the greenhouse facility.
5. The PI shall report any greenhouse accidents involving the inadvertent release or spill of microorganisms to the Greenhouse Director, IBC, BioSafety Officer, and the NIH/OBA.
6. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
7. Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatment should

be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

8. A program shall be implemented to control undesired species (e.g., weeds, rodents, or arthropods pests and pathogens).
9. Arthropods and other motile macro organisms shall be housed in appropriate cages. If macro organisms (e.g., flying arthropods or nematodes) are released within the green house, precautions shall be taken to minimize escape from the greenhouse facility.
10. Experiments involving other organisms that require a containment level lower than PBL-2 may be conducted in the greenhouse concurrently provide that all work is conducted in accordance with PBL-2 greenhouse practices.
11. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: the name of the responsible individual, the plants in use, any special requirements for using the area.
12. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
13. If there is a risk to human health, a sign shall be posted incorporation the universal biosafety symbol.
14. Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.
15. A greenhouse practices manual shall be prepared or adopted. This manual shall: advise personnel of the potential consequences if such practices are not followed and outline contingency plans to be implemented in the event of the unintentional release of organisms.

Biosafety Level 3 (BSL-3)

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

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

A. Standard Microbiological Practices
    1. Work is confined to Class II biological safety cabinets.
    2. Access to the laboratory is double-door access controlled with all penetrations sealed. Lockable doors are required.
    3. Negative pressure with HEPA filters. Air is not recirculated. Audio alarms are installed in case the negative pressure is low or the system malfunctions.
    4. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the laboratory.
6. Mouth pipetting is prohibited.
7. All procedures are performed carefully to minimize splashes or aerosols. This may include centrifuging, centrifuge safety cups, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers, inoculating animals intranasally, and infected tissues from animals or embryonate eggs. All devices must exhaust air through HEPA filters before discharge into the laboratory.
8. Vacuum lines/portable vacuum pumps are protected with liquid disinfectant traps and HEPA filters.
9. Eyewash station(s) are mandatory in the laboratory.
10. Work areas/surfaces are decontaminated at least once per day and after any spill of viable materials.
11. All cultures, stocks, and other regulated waste are autoclaved before disposal or placed in a durable, leakproof container and closed for transport. All potentially contaminated waste from the laboratory is decontaminated before disposal or reuse.
12. A biohazard sign is placed at the entrance of the laboratory.
13. Agents posted and the door.
14. Required immunizations are posted on the door.
15. If needed before entry, respirators or other personal protective measure are posted on the door.
16. Laboratory coats, gowns or uniforms are worn to prevent contamination to street clothes. Clothing cannot be taken home by personnel to be cleaned. Clothing is decontaminated before being laundered.
17. Gloves must be worn. Always-double glove. Frequent changing of gloves accompanied by hand washing is required.
18. Protective eyewear must be worn.
19. All windows are closed and sealed. The windows cannot be opened.
20. Laboratory must have a sink for hand washing.
21. Laboratory must have bench tops impervious to water and resistant to laboratory chemicals.
22. Sharps containers must be available in the laboratory.
23. Needles, syringe, or other sharp instruments should be restricted and used only when there is no alternative. Only use needle-locking syringes or disposable syringe units. Do not allow recapping of the needle.
24. Spills and accidents that result in overt exposure to infectious material are immediately reported to the PI and BioSafety Officer and followed-up with medical evaluation, surveillance and treatment.
25. Animals or plants not involved in the work being performed are not permitted in the laboratory.
26. Respiratory and face protection are required when in rooms containing infectious animals.
27. BSL-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 must be re-verified annually or after modifications to the laboratory.

Animal Biosafety Level 3 (ABSL-3)

ABSL-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.

The following additional standard and special practices must be added to BSL-3 inside the animal facility:

A. Additional Standards Microbiological Practices

1. Only the fewest number of persons required for the program or support purposes are authorized to enter the facility. Personnel who must enter the room for the program or service purposes are advised of the potential hazard.
2. Doors to animal rooms open inward, are self-closing, and are kept closed.
3. Windows must be resistant to breakage, sealed or fitted with screens.
4. If floor drains are provided, traps are always filed with water and/or appropriate disinfectant.
5. Rooms are kept under negative pressure.
6. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned.
7. 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. The outer surface is disinfected prior to moving the material. All material is autoclaved prior to incineration.
8. An autoclave is available in the animal facility to decontaminate infectious waste.
9. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
10. A biohazard sign must be posted on the entrance. In addition, the infectious agents in use, the name(s) and telephone numbers of contact personnel, and special requirements (e.g., the need for immunizations and respirators) for entering the room are posted.
11. The wearing of laboratory coats, gowns, and/or uniforms in the facility are required. Laboratory coats, gowns, and/or uniforms are not worn outside the facility.
12. All personnel entering animal rooms wear appropriate face/eye and respiratory protection.
13. Boots, shoe covers, or other protective footwear, and disinfectant footbaths are available.
14. When needed, animals are housed in primary Biosafety containment equipment appropriate for the animal species. Filter cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.
15. Spill procedure is posted.
16. Personnel entering the animal room wear uniforms or scrub suits. Wrap-around or solid-front gowns must be worn over this clothing. Front-button laboratory coats

are unsuitable. The gown must be removed and left in the animal room and appropriately contained and decontaminated prior to laundering or reuse.

17. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.

Plants Biosafety Level 3 (PBL-3)

1. Access to the green house shall be restricted to individuals who are required for the program or support purposes. The discretion of the Greenhouse Director shall determine those individuals who are authorized to enter the greenhouse facility.
2. Prior to entering the green house, personnel shall be required to read and follow instructions on PBL-3 greenhouse practices and procedures.
3. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
4. A record shall be kept of experimental plants currently in progress in the greenhouse facility.
5. The PI shall report any greenhouse accidents involving the inadvertent release or spill of microorganisms to the Greenhouse Director, IBC, BioSafety Officer, and the NIH/OBA.
6. Experimental organisms shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.
7. A program shall be implemented to control undesired species (e.g., weeds, rodents, or arthropods pests and pathogens).
8. Arthropods and other motile macro organisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.
9. Experiments involving other organisms that require a containment level lower than PBL-3 may be conducted in the greenhouse concurrently provide that all work is conducted in accordance with PBL-3 greenhouse practices.
10. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: the name of the responsible individual, the plants in use, any special requirements for using the area.
11. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
12. If there is a risk to human health, a sign shall be posted incorporation the universal biosafety symbol.
13. Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable sealed primary container then enclosed in a non-breakable, sealed secondary container. At the time of transfer, if the same plant species, hosts, or vectors are present within the effective dissemination distance of

propagules of the experiment organism, the surface of the secondary container shall be decontaminated.

14. A greenhouse practices manual shall be prepared or adopted. This manual shall: advise personnel of the potential consequences if such practices are not followed and outline contingency plans to be implemented in the event of the unintentional release of organisms.
15. Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of the potential dissemination of the experiment microorganisms.
16. Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.
17. Personnel are required to thoroughly wash their hands upon exiting the greenhouse.
18. All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and experimental manipulations.

Biosafety Level 4 (BSL-4)

Currently BSL-4 & ABSL-4 is not approved at this campus. Additional requirements regarding Primary and Secondary barriers are not listed below. Before applying for a grant application, contact the BioSafety Officer and IACUC for all of the facilities upgrades that must be in place before approval can be approved.

BSL-4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infectious and life-threatening disease.

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

A. Standard Microbiological Practices
    1. All work is confined to Class III biological safety cabinets. There will be supplied air to and exhaust air from the cabinet. Cabinets will be tested annually.
    2. Access to the laboratory is double-door access and limited entry with all penetrations sealed. Lockable and self-closure doors are required. Only persons whose presence in the laboratory room for support purposes have authorized enter.
    3. Personnel enter and leave the laboratory only through the clothing change and shower rooms. A shower is taken each time they leave the laboratory. Personnel use the airlocks to enter or leave the laboratory only in an emergency.
    4. Personal clothing is not allowed into the laboratory and laboratory clothing is not allowed to leave.
    5. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. Both doors cannot be opened at the same time.

6. Negative pressure with HEPA filters. Air is not recirculated. Audio alarms are installed in case the negative pressure is low or the system malfunctions.
7. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the laboratory.
8. All procedures are performed carefully to minimize splashes or aerosols. This may include centrifuging, centrifuge safety cups, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers, inoculating animals intranasally, and infected tissues from animals or embryonate eggs. All devices must exhaust air through HEPA filters before discharge into the Class III biological safety cabinet.
9. Vacuum lines/portable vacuum pumps are protected with liquid disinfectant traps and HEPA filters.
10. Work areas/surfaces are decontaminated at least once per day and after any spill of viable materials.
11. Biological materials to be removed from the Class III cabinet or from the BSL-4 in a viable or intact state are transferred to a non-breakable sealed primary container and then enclosed in a non-breakable, sealed secondary container. This is removed from the facility through a disinfectant tank, fumigation chamber, or an airlock designed for this purpose.
12. Double-door autoclaves are provided for decontaminating materials passing out of the Class III biological safety cabinet. Waste is placed in a durable, leakproof container and closed for transport. All potentially contaminated waste from the laboratory is decontaminated before disposal or reuse.
13. A biohazard sign is placed at the entrance of the laboratory.
14. Agents posted and the door.
15. Required immunizations are posted on the door.
16. If needed before entry, respirators or other personal protective measure are posted on the door.
17. All windows are break-resistant, closed and sealed. The windows cannot be opened.
18. Laboratory must have a hands-free or automatically operated hand-washing sink.
19. Laboratory must have bench tops impervious to water and resistant to laboratory chemicals.
20. Sharps containers must be available in the biological safety cabinet.
21. Needles, syringe, or other sharp instruments should be restricted and used only when there is no alternative. Only use needle-locking syringes or disposable syringe units. Do not allow recapping of the needle.
22. A spill procedure is developed and posted within the laboratory.
23. Spills and accidents that result in overt exposure to infectious material are immediately reported to the PI and BioSafety Officer and followed-up with medical evaluation, surveillance and treatment.
24. A system is established for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses.

25. Animals, plants, or clothing not involved in the work being performed are not permitted in the laboratory.
26. BSL-4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities must be re-verified annually or after modifications to the laboratory.
27. Appropriate communication systems are provided between the laboratory and the outside.

Animal Biosafety Level 4 (ABSL-4)

ABSL-4 involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening disease, aerosol transmission, or related agents with unknown risk of transmission.

The following additional standard and special practices must be added to BSL-3 inside the animal facility:

A. Additional Standards Microbiological Practices

1. Only the fewest number of persons required for the program or support purposes are authorized to enter the facility. Personnel who must enter the room for the program or service purposes are advised of the potential hazard and must work in pairs.
2. A medical surveillance program is mandatory for all personnel entering animal rooms.
3. Doors to animal rooms open inward, are self-closing, and are kept closed.
4. Windows must be resistant to breakage, sealed or fitted with screens.
5. If floor drains are provided, traps are always filed with water and/or appropriate disinfectant.
6. Rooms are kept under negative pressure.
7. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned.
8. 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. The outer surface is disinfected prior to moving the material. All materials are sterilized in a double-door autoclaved prior to incineration.
9. An autoclave is available in the animal facility to decontaminate infectious waste.
10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
11. A biohazard sign must be posted on the entrance. In addition, the infectious agents in use, the name(s) and telephone numbers of contact personnel, and special requirements (e.g., the need for immunizations and respirators) for entering the room are posted.
12. The wearing of laboratory coats, gowns, and/or uniforms in the facility are required. Laboratory coats, gowns, and/or uniforms are not worn outside the facility.

13. All personnel entering animal rooms wear appropriate face/eye and respiratory protection.
14. Boots, shoe covers, or other protective footwear, and disinfectant footbaths are available.
15. When needed, animals are housed in primary Biosafety containment equipment appropriate for the animal species. Filter cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.
16. Spill procedure is posted.
17. Personnel entering the animal room wear uniforms or scrub suits. Wrap-around or solid-front gowns must be worn over this clothing. Front-button laboratory coats are unsuitable. The gown must be removed and left in the animal room and appropriately contained and decontaminated prior to laundering or reuse.
18. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.
19. Only work with anesthetized animals.

Plants Biosafety Level 4 (PBL-4)

1. Access to the green house shall be restricted to individuals who are required for the program or support purposes. The discretion of the Greenhouse Director shall determine those individuals who are authorized to enter the greenhouse facility.
2. Prior to entering the green house, personnel shall be advised of the potential environmental hazards and instructed on the appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.
3. Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.
4. Prior to entering the green house, personnel shall be required to read and follow instructions on PBL-4 greenhouse practices and procedures.
5. A record shall be kept of experimental materials brought into or removed from the greenhouse facility.
6. A record shall be kept of experimental currently in progress in the greenhouse facility.
7. A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.
8. The PI shall report any greenhouse accidents involving the inadvertent release or spill of microorganisms to the Greenhouse Director, IBC, BioSafety Officer, and the NIH/OBA.
9. Experimental organisms shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in

contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

10. A chemical control program shall be implemented to eliminate undesired pests and pathogens.
11. A program shall be implemented to control undesired species (e.g., weeds, rodents, or arthropods pests and pathogens).
12. Arthropods and other motile macro organisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.
13. Experiments involving other organisms that require a containment level lower than PBL-4 may be conducted in the greenhouse concurrently provide that all work is conducted in accordance with PBL-4 greenhouse practices.
14. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: the name of the responsible individual, the plants in use, any special requirements for using the area.
15. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
16. If there is a risk to human health, a sign shall be posted incorporation the universal biosafety symbol.
17. Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable sealed primary container then enclosed in a non-breakable, secondary container. These containers shall be removed through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.
18. Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriate decontaminated between each use.
19. A greenhouse practices manual shall be prepared or adopted. This manual shall: advise personnel of the potential consequences if such practices are not followed and outline contingency plans to be implemented in the event of the unintentional release of organisms.
20. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.
21. Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.
22. Personnel are required to thoroughly wash their hands upon exiting the greenhouse.
23. All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and experimental manipulations.

CHAPTER 5. Risk Assessment

5.1 General

"Risk" implies the probability that harm, injury, or disease will occur. A conservative approach is generally advisable when insufficient information forces subjective judgment. Universal precautions are always required.

5.2 Risk Factors

One factor is the pathogenicity of the infectious or suspected infectious agent, which includes the disease incidence and severity. The more severe the potentially of the disease is to acquire, the higher the risk.

A second factor to consider is the route of transmission. The route of transmission of newly isolated agents may not be definitively established. Agents that can be transmitted by the aerosol route have caused most laboratory infections. The greater is the agent aerosol potential, the higher the risk.

A third factor is the agent stability. The agent stability is a consideration that involves not only aerosol infectivity, but also the agent's ability to survive over time in the environment. Factors such as desiccation, exposure to sunlight, or exposure to chemical disinfectants must be considered.

A fourth factor is the infectious dose of the agent. The infectious dose can very from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized worker and may pose a serious risk to those with lesser resistance. The worker's immune status is directly related to his/her susceptibility to disease when working with an infectious agent.

The fifth factor is concentration/volume. The concentration (number of infectious organisms per unit volume) will be important in determining the risk. The volume of concentrated material being handled is also important. In most instances, the risk factor increase as the working volume of high-titered microorganisms increases, since additional handling of the materials is often required.

The sixth factor is origin. The origin of the potentially infectious material is also critical. "Origin" may refer to geographic location (e.g., domestic or foreign); host (e.g., infected or uninfected human or animal); or nature of source (potential zoonotic or associated with a disease outbreak). From another perspective, this factor can also consider the potential of agents to endanger livestock and poultry.

Caution must always be exercised in translating infectivity data from one species of animals to another species.

The seventh factor is the established availability of an effective prophylaxis or therapeutic intervention. The most common form of prophylaxis is immunization with an effective vaccine. It is important to understand that immunization only serves as an additional layer of protection beyond

engineering controls, proper practices and personal protective equipment. Immunization or therapeutic intervention may be particularly important in field conditions.

Risk Factors Based on BioSafety Level Currently in Use

Risk Factors	Low									High
Pathognicity	1	2	3	4	5	6	7	8	9	10
Transmission	1	2	3	4	5	6	7	8	9	10
Stability	1	2	3	4	5	6	7	8	9	10
Infectious dose	1	2	3	4	5	6	7	8	9	10
Concentration/volume	1	2	3	4	5	6	7	8	9	10
Origin	1	2	3	4	5	6	7	8	9	10
Prophylaxis or therapeutic intervention	1	2	3	4	5	6	7	8	9	10

Any Risk Factor that has an indication of 6 or higher (from the above table) must be evaluated to see if the BioSafety Level must be increase to the next higher level for safety considerations. Reasons for not increasing the BioSafety Level must be documented.

Medical surveillance ensures that the safeguards decided upon produces the expected health outcome. The medical surveillance is part of the risk management. It may include serum banking, monitoring employee health, and participating in post-exposure management.

Risk assessment must also include an evaluation of the experience and skill level of the at-risk personnel such as laboratory worker's, maintenance personnel, housekeeping, and animal care personnel.

5.3-1 Risk Group 1 (BSL-1 and ABSL-1) Agents

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

Those agents not listed in Risk Groups (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.

5.3-2a Risk Group 2 (BSL-2 and ABSL-2) Agents

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

Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)
--Actinobacillus
--Actinomyces pyogenes (formerly Corynebacterium pyogenes)
--Aeromonas hydrophila
--Amycolata autotrophica
--Archanobacterium haemolyticum (formerly Corynebacterium haemolyticum)

--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 Risk Group 3
--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. pseudotuberculosis, 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 Risk Group 3) including M. avium complex, M. asiaticum, M. bovis BCG vaccine strain, M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi
--Mycoplasma, except M. mycoides and M. agalactiae which are restricted animal pathogens
--Neisseria gonorrhoeae, 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 pallidum, T. carateum
--Vibrio cholerae, V. parahemolyticus, V. vulnificus
--Yersinia enterocolitica

5.3-2b Risk Group 2 (BSL-2 and ABSL-2) Fungal Agents
--Blastomyces dermatitidis
--Cladosporium bantianum, C. (Xylohypha) trichoides
--Cryptococcus neoformans

--Dactylaria galopava (Ochroconis gallopavum)
--Epidermophyton
--Exophiala (Wangiella) dermatitidis
--Fonsecaca pedrosoi
--Microsporum
--Paracoccidioides braziliensis
--Penicillium marneffei
--Sporothrix schenckii
--Trichophyton

5.3-2c Risk Group 2 (BSL-2 and ABSL-2) Parasitic Agents
--Ancylostoma human hookworms including A. duodenale, A. ceylanicum
--Ascaris including Ascaris lumbricoides suum
--Babesia including B. divergens, B. microti
--Brugia filaria worms including B. malayi, B. timori
--Coccidia
--Cryptosporidium including C. parvum
--Cysticercus cellulosae (hydatid cyst, larva of T. solium)
--Echinococcus including E. granulosis, E. multilocularis, E. vogeli
--Entamoeba histolytica
--Enterobius
--Fasciola including F. gigantica, F. hepatica
--Giardia including G. lamblia
--Heterophyes
--Hymenolepis including H. diminuta, H. nana
--Isospora
--Leishmania including L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica
--Loa loa filaria worms
--Microsporidium
--Naegleria fowleri
--Necator human hookworms including N. americanus
--Onchoerca filaria worms including, O. volvulus
--Plasmodium including simian species, P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax
--Sarcocystis including S. sui hominis
--Schistosoma including S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi
--Strongyloides including S. stercoralis
--Taenia solium
--Toxocara including T. canis
--Toxoplasma including T. gondii
--Trichinella spiralis
--Trypanosoma including T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi
--Wuchereria bancrofti filaria worms

5.3-2d Risk Group 2 (BSL-2 and ABSL-2) Viruses

Adenoviruses, human - all types
Alphaviruses (Togaviruses) - Group A Arboviruses
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strain TC-83
--Western equine encephalomyelitis virus
Arenaviruses
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in "Biosafety in Microbiological and Biomedical Laboratories"
Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in "Biosafety in Microbiological and Biomedical Laboratories"
Calciviruses
Coronaviruses
Flaviviruses (Togaviruses) - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Yellow fever virus vaccine strain 17D
--Other viruses as listed in "Biosafety in Microbiological and Biomedical Laboratories"
Hepatitis A, B, C, D, and E viruses
Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Risk Group 4 (RG4) -Viral Agents)
--Cytomegalovirus
--Epstein Barr virus
--Herpes simplex types 1 and 2
--Herpes zoster
--Human herpesvirus types 6 and 7
Orthomyxoviruses
--Influenza viruses types A, B, and C
--Other tick-borne orthomyxoviruses as listed in "Biosafety in Microbiological and Biomedical Laboratories"
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)
Picornaviruses
--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 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)
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)

Arboviruses and Arenaviruses

Acado
Acara
Aquacate
Alfuy
Almpiwar
Amapari
Ananindeus
Anhanga
Anhembi
Anopheles A
Anopheles B
Apeu
Apoi
Aride
Arkonam
Aroa
Aruac
Arumowot
Aura
Avalon
Abras
Abu Hammad
Babahoyo
Bagaza
Bahig
Bakau
Baku
Bandia
Bangoran
Bangui
Banzi
Barmah Forest
Barur
Batai
Batama
Bauline
Bebaru
Belmont
Benevides
Benfica
Bertioga
Bimiti
Birao
Bluetongue*
Boraceia
Botambi
Botekc
Bouboui
Bujaru
Bunyamwera
Bunyip Creek
Burg El Arab
Bushbush
Bussuquara
Buttonwillow
Bwamba
Cacao
Cache Valley
Caimito
California enc.
Calovo
Candiru
Cape Wrath
Capim
Caraparu
Carey Island
Catu
Chaco
Chagres
Chandipura
Changuinola
Charleville
Chenuda
Chilibre
Chobar gorge
Clo Mor
Colorado tick fever
Corriparta
Cotia
Cowbone Ridge
Csiro Village
Cuiaba
D'Aguilar
Dakar Bat
Dengue-1
Dengue-2
Dengue-3
Dengue-4
Dera Ghazi Khan
East equine
Encephalitis
Edge Hill
Entebbe Bat
Ep. Hem. Disease
Erve
Eubenangee
Eyach
Flanders
Fort Morgan
Frijoles
Gamboa
Gan Gan
Gomoka
Gossas
Grand Arbaud
Great Island
Guajara
Guama
Guaratuba
Guaroa
Gumbo Limbo
Hart Park
Hazara
Highlands J
Huacho
Hughes
Icoaraci
Ieri
Ilesha
Ilheus
Ingwavuma
Inkoo
Ippy
Irituia
Isfahan
Itaporanga
Itaqui
Jamestown Canyon
Japanaut
Johnson Atoll
Joinjakaka
Juan Diaz

Jugra
Jurona
Jutiapa
Kadam
Kaeng Khoi
Kaikalur
Kaisodi
Kamese
Kammavanpettai
Kannamangalam
Kao Shuan
Karimabad
Karshi
Kasba
Kemerova
Kern Canyon
Ketapang
Keterah
Keuraliba
Keystone
Kismayo
Klamath
Kokobera
Kolongo
Koongol
Kotonkan
Kowanyama
Kunjin
Kununurra
Kwatta
La Crosse
La Joya
Lagos Bat
Landjia
Langat
Lanjan
Las Maloyas
Latino
Le Dantec
Lebombo
Lednice
Lipovnik
Lokern
Lone Star
Lukuni
M'poko
Madrid
Maguari
Mahogany hammock
Main Drain
Malakal
Manawa
Manitoba
Manzanilla
Mapputta
Maprik
Marco
Marituba
Marrakai
Matariya
Matruh
Matucare
Melao
Mermet
Minatitlan
Minnal
Mirim
Mitchell River
Modoc
Moju
Mono Lake
Mont. Myotis leuk
Moriche
Mosqueiro
Mossuril
Mont Elgon bat
Murutucu
Mykines
Navarro
Nepuyo
Ngaingan
Nique
Nkolbisson
Nola
Ntaya
Nugget
Nyamanini
Nyando
O'nyong-nyong
Okhotskiy
Okola
Olifantsvlei
Oriboca
Ossa
Pacora
Pacui
Pahayokee
Palyam
Parana
Pata
Pathum Thani
Patois
Phnom-Penh bat
Pichinde
Pixuna
POngola
Ponteves
Precariious Point
Pretoria
Prospect Hill
Puchong
Punta Salinas
Punta Toro
Qalyub
Quaranfil
Restan
Rio Bravo
Rio Grande
Ross River
Royal Farm
Sabo
Saboya
Saint Floris
Sakhalin
Salehabad
San Angelo
Sandfly fever (Naples)
Sandfly fever (Sicilian)
Sandjimba
Sango
Sathuperi
Sawgrass
Sebokele
Seletar
Sembalam

Serra do Navio
Shamonda
Shark River
Shuni
Silverwater
Simbu
Simian hem. Fever
Sindbis
Sixgun City
Snowshoe hare
Sokuluk
Soldado
Sororoca
Stratford
Sunday Canyon
Tacaiuma
Tacaribe
Taggert
Tahyna
Tamiami
Tanga
Tanjong Rabok
Tataguine
Tehran
Tembe
Tembusu
Tensaw
Tete
Tettnang
Thimiri
Thottapalayam
Tibrogargan
Timbo
Timboteua
Tindholmur
Toscana
Toure
Tribec
Triniti
Trivittatus
Trubanaman
Tsuruse
Turlock
Tyuleniy
Uganda S
Umatilla
Umbre
Una
Upolu
Urucuri
Usutu
Uukuniemi
Vellore
Venkatapuram
Vinces
Virgin River
VS-Indiana
VS-New Jersey
Wad Medani
Wallal
Wanowrie
Warrego
West. Equine enc.
Whataroa
Witwatersrand
Wongal
Wongorr
Wyeomyia
Yaquina Head
Yata
Yogue
Zaliv Terpeniya
Zegla
Zika
Zirqa

*Export permit required by Department of Commerce.

5.3-3a Risk Group 3 (BSL-3 and ABSL-3) Agents

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

Risk Grou

p 3 (RG3) - Bacterial Agents Including Rickettsia

--Bartonella
--Brucella including B. abortus, B. canis, B. suis
--Burkholderia (Pseudomonas) mallei, B. pseudomallei
--Coxiella burnetii
--Francisella tularensis
--Mycobacterium bovis (except BCG strain, see 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, sibcrica, R. tsutsugamushi, R. typhi (R. mooseri)
--Yersinia pestis

5.3-3b Risk Group 3 (BSL-3 and ABSL-3) Fungal Agents

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

5.3-3c Risk Group 3 (BSL-3 and ABSL-3) Parasitic Agents

None

5.3-3d Risk Group 3 (BSL-3 and ABSL-3) 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 RG2)
--Other viruses as listed in "Biosafety in Microbiological and Biomedical Laboratories"
Arenaviruses
--Flexal
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus
Flaviviruses (Togaviruses) - Group B Arboviruses
--Japanese encephalitis virus
--Yellow fever virus
--Other viruses as listed in "Biosafety in Microbiological and Biomedical Laboratories"
Poxviruses
--Monkeypox virus
Prions
--Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see "Biosafety in Microbiological and Biomedical Laboratories" for containment instruction)

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

Arboviruses and Certain Other Viruses (Based on insufficient experience)

Adelaide River
Agua Preta
Alenquer
Almeirim
Altamira
Andasible
Antequera
Araguari
Aransas Bay
Arbia
Arboledas
Babanki
Batken
Belem
Berrimah
Bimbo
Bobaya
Bobia
Bozo
Buenaventura
Cabassou
Cacipacore
Calchaqui
Canancia
Caninde
Chim
Coastal Plains
Connecticut
Corfou
Dadakala
Douglas
Enscada
Estero Real
Fomede
Forecariah
Fort Sherman
Gabek Forest
Gadgets Gully
Garba
Gordil
Gray Lodge
Gurupi
Iaco
Ibaraki
Ife
Iguape
Inhangapi
Inini
Issyk-Kul
Itaituba
Itimirim
Itupiranga
Jacareacanga
Jaman xi
Jari
Kedougou
Khasan
Kindia
Kyzylagach
Lake Clarendon
Liano Seco
Macaua
Mapuera
Mboke
Meaban
Mojui Dos Compos
Monte Dourado
Munguba
Naranjal
Nariva
Nasoule
Ndelle
New Minto
Ngari
Ngoupe
Nodamura
Northway
Odrenisrou
Omo
Oriximina
Ouango
Oubangui
Oubi
Ourem
Palestina
Palma
Para
Paramushir
Paroo River
Perinet
Petevo
Picola
Playas
Pueblo Viejo
Purus
Radi
Razdan
Resistencia
Rochambeau
Salanga
San Juan
Santa Rosa
Santarem
Saraca
Saumarez Reef
Sedlec
Sena Madureira
Sepik
Shokwe
Slovakia
Somone
Sripur
Tai
Tamdy
Telok Forest
Termeil
Thiafora
Tilligerry
Tinaroo
Tlacotalpan
Tonate
Utinga
Xiburema
Yacaaba
Yaounde
Yoka
Yug Bogdanovac

Arboviruses and Certain Other Viruses

Aino
Akabane
Banna
Bhanja
Central Eur. TBE (Kumlinge. Hypr, Hanzalova, Absettarov)
Chikungunya
Cocal
Dhori
Dobrava-Belgrade
Dugbe
Everglades
Flexal
Germiston
Getah
Hantaan
Israel Turkey mening.
Japanese enc.
Junin
Kairi
Kimberley
Koutango
Kumlinge (Cent. Eur. TBE)
Louping III
Mayaro
Middelburg
Mobala
Mopeia
Mucambo
Murray Valley enc.
Nairobi sheep
Deisease
Ndumu
Negishi
Oropouche
Orungo
Peaton
Piry
Powassan
Puumala
Rift Valley fever
Rocio
Sagiyama
Sal Vieja
San Perlita
Semliki Forest
Scoul
Sin Nombre
Spondweni
St. Louis enc.
Thogoto
Turuna
Venezuelan equine encephalitis (Alagoas)
Wesselsbron
West Nile
Yellow fever
Zinga

5.3-4a Risk Group 4 (BSL-4 and ABSL-4) Agents
RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.
Risk Group 4 (RG4) - Bacterial Agents
None

5.3-4b Risk Group 4 (BSL-4 and ABSL-4) Fungal Agents
None

5.3-4c Risk Group 4 (BSL-4 and ABSL-4) Parasitic Agents
None

5.3-4d Risk Group 4 (BSL-4 and ABSL-4) Viral Agents
Arenaviruses
--Guanarito virus
--Lassa virus
--Junin virus
--Machupo virus
--Sabia
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, and Russian spring-summer encephalitis viruses
Herpesviruses (alpha)
--Herpesvirus simiae (Herpes B or Monkey B virus)
Paramyxoviruses
--Equine morbillivirus
Hemorrhagic fever agents and viruses as yet undefined

5.4 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 cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.
Baculoviruses
Herpesviruses
--Herpesvirus ateles

--Herpesvirus saimiri
--Marek's disease virus
--Murine cytomegalovirus
Papovaviruses
--Bovine papilloma virus
--Polyoma virus
--Shope papilloma virus
--Simian virus 40 (SV40)
Retroviruses
--Avian leukosis virus
--Avian sarcoma virus
--Bovine leukemia virus
--Feline leukemia virus
--Feline sarcoma virus
--Gibbon leukemia virus
--Mason-Pfizer monkey virus
--Mouse mammary tumor virus
--Murine leukemia virus
--Murine sarcoma virus
--Rat leukemia virus
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 retrovirus can be maintained, handled, and administered, under BL1 containment.
*List taken from May 1998 NIH *Guidelines for Research Involving Recombinant DNA Molecules*. See the most current NIH *Guidelines* for current classifications, or the American Biological Safety Association *Risk Group Classification for Infectious Agents*.

5.5 Regulated Select Agents
Viruses
Crimean-Congo haemorrhagic fever virus
Eastern Equine Encephalitis virus
Ebola viruses
Equine Morbilli virus
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

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 above, or their toxic subunits.
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) are exempt.

Bacteria
Bacillus anthracis
Brucella abortus, B. melitensis, B. suis
Burkolderia (Pseudomonas) mallei
Burkolderia (Pseudomonas) pseuodomallei
Clostridium botulinum
Francisella tularensis
Yersinia pestis

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 above, or their toxic subunits.

5.6 Exemptions
Vaccine strains as described in Title 9 CFR, 78.1 are exempt.
Rickettsiae
Coxiella burnetii
Rickettsia prowazekii
Rickettsia rickettsii

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 above, or their toxic subunits.

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 above, or their toxic subunits.

Toxins
Abrin
Abrin A
Abrin B
Abrin C
Abrin D
Abrin reconstituted (A+B mix)
Aflatoxins
Aflatoxin 495
Aflatoxin B
Aflatoxin B1
Aflatoxin B1 mixed with G1
Aflatoxin B1 dichlorides, oxides, epoxides
Aflatoxin B2 dihydro B1
Aflatoxin G1
Aflatoxin G2 dihydro G1
Aflatoxin M1 4-hydroxy B1
Aflatoxin M2 4-hydroxy B2
Aflatoxin P1
Aflatoxin Q1
Aflatoxin Ro
Botulinum toxins
Clostridium botulinum
C. Botulinum neurotoxin
C. Botulinum toxin A
C. Botulinum toxin B
C. Botulinum toxin C1
C. Botulinum toxin C2
C. Botulinum toxin D
C. Botulinum toxin E
C. Botulinum toxin F
Clostriduium perfringens epsilon toxin
Conotoxins
Diacetoxyscirpenol
Ricin
Ricin A
Ricin A chain
Ricin B
Ricin C
Ricin D
Ricin D alanine-chain protein
Ricin D isoleucine-chain reduced
Ricin nitrogen
Ricin, reduced
Recin, total hydrolysate

Ricin toxin -Con A
Saxitoxin
Saxitoxin hydrate
Saxitoxin dihydrochloride hydrochloride
Saxitoxin p-bromobenzenesulfonate
Shigatoxin
Shigella shigae neurotoxin
Staphylococcal enterotoxins
Staphylococcus enterotoxin A
Staphylococcus enterotoxin B
Staphyloccus enterotoxin F
Tetrodotoxin
T-2 toxin
T-2 toxin tetraol
T-2 hemisuccinate
Tetrodotoxin
Tetrodotoxin citrate, 2-hydroxy
Tetrodotoxin 4,9-anhydro
Tetrodotoxin 4,9 anhdro, 8,3-diacetate
Tetrodotoxin 4-amino-4-deoxy
Deoxytetrodotoxin
Methoxytetrodotoxin
Ethoxytetrodotoxin

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 above, or their toxic subunits

Regulated Plant Pest List

AGENTS

African soybean dwarf agent
Apple rinspot agent
Cherry rusty mottle (European) agent
Chlorotic ringspot agent (associated with Jasminum spp)
Cotton anthocyanosis agents
Cotton small leaf agent
Euonymus mosaic agents
Grapevine Brarislava mosaic agent
Grapevine chasselas latent agent
Grapevine little leaf agent
Grapevine vein mosaic agent
Grapevine vein necrosis agent
Hibiscus leaf curl agent
Horsechestnut variegation
Horsechestnut yellow mosaic agent
Jasmine variegation agents
Ligustrum mosaic agents
Maple mosaic agent
Maple variegation agent
Mountain ash ringspot mosaic agent
Mountain ash variegation agent
Mulberry mosaic agent
Okra mosaic agents
Okra yellow leaf curl agent
Pear bud drop agent
Phyllody agent (associated with Jasminum spp.)
Quince sooty ringspot agent
Quince stunt agent
Quince yellow blotch agent
Rose wilt agent
Sampaguita yellow ringspot mosaic agent
Yellow ring mosaic agent (associated with Jasminum spp.)

BACTERIUM

Bacillus spp. (associated with beekeeping and honey production)
Erwinia salicis
Grapevine infectious necrosis bacterium
Grapevine yellow disease bacterium
Lieberobacter africanium
Lieberobacter asiaticum
Potato leaflet stunt
Pseudomonas lignicola
Wheat yellowing stripe bacterium
Xanthomonas acernea
Xanthomonas ampelina
Xanthomonas axonopodis pv. Citri
Xanthomonas campestris pv oryzicola
Xanthomonas campestris pv vasculorum
Xanthomonas manihotis
Xanthomonas populi

FUNGUS

Aecidium hydrangeae-paniculatea
Aecidium mori
Beauveria spp.
Ceratocystis fimbria, cocoa isolates
Cercospora batatae
Chrysomyxa abietis
Chrysomyxa himalensis
Chrysomyxa ledi var. rhododendri
Cordyceps spp.
Crinipellis perniciosa
Cronartium flaccidium
Diaporthe mali
Elsinoe australis
Elsinoe batatas
Entomophthora spp.
Entyloma oryzae
Fusarium fuliginosporum
Guignardia piricola
Gymnosporangium asiaticum
Hemileia vastatrix
Lachnellula willkommil
Melanomma glumarum
Monilinia fructigena
Moniliophthora rorei
Oncobasidum theobromae
Oospora oryzetorum
Peronosclerospora maydis
Peronosclerospora sacchari
Pestalotiopsis disseminate
Phacidiopycnis pseudotsuga

Phialophora cinerescens
Phytophthora fragariae (foreign strains)
Pseudopezicula tracheiphila
Puccinia gladioli
Puccinia horiana
Puccinia mccleanii
Pucciniastrum actinidae
Pucciniastrum areolatum
Rhacodiella vitis
Rosellinia necatrix
Septoria melanosa
Stephanoderes hampei
Stereum hirsutum
Stigmina deflectans
Synchytrium endobioticum
Tilletia indica
Trachysphaera fructigena
Uredo dioscoreae-alatae
Uredo gladioli-buettneri
Urocystis agropyri foreign strains
Urocystis tritici
Uromyces gladioli
Uromyces nyikensis
Uromyces transversalis
Uromycladium tepperianum
Prodenia litura

INSECT-Acrolepiidae
Acrolepiopsis assectella
INSECT-Aleyrodidae
Aleurocanthus spiniferus
Neomaskellia bergii
INSECT-Alydidae
Leptocorisa acuta
INSECT-Apidae
Apis mellifera capensis
Apis mellifera scuttellata
INSECT-Carposinide
Carposina niponensis
INSECT-Cerambycidae
Anoplophora glabripennis
INSECT-Chrysididae
Chrysis spp.
INSECT-Chrysomelidae
Exosoma lusitanca

INSECT-Coccidae
Coccus viridis
INSECT-Coreidae
Leptoglossus chilensis
INSECT-Cossidae
Dyspessa ulula
INSECT-Crambidae
Maruca vitrata
INSECT-Curculiondae
Brachycerus spp.
Conotrachelus aguacatae
Conotrachelus spp.
Copturus aguacatae
Cryptorhynchus mangiferae
Curculio elephas
Curculio nucum
Elytroteinus subtruncatus
Euscepes postfasciatus
Hepilipus lauri
Listroderes subcinctus
Megalometis chilensis
Metamasius spp.
Naupactus xanthographus
Rhabdoscelus obscurus
Sternochetus mangiferae
INSECT-Cynipidae
Dryocosmus kuriphilus
INSECT-Dermestidae
Trogoderma granarium
INSECT-Diaspididae
Furcaspis oceanica
INSECT-Elachistidae
Stenoma catenifer
INSECT-Elateridae
Conoderus rufangulus
INSECT-Formicidae
Solenopsis invicta
Solenopsis richteri
Solenopsisrichteri X Solenopsis invicta hybrid
INSECT-Gelechiidae
Pectinophora gossypiella
Pectinophora scutigera
INSECT-Gracillariidae
Conopomorpha cramerella
INSECT-Hieroxestidae

Opogona sacchari
INSECT-Lycaenidae
Lampides boeticus
INSECT-Lymantriidae
Lymantria dispar
INSECT-Lyonetiidae
Leucoptera malifoliella
INSECT-Margarodidae
Icerya aegyptiaca
INSECT-Megachilidae
Coelioxys spp.
INSECT-Noctuidae
Earias fabia
INSECT-Phlaeothripidae
Haplothrips chinensis
INSECT-Plutellidae
Prays endocarpa
INSECT-Pseudococcidae
Phenococcus manihoti
INSECT-Pyralidae
Chilo suppressalis
Conogethes punctiferalis
Omphisa anastomosalis
INSECT-Scarabaeidae
Adoretus sinicus
Adoretus spp.
Anomala sulcatula
Holotrichia mindanaona
Phyllophaga spp.
Popillia japonica
INSECT-Scolytidae
Hypothenemus hampei
Tomicus piniperda
Xyleborus spp.
INSECT-Sminthuridae
Sminthurus viridus
INSECT-Tephritidae
Anastrepha fraterculus
Anastrepha grandis
Anastrepha ludens
Anastrepha obliqua
Anastrepha serpentina
Anastrepha striata
Anastrepha suspensa
Bactrocera cucurbitae
Bactrocera dorsalis
Bactrocera tryoni
Ceratitis capitata
Ceratitis spp.
Pterandrus spp.
Toxotrypana curvicauda
INSECT-Tortricidae
Adoxophyes orana
Argyotaenia pulchellana
Capua tortrix
Cryptophlebia leucotreta
Cydia funebrana
Cydia splendana
Epiphyaspostvittana
Hemimene Juliana
Laspeyresia spp.
Lobesia botrana
Pammene fasciana
Proculia spp.
INSECT-Eriophytidae
Eriophyes gossypii
Eriophyes litchi
INSECT-Laelapidae
Tropilaelaps clareae
INSECT-Tarsonemidae
Acarapis woodi
INSECT-Tenuipalpidae
Brevipalpus chilensis
INSECT-Tetranychidae
Amphitetranychus viennensis
Mononychellus tanajoa
INSECT-Varroidae
Euvarroa sinhai
Varroa jacobsoni
INSECT-Heteroderidae
Globodera pallida
Globodera rostochiensis
Phytoplasma
Apple proliferation
Australian grapevine yellows
Black wood (bois-noir)
Cotton virescence
European aster yellows
European stone fruit yellows
Flavescence-doree

Grapevine vein yellows and leaf roll
Grapevine vergelbungskrankheit
Groundnut witches broom
Mulberry dwarf
Parastolbur
Potato marginal flavescence
Potato purple top roll
Potato witches broom (European and Asian pathogens)
Rice yellow dwarf
Rubus stunt
Stolbur
Sugarcane white leaf phytoplasma
Sweetpotato witches broom (little leaf)

Viroid

Coconut cadang-cadang viroid
Pear blister canker viroid

Virus

Alfalfa enation virus
Andean potato latent virus
Andean potato mottle virus
Arabis mosaic virus and its strains
Arracacha Virus B
Artichoke Italian latent virus
Azuki bean mosaic virus
Banana streak virus
Barley yellow mosaic virus
Barley yellow strate mosaic virus
Bhendi yellow vein mosaic virus
Black current reversion virus
Brome streak mosaic virus
Cassava African mosaic virus
Cassava brown streak virus
Cassava common mosaic virus
Cassava latent virus
Cereal chlorotic mosaic virus
Cocksfoot mild mosaic virus
Cocoa mottle leaf virus
Cocoa necrosis virus
Cocoa swollen shoot virus
Cocoa yellow mosaic virus
Cotton leaf curl virus
Cowpea mild mottle virus
Cynodon chlorotic streak virus
Cynosurus mottle virus
Datura Colombian virus
Datura distortion virus
Datura enation mosaic virus
Dulcamara mottle virus
Echinochloa ragged stunt virus
Elm mottle virus
European wheat striate mosaic virus
French bean mosaic virus
Grapevine Algerian latent virus
Grapevine berry inner necrosis virus
Grapevine Bulgarian latent virus
Grapevine Tunisian ringspot virus
Groundnut chlorotic leaf streak virus
Groundnut chlorotic spotting virus
Groundnut rosette viruses
Horsegram yellow mosaic virus
Hungarian chrome mosaic virus
Indian peanut clump virus
Indonesian soybean dwarf virus
Iranian maize mosaic virus
Kashmir virus (associated with honeybees)
Lima bean mosaic virus
Lucerne Australian symptomless virus
Lucerne vein yellowing virus
Maize mottle/chlorotic stunt virus
Maize rough dwarf virus
Maize streak virus
Mung bean yellow mosaic virus
Northern cereal mosaic virus
Oak red streak mosaic virus
Oat sterile dwarf virus
Okra mosaic virus
Peanut clump virus
Plum bark split virus
Plum pox virus
Potato mop top virus
Potato virus T
Potato virus U
Potato virus V
Potato virus Y, tobacco veinal necrosis strain
Potato yellow vein virus
Potato yellowing virus
Raspberry ringspot virus and its strains
Red clover mottle virus
Rice dwarf virus

Rice gall dwarf virus
Rice tungro virus
Rice wilted stunt virus
Rice yellow mottle virus
Strawberry latent ringspot virus and its strains
Tobacco ringspot virus (Andean potato calico strain)
Tomato blackring virus and its strains
Wheat yellow leaf virus

WEEDS–Acanthaceae
Hygrophila polysperma

WEEDS–Alismataceae
Sagittaria sagittifolia

WEEDS–Amaranthaceae
Alternanthera sessilis

WEEDS–Apiaceae
Heracleum mantegazzianum

WEEDS–Asteraceae
Ageratina adenophora

WEEDS–Asteraceae
Carthamus oxyacanthus
Crupina vulgaris
Mikania cordata
Mikania micrantha
Tridax procumbens

WEEDS–Azollaceae
Azolla pinnata

WEEDS–Cactaceae
Opuntia aurantiaca

WEEDS–Caryophyllaceae
Drymaria arenarioides

WEEDS–Caulerpaceae
Caulerpa taxifolia

WEEDS–Chenopodiaceae
Salsola vermiculata

WEEDS–Commelinaceae
Commelina benghalensis

WEEDS–Convolvulaceae
Ipomoea aquatica

WEEDS–Cuscutaceae
Cuscuta spp.

WEEDS–Fabaceae
Galega officinalis
Mimosa diplotricha
Mimosa pigra var. pigra
Prosopis alpataco
Prosopis argentina
Prosopis articulate
Prosopis burkartii
Prosopis caldenia
Prosopis calingastana
Prosopis campestris
Prosopis castellanosii
Prosopis denudans
Prosopis elata
Prosopis farcta
Prosopis ferox
Prosopis fiebrigii
Prosopis hassleri
Prosopis humilis
Prosopis kuntzei
Prosopis pallida
Prosopis palmeri
Prosopis reptans
Prosopis rojasiana
Prosopis ruizlealii
Prosopis ruscifolia
Prosopis sericantha
Prosopis strombulifera
Prosopis torquata

WEEDS–Hydrocharitaceae
Hydrilla verticillata
Lagarosiphon major
Ottelia alismoides

WEEDS–Liliaceae
Asphodelusfistulosus

WEEDS–Melastomataceae
Melastoma malabathricum

WEEDS–Myrteae
Melalcuca quinquenervia

WEEDS–Orobanchaceae
Aeginetia spp.
Orobanche spp.

WEEDS–Poaceae
Avena sterilis
Chrysopogon aciculatus
Digitaria abyssinica
Digitaria velutina
Imperata brasiliensis
Imperata cylindrical

Ischaemum rugosum
Leptochloa chinensis
Nassella trichotoma
Oryza longistaminata
Oryza punctata
Oryza rufipogon
Paspalum scrobiculatum
Pennisetum clandestium
Pennisetum macrourum
Pennisetum pedicellatum
Pennisetum polystachion
Rottboellia cochinchinensis
Saccharum spontaneum
Setaria pallide-fusca
Urochloa panicoides

WEEDS–Polygonaceae

Emex australis
Emex spinosa

WEEDS–Pontederiaceae

Eichhornia azurea
Monochoria hastate
Monochoria vaginalis

WEEDS–Rosaceae

Rubus fruticosus (complex)
Rubus moluccanus

WEEDS–Rubiaceae

Spermacoce alata

WEEDS–Salviniaceae

Salvinia auriculata (complex)
Salvinia biloba
Salvinia herzogii
Salvinia molesta

WEEDS–Scrophulariaceae

Alectra spp.
Limnophila sessilifora
Striga spp.

WEEDS–Solanaceae

Lycium ferocissimum
Solanum tampicense
Solanum torvum
Solanum viarum

WEEDS–Sparganiaceae

Sparganium erectum

Chapter 6.0 Recombinant DNA (rDNA)

The "Guidelines for Research Involving Recombinant DNA Molecules", (NIH Guidelines) outline the procedures required for use of rDNA, and describe the roles and responsibilities of the University and the principal investigator (P.I.). The University is responsible for ensuring that the rDNA activities comply with the provisions of the NIH Guidelines. (A complete description of the University's responsibilities can be found in Section IV-B of the NIH Guidelines).

Proposals for non-exempt rDNA work are submitted to the BioSafety Committee for review prior to initiation. The Committee is responsible for review of all rDNA experiments for compliance and for assessing the containment level, facilities, procedures, practices, and expertise and training of research personnel. Committee results are communicated to the P .I. describing the containment level and any additional precautions. The Committee will also periodically review rDNA research at the University to ensure compliance with the NIH Guidelines.

The PI is ultimately responsible for compliance with the NIH Guidelines and for the safe conduct of rDNA experiments. S/he must perform an initial risk assessment for rDNA work and identify an appropriate containment level for the experiment. In addition, the PI must ensure that all personnel involved in the experiment are trained in safe working procedures. (A complete list of PI responsibilities can be found in Section IV-B-7 of the NIH Guidelines and are referenced below). These responsibilities are also outlined on the rDNA registration form). Experiments that require the Institutional BioSafety Committee (IBC) approval may not be initiated or modified until the Committee has provided approval.

According to NIH Recombinant DNA Molecules are defined as either:

(1) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or
(2) molecules that result from the replication of those described in (1) above.

Experiments involving rDNA are classified on the basis of hazard and fall into three major categories:

(A) Experiments that require IBC approval prior to initiation,
(B) Experiments that require IBC notice simultaneous with initiation,
(C) Exempt experiments (experiments that are of minimal hazard and do not require registration.

6.1 (A) Experiments that require Institutional BioSafety Committee approval prior to initiation (Section III-A, III-B, III-C, and III-D)

III-A-1-a Deliberate transfer of a drug trait to a microorganism not known to acquire it naturally.

III-B-1 Cloning of DNA encoding molecules lethal to vertebrates at an LD 50 of <100ug/kg body weight.

III-C-1 Human gene transfer experiments.

III-D-1 Cloning using human or animal pathogens as host-vector systems. (Refer to the "Classification of Etiologic Agents on the Basis of Hazard" from Appendix B, of the NIH Guidelines).

III-D-2 Cloning of DNA from all Class 3, 4, or 5 human or animal pathogens (including HIV and related viruses, and human tumor viruses).

III-D-3 Experiments using more than 2/3 of the genome of infectious animal or plant viruses or defective viruses grown in the presence of helper virus.

III-D-4 Recombinant DNA experiments involving whole animals. Note that transgenic or knockout rodent experiments that require BL1 containment can be initiated simultaneously with the BioSafety Committee notice. The purchase of transgenic or knockout rodents for BL1 experiments is exempt from NIH Guidelines.

III-5 Recombinant DNA experiments involving whole plants.

III-6 Large-scale DNA projects (>10 liters of culture)

6.2 (B) Experiments that require Institutional BioSafety Committee notice simultaneous with initiation (Section III-E)

III-E-1 Experiments using as vectors 2/3 of the genome of a eukaryotic virus, free of helper virus.

III-E-2 Low risk rDNA plant experiments

III-E-3 Transgenic or knockout rodent experiments that require BL1 containment.

6.3 (C) Exempt experiments (experiments that are of minimal hazard and do not require registration.

- rDNA containing less ½ of an eukaryotic viral genome propagated in cell culture (with the exception of DNA from class 3, 4, or 5 agents).
- rDNA work involving E. coli K12, S. cerevisiae, and B. subtilis host-vector systems (with the exception of DNA from class 3, 4, or 5 agents.
- The purchase or transfer of transgenic rodents for experiments that require BL1 containment.

Synthetic DNA segments, which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their

natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

Principal Investigator (PI)
In addition to the requirement previously mentioned, the PI is responsible for full compliance with NIH Guidelines in the conduct of recombinant DNA research. The PI is responsible for ensuring that all reporting requirements are fulfilled. Report any significant problems of NIH Guidelines to the Biosafety Officer and to NIH/Office of Biotechnology Activities.

Exemption of Natural Exchangers
Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent are exempt from NIH Guidelines.

Sublist A
Genus Escherichia
Genus Shigella
Gen Salmonella – including Arizona
Genus Enterobacter
Genus Citrobacter – including Levinea
Genus Klebsiella – including oxytoca
Genus Erwinia
Pseudomonas aeruginosa
Pseudomonas putida
Pseudomonas fluorescens
Pseudomonas mendocina
Serratia marcescens
Yersinia enterocolitica

Sublist B
Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus aterrimus

Sublist C
Streptomyces aureofaciens

Streptomyces rimosus
Streptomyces coelicolor

Sublist D
Streptomyces griseus
Streptomyces cyancus
Streptomyces venezuelae

Sublist E
One way transfer of Streptococcus mutans or Streptoccus lactis DNA into Streptococcus sanguis

Sublist F
Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

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 cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

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

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

Retroviruses
-Avian leucosis 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

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 retrovirus can be maintained, handled, and administered, under BL1 containment.

Recombinant DNA Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical), that are propagated and maintained in cells in tissue culture are exempt from NIH Guidelines with the following exceptions:

1. Experiments described in Section III-A, which require the IBC approval, RAC review, and NIH Director approval before initiation.
2. Experiments described in Section III-B, which require NIH/OBA and the IBC approval before initiation.
3. Experiments involving DNA from Risk Groups 3,4 or restricted organisms or cells known to be infected with agents.
4. Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates.
5. Whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

Escherichia coli K-12 Host-Vector Systems
See the NIH Guideline in Appendix C-II.

Saccharomyces Host-Vector Systems
See the NIH Guideline in Appendix C-III.

Bacillus subtills or Bacillus licheniformis Host-Vector Systems
See the NIH Guideline in Appendix C-IV.

Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms propagated and maintained in the organisms listed below are exempt from NIH Guidelines.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria moncytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylococcus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremotis
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior

Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarious
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Except those listed in 6.1, 6.2, and 6.3 above.

Appendix/Procedures

ANY CHANGES MADE TO THE APPENDIXES MUST BE APPROVED BY THE BIOSAFETY COMMITTEE AND/OR BIOSAFETY SAFETY OFFICER. THESE CHANGES WILL BE USED TO INCORPORATE NEW REGULATORY CHANGES TO BETTER MAINTAIN NUCLEAR REGULATORY REQUIREMENTS AND/OR PROCEDURAL CHANGES. THE APPENDIXES CONTAIN FORMS AND PROCEDURES USED BY THE UNIVERSITY.

University of Puerto Rico
Medical Sciences Campus
INSTITUTIONAL BIOSAFETY COMMITTEE

INSTRUCTIONS FOR COMPLETING THE REQUEST FORM FOR THE EVALUATION OF BIOLOGICAL AND CHEMICAL SAFETY IN RESEARCH PROPOSALS

1. Complete all parts of this form.

2. **All Biological and Chemical research investigations require IBC approval before starting. Remember that all research projects utilizing human subjects, experimental animals or radioactive materials MUST obtain their approval of the IRB, IACUC or Institutional Radiation Safety Committee, respectively.**

3. Incomplete Request Forms that do not have all the required documentation will not be evaluated until they are completed.

Please review the following checklist before submitting the Request Form to the IBC. You should attach the required documents to this application.

- ☐ One (1) original and 2 copies of the Request Form.
- ☐ Three (3) copies of the research proposal
- ☐ Transmittal Letter addressed to the IBC Chair
- ☐ Safety Training certificates
  Please include copies of all relevant Biosafety training certificates or of the Online Biosafety tests.
- ☐ Standard Operating Procedures (SOP)
  If you do not have detailed SOP'S relevant to your project in the IBC's files, please include them in this application.

University of Puerto Rico
Medical Sciences Campus
Institutional Biosafety Committee

IBC approved_____
University of Puerto Rico, MSC
From_____ to_____
_____
IBC Chairperson

FOR NEW PROPOSALS

REQUEST FORM FOR THE EVALUATION OF BIOLOGICAL AND CHEMICAL SAFETY IN RESEARCH PROPOSALS

Instructions:

Submit this form in conjunction with research proposals of projects dealing with:

- **Infectious Agents**
- **Toxic chemicals**
- **Use of Human and Non-human Primates' Blood, Body fluids or Tissues**
- **Non-Exempt Recombinant DNA (rDNA) Work**

Investigator's name: (Last, First, MI)

School School of Pharmacy **Department** **Office Num.**

PI's E-mail Address

Proposal Title

Funding Agency

Proposed Start Date (MM/DD/YY) **Proposed End Date (MM/DD/YY)**

Location of Work (List Building (s) and Room Number (s))

Lab Supervisor or Key Senior Personnel (to Answer Questions in PI's Absence)

Is this a new project or an amendment to an existing project? New

Write a brief overview of the project and its goals. This description needs to be understood by scientists outside your field and lay persons that are members of the committee.

Please answer all of the following:

1. **Will this project utilize infectious agents (excluding hosts for recombinant DNA)**
☐ Yes ☒ No

If 'Yes, complete Section A- (Use of Infectious Agents) , Section E (Safety Training) and Section F (Procedures in Case of Accidents)

2. **Will this project utilize hazardous chemicals (carcinogens, teratogens), or other toxic chemical agents?**
☐ Yes ☐ No

If 'Yes', complete Section B- (Use of Hazardous Chemicals) , Section E- (Safety Training) and Section F (Procedures in Case of Accidents)

3. **Will this project utilize human or non-human primates' blood, body fluids or tissues?**
☐ Yes ☐ No

If 'Yes', complete Section C- (Use of Human and Non-human Primates' Blood, Body Fluids or Tissues) , Section E- (Safety Training) and Section F (Procedures in Case of Accidents)

4. **Will this project involve non-exempt recombinant DNA (rDNA) work?**
☐ Yes ☐ No

If 'Yes,' complete Section D-(Use of Non-Exempt Recombinant DNA), Section E- (Safety Training) and Section F (Procedures in Case of Accidents)

It is your responsibility to classify your work correctly. If you are unsure whether your research is exempt or not, please do not hesitate to contact the Compliance Office and consult the NIH Recombinant DNA Guidelines at: http://www4.od.nih.gov/oba/Rdna.htm

5. **Are detailed Standard Operating Procedures (SOP's) relevant to this project in the files of the Institutional Biosafety Committee (IBC) ?**
☐ Yes ☐ No

A) **If 'No' please submit all relevant SOP's with this proposal**

B) **If 'Yes', does this proposal require new SOP's?** ☐ Yes ☐ No

C) **If new SOP's are needed, please list them below and provide copies of them in this application.**

Section A- **Use of Infectious Agents**

Provide the following information for all agents that you will use in this project.

Note: It is your responsibility to ensure that work with the agent is conducted in accordance with the biosafety level for which you are approved to use that agent.

Reminder: If your project involves the use of laboratory animals, human subjects or radioisotopes, you must also obtain approval from the Institutional Animal Care and Use Committee (IACUC), the Institutional Review Board (IRB) and the Radiation Safety Committee (RSC), respectively.

Name of Agent	Strains	Used in Vitro? Enter Biosafety level	Used in Vivo? Enter Species and Biosafety level
1.		BSL 1	BSL 1
2.		BSL 1	BSL 1
3.		BSL 1	BSL 1
4.		BSL 1	BSL 1
5.		BSL 1	BSL 1
6.		BSL 1	BSL 1
7.		BSL 1	BSL 1
8.		BSL 1	BSL 1
9.		BSL 1	BSL 1
10.		BSL 1	BSL 1
11.		BSL 1	BSL 1
12.		BSL 1	BSL 1
13.		BSL 1	BSL 1
14.		BSL 1	BSL 1

Describe the method of decontamination and disposal of the waste generated from the use of the agents listed above. (Gloves, plastic ware, materials, fluids, etc.)

Section B- Use of Hazardous Chemicals

Categories of Hazardous Chemicals:

1. Toxic Chemicals (including heavy metals)
2. Flammable/explosive/corrosive chemicals
3. Carcinogenic/mutagenic/teratogenic chemicals
4. Acetyl cholinesterase inhibitors/neurotoxins
5. Controlled substances (refer to http://www.dea.gov/concern/concernhtm)

Chemical List

Name of Chemical and Category Numbers•	MSDS on File	*PPE	**SES	***SEH	Decontamination and Disposal Method
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				
	Yes				

***Personnel Protection Equipment= includes gloves, respiratory protection, eye protection, etc.**
****Safety Equipment for Storage: Location= cabinets for chemicals and/or flammables, safety box for controlled substances, etc.**
*****Safety Equipment for Handling: Location= chemical/fume hoods.**
•Corresponds to any of the category numbers (1-5) of the hazardous chemicals described above.

Section C- Use of Human or Non Human Primate's Blood, Body Fluids or Tissues

Describe in the space provided below the sources of blood, body fluids or tissues to be used in your project and any information relevant to determining its infectious or otherwise hazardous potential.

Reminder: In many cases, use of human- origin material also requires approval of the Institutional Review Board. Use of non-human primate material also requires IACUC approval.

Have these materials been tested for infectious agents prior to use in your laboratory? Yes☐ No ☐

Describe the methods for decontamination and disposal of the waste generated from the use of the materials listed above. (Gloves, plastic ware, materials, fluids, etc.)

Enter the names of all personnel that will be handling the human-origin material and whether they have received vaccination for Hepatitis B virus, and if that personnel have taken the required course about blood-borne pathogens. The University of Puerto Rico Medical Sciences Campus policy is that all such personnel must take the required course about blood-borne pathogens and be offered immunization against hepatitis B virus.

Name	Blood-Borne Pathogens Course	Hepatitis B Virus Vaccination
	N/A	N/A
	N/A	N/A
	N/A	N/A
	N/A	N/A
	N/A	N/A
	N/A	N/A
	N/A	N/A
	N/A	N/A

Describe how personnel have been trained in the handling of potential hazardous materials/agents to be used.

Section D- Use of Non-Exempt Recombinant DNA (rDNA)*

Provide a brief description of the non-exempt rDNA work to be conducted for this project.

Please provide the following information on the use of non-exempt rDNA use:

Source(s) of DNA
Vector(s)
Host(s) for propagation
Name of protein(s) to be expressed (enter "none" if not applicable)

Is the expressed protein toxic to vertebrates? (enter NO if not applicable) Yes ☐ No ☐

Does recombinant contain 2/3rds of a viral genome? Yes ☐ No ☐

Which of the following will serve as hosts for the rDNA? (enter animal or plants species and population of humans if appropriate)

☐ Cultured Cells:
☐ Animals:
☐ Whole Plants:
☐ Humans:
☐ Other:

***For definition of Non-exempt DNA refer to:**
http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html NIH Guidelines for Research Involving Recombinant DNA molecules (Section IB=Definition of Recombinant DNA molecules).

Describe the methods for the decontamination and disposal of the waste generated from the use of the potentially hazardous materials/agents listed above. (Gloves, plastic ware, materials, fluids, etc.)

Section E- Safety Training

Describe how personnel have been trained in the handling of the following: rDNA, infectious agents, biological toxins, chemical toxins, human or non-human primates' blood components, body fluids and tissues, when applicable.

Indicate the name of the personnel that will participate in this project and their training dates. For training of personnel, courses are routinely offered by the MSC/UPR Office for the Safety in Research Laboratories, and you may also take the test for Biosafety at the following web page: www.practicingsafescience.org. Provide a copy of the certificates of the training and/or test and date taken, with this form.

Name of personnel	Training Date

Section F- Procedures in case of Accidents

Describe the procedures that will be performed in the event an employee, student or co-worker becomes ill, is accidentally exposed, and/or exhibits symptoms and signs consistent with exposure to any hazardous agents described in this form.

ASSURANCE

I agree to fully comply with the policies and procedures established by the University of Puerto Rico Medical Sciences Campus as well as all applicable rules and regulations. I certify that all personnel involved in this project have been trained in all applicable safety procedures and has been made aware of all risks involved in this project. The information provided is accurate and complete.

_____

Investigator's Signature Date

_____

Faculty Advisor's Signature Date
(If investigator is a student)

Revised by Teresa Soto and Dr. Julio Lavergne 15/10/06

Appendix A, Applications for Authorized Users **Protocol ID#:_____**

APPLICATION FOR POSSESSION AND USE OF BIOHAZARDOUS/INFECTIOUS MATERIAL

General information:

Name of Applicant: _____ Date: ___/___/___
First Middle Last MM/DD/YY

Department _____ e-mail: _____

Telephone# _____ Extension: _____ Room number: _____

Laboratory Supervisor: _____

Individual authorized to handle material:

_____

_____

_____

_____

1. rDNA/DNA/RNA/Biohazard/ infectious material	2. Type (Agent, Fungal, Parasitic, Virus, etc.)	3. Host	4. BLS Level (1-4)
a.			
b.			
c.			
d.			
e.			
f.			
g.			

5. Biohazardous /infectious material is for:

Yes/ No		Biohazardous material (list as 7a, 7c, etc.)
☐	☐ LABORATORY USE	_____
☐	☐ ANIMAL USE**1	_____
☐	☐ HUMAN USE**2	_____

(*** Requires additional approval by appropriate committees, **1 IACUC, **2 IRB) If yes, provide information if this material has been used in humans or animal and attach appropriate research information.

6. Description of Project

A. Title of Project:

B. Abstract of Project: (Please attach to the application)(Brief overview of the project and its goals. This description needs to be understood by scientists outside your field and members of the general public that are members of the committee)

C. Detailed Laboratory Procedures involving Biohazardous/Infectious Materials: (Please Attached to the application)

D. Check the appropriate registration category for experiments covered by the NIH Guidelines: *All categories are defined in the NIH Guidelines.*

(A) Experiments, which are exempt and do not require registration.

- ❑ If work is exempt, then go to "STATEMENT OF TRAINING AND EXPERIENCE"

(B) Experiments that Require IBC Approval, Recombinant DNA Advisory Committee Review, and NIH Director Approval Before Initiation

- ❑ Major Actions (See Section III-A-1 of the NIH Guidelines)
- ❑ Deliberate transfer of a drug resistance trait to a microorganism that is not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture

(C) Experiments that Require NIH/ORDA abd IBC Approval Befoe Initiation

- ❑ Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight

(D) Experiments that Require IBC Approval, Human Subjects Approval, and NIH/ORDA Registration Before Initiation. Submit completed Appendix M, I-V from the NIH Guidelines along with this document

- ❑ Experiments involving the deliberate transfer of rDNA or DNA or RNA derived from rDNA into one or more human subjects (human gene transfer)

(E) Experiments that IBC Approval Before Initiation

- ❑ Experiments using Risk Group 2, 3, 4, or Restricted Agents as Host-Vectpr Systems
- ❑ Experiments in which DNA from Risk Group 2, 3, 4, or Restricted Agents is cloned into nonpathogenic prokaryotic or lower eukaryotic Host-Vector Systems
- ❑ Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems

- ❑ Experiments involving rDNA in animals or transgenic whole animals
- ❑ Experiments involving whole plants
- ❑ Experiments involving more than 10 liters of culture

(F) Experiments that Require IBC Notice Simultaneous with Initiation

- ❑ Experiments involving the formation of rDNA molecules containing no more than 2/3rds of the genome of any eukaryotic virus
- ❑ Experiments involving whole plants (if not included in Category E% above)

7. Biohazard/infectious material	AMOUNT USED PER EXPERIMENT OR PROCEDURE	Specimen type:
a.		
b.		
c.		
d.		
e.		
f.		
g.		

E. Safety precautions to be taken in these experiments to preclude or reduce exposure to individuals. Please note that the consumption or storage of food/beverages or smoking is strictly forbidden within the laboratory or other areas (e.g., cold storage rooms) where Biohazardous/Infectious materials are authorized.

8. Methods for medical surveillance, monitoring and frequency. (Required for all BSL 2-4)

❑ Not required (BSL 1 only)

9. WASTE

A. METHOD FOR DISPOSAL OF BIOHAZARDOUS/INFECTIOUS WASTE:

- ❑ Incineration
  - o By UPR personnel.
  - o By a licensed vender.
- ❑ Autoclaved
- ❑ Other (explain)

_____
_____
_____

WILL YOU PRODUCE MIXED WASTE?

- ❑ No
- ❑ If yes, continue

(Waste that contains chemical compounds, biohazardous/infectious materials and/or radioactive materials) List types of mixed waste:

_____
_____
_____
_____
_____
_____

List methods for disposal of mixed waste:

_____
_____
_____
_____
_____
_____

STATEMENT OF TRAINING AND EXPERIENCE

List all training regarding the use of biohazardous/infectious materials. Indicate whether the training was of a formal nature or on-the-job training as would be gained through experience under a preceptor. Indicate the nature and duration of experience with Biohazardous and infectious materials. If you have never been a Principal Investigator at the Medical Science Campus, include a preceptor letter. Use additional pages if necessary.

TYPE OF TRAINING WHERE RECEIVED LENGTH FORMAL COURSE (Y OR N)

EXPERIENCE WITH BIOHAZARDOU/INFECTIOUS MATERIALS

Biohazardous and Infectious Material	Maximum Amount used	Facility/University	Facility/University Phone #	# of years working with the materials

If Radioactive Materials or Chemicals are used with your experiments. If YES, please attach the "Radioactive Material Authorization" form and/or list all chemicals used in your protocol to this application. Failure to do so will delay the approval process. Please check the appropriate box(es).

- ☐ Radioactive Materials
- ☐ Chemicals
- ☐ Not applicable

CERTIFICATION: I certify that all the information contained in this form is true and that all procedures dealing with the use of biohazardous/infectious materials. This material will be used in accordance with current federal regulations and the BioSafety Manual. Furthermore, I understand that failure to comply with the above requirements could result in the withdrawal of any authorization to use biohazardous/infectious materials.

_____

APPLICANT Date

_____

RSC CHAIRPERSON Date

UNIVERSITY OF PUERTO RICO
MEDICAL SCIENCES CAMPUS
INSTITUTIONAL BIOSAFETY COMMITTEE

REQUEST FORM FOR MINOR MODIFICATIONS OR RENEWAL OF PREVIOUSLY APPROVED PROTOCOLS

IBC Protocol Code:

Name of PI:

Lab Number:

Telephone:

E-mail:

Title of the Project	Funding Source

Describe changes in procedures to the original Project and specify the names of new personnel, biological or chemical agents used, how they will be handled and disposed and attach to this page.

1. Changes in Biological Agents*: **Yes** **No**

2. Changes in Chemical Agents*: **Yes** **No**

3. Changes in Recombinant DNA*: **Yes** **No**

4. Changes in Procedures*: **Yes** **No**

5. Change in Location: **Yes** **No**

6. Changes in Personnel: **Yes** **No**

* These are Mayor Modifications. Please fill the **REQUEST FORM FOR THE EVALUATION OF BIOLOGICAL AND CHEMICAL SAFETY IN RESEARCH PROPOSALS.**

Signature of PI:_____ **Date:**_____

Signature of Biological and Chemical Safety Officer:
_____ **Date**_____

Signature of the Biological and Chemical safety Committee Chair:

_____

Appendix B, Enforcement Policy

1. NONCOMPLIANCE POLICY

2. CITATION NOTICE

Policy on Enforcement Actions for Items of Noncompliance

All Principal Investigators

1. The Medical Science Center's BioSafety Committee has formulated and approved the following enforcement policy for items of noncompliance with Federal Regulations and the BioSafety Manual in laboratories or other rooms approved for the use or storage of biohazardous/infectious materials.

   A. Instances of noncompliance will be noted on laboratory/room surveys or during other spot checks. Laboratory personnel will be given a verbal warning and instructed on corrective actions.

   B. The issuance of a second verbal citation for the same item of noncompliance will result in a warning letter from the BioSafety Office.

   C. The issuance of a third verbal citation for the same item of noncompliance will result in a second warning letter from the BioSafety Office suspending the authorization to order, receive, and use of biohazardous/infectious material in the laboratory.

2. License reinstatement will require the following:

   A. A meeting with the BioSafety Officer.

   B. A written reapplication for the use of biohahazrdous/infectious material to be approved by the BioSafety Committee to include measures taken to prevent recurrence of the instance or instances of noncompliance.

   C. A meeting with the BioSafety Committee.

3. Please direct any questions on this or other policies to BioSafety Office.

BioSafety Office

Citation Notice

1. This is to formally notify you that you are being cited for violations of the Medical Sciences Campus BioSafety Policies and Procedures as described below.

2. Additionally, you and your laboratory personnel must attend a waste policy training course scheduled for _____ at _____) or take the following corrective actions to prevent recurrence.

You are requested to reply in writing within 10 days. Provide your corrective actions related to the training of your laboratory staff to prevent recurrence.

_____

BioSafety Officer

BIOHAZARDOUS/INFECTIOUS WASTE POLICY - GENERAL REQUIREMENTS

Hazard Reduction

Biohazardous/infectious waste containing chemical or radioactive material must be treated to reduce the potential hazard from all hazardous materials. The goal is two fold: to minimize the hazards for those persons who handle the waste at each step of the disposal process and to minimize the potential impact on the environment during the lifetime of the disposal facility. Accordingly, the BioSafety Office asks you to do the following procedures:

1. Adjust the pH of all aqueous as close to neutral as possible. For aqueous waste, the pH should be between 7 and 9. If necessary, solutions should be buffered to maintain this pH.

2. Hazardous waste mixed with radioactive waste must be kept separated from all other wastes. Contact the Radiation Safety Office as well as the Chemical Safety Office for the proper disposal method.

3. Autoclave or chemically treat pathogenic and infectious material. Red bags can not be placed in or used for radioactive waste or chemical waste.

4. If practical, treat carcinogens, teratogens, and other highly toxic materials to reduce their impact on the environment.

5. PACKAGE SYRINGE NEEDLES AND OTHER SHARP OBJECTS IN SHARP CONTAINERS SO AS TO PREVENT INJURY.

Record Keeping

All materials designated, as infectious waste must be identified.
Unidentified material cannot be processed. Identification means stating:

1. Authorized individual's name
2. Biohazardous material (if above BSL-1)
3. Date
4. Room number

Biohazardous labels should be completed and attached to each container. Do not over label with biohazardous signs.

Segregation by BSL

Segregate Liquid Waste from Dry Waste

Segregation by Form

Dry waste must not contain any bulk liquids.

Aqueous and organic liquids must be collected separately. Disposal methods may differ greatly.

Disposal of Animal Carcasses

Once animals have been injected with biohazardous/infectious material, they become a source for personnel exposure and for potential contamination of equipment and facilities. Special attention must be given to avoid both.

If a protocol does not require maintenance of an animal following its treatment with biohazardous/infectious substances, the carcasses must be placed in strong leak proof bags and securely fastened. Large animal, such as sheep or dogs, must be frozen in such a manner that the least amount of freezer space is required.

Smaller animals like rabbits or cats, should also be individually bagged. The waste card should be attached to the side of the bag. Mice, rat or similarly sized animals may be combined in a single bag.

Carcasses must remain frozen until disposal is possible by incineration. Other carcasses not fitting these parameters must be shipped for burial. The BioSafety Office prepares the carcasses for shipment.

Under no circumstances should materials other than carcasses or tissue be placed in bags and frozen. Sharp objects, such as needles, or scalpel blades, absorbent paper and litter must be discarded as solid waste and packaged separately. (Follow the IACUC policies and procedures and use the Notice "Animals In This Room Have Received Biohazardous/infectious Materials" at the end of this Section.)

Maintain accurate records of activity and radioisotope in the waste. Each liquid waste container must have a waste card attached.

Waste Collection

Follow the University policies and procedures.

Design and Sign of Waste Containers

It is the responsibility of the principal investigator for the proper containers and signs needed in the research rooms.

NOTICE

ANIMALS IN THIS ROOM HAVE RECEIVED BIOHAZARDOUS/INFECTIOUS SUBSTANCES

Name of Authorized User:	
Phone Number(s) Office, Cellular, Home:	
Planned termination date of the Experiment:	

BSL:		Room #		Species:		# of Animals:	

Date of Administration_____
Route of Administration_____

Biohazardous/infectious Substance:

Method of Disposal of Excreta:

Method of Disposal of Animals Found Dead:

Any Special Instruction:

_____
Signature

_____
Date

IN CASE OF EMERGENCY, NOTIFY THE PRINCIPAL INVESTIGATOR OR BIOSAFETY OFFICE.
THIS NOTICE MUST BE ATTACHED TO THE DOOR OF THE ANIMAL ROOM
When the room is Cleared of BIOHAZARDOUS/INFECTIOUS MATERIAL, notify the BioSafety Office to remove the sign.
PLEASE NOTE: The BioSafety Office requests a copy of this form for informational purposes.

Appendix D, Shipping form/Transfer form

	From:	To:
Name:		
Address:		
City, State		
Zip code		
Phone #		
Fax #		
BSO Name	**********	
BSO Phone	**********	

Biohazardious/Infectious Material	Listed in 42 CFR 72.3(f)	BSL	Form	Volume (ml)
	Yes No	1 2 3 4	S L G	
	Yes No	1 2 3 4	S L G	
	Yes No	1 2 3 4	S L G	
Comments:				

	Proper Shipping Name	Hazard Class	Id Number	Passenger aircraft limit	Cargo aircraft limit
	Diagnostic specimen	6.2		4 L or 4 kg	4 L or 4 kg
	Toxin, from living sources, liquid, n.o.s.,	6.1	UN3172	1 L of PG I 5 L of PG II 60 L of PG III	30 L of PG I 60 L of PG II 220 L of PG III
	Toxin, from living sources, solid, n.o.s.,	6.1	UN3172	5 kg of PG I 25 kg of PG II 100 kg of PG III	50 kg pf PG I 100 kg of PG II 200 kg of PG III
	Infectious substances, affecting animals only.	6.2	UN2900	50 ml or 50 g	4 L or 4 kg
	Infectious substances, affecting humans.	6.2	UN2814	50 ml or 50 g	4 L or 4 kg
	Regulated medical waste	6.2	UN3291	No limit	No limit

Air Cargo labels required? _____ Yes _____ No

Dry Ice _____ Yes _____ No

Registered mail or equivalent tracking number: _____

Notice of delivery; failure to receive (Listed in 42 CFR 72.3(f)):

_____

Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, Georgia 30333
(404) 633-5313

Carrier:_____

Date:_____ Time:_____

BIOHAZARDOUS/INFECTIOUS MATERIAL TRANSFER/RECEIPT AND DISPOSAL RECORD
(BETWEEN UPRMSC AUTHORIZED INVESTIGATORS)

FROM
INVESTIGATOR _____ BSO LOG NUMBER _____
Biohazardous/infectious Material: _____ Amount _____

TO
INVESTIGATOR _____

BSO APPROVAL BY _____ NEW BSO LOG NUMBER _____

Transfer of Biohazardous/Infectious Material -

1. Individuals must obtain the permission from the BioSafety Office to receive and/or transfer biohazardous/infectious material to others. Additionally, any material that is received must be approved prior to receipt.

2. When transferring material, use the following as a guide:

Up to 50 ml volume or 5grams

a. Use a strong, tight inner container with a secure watertight/leak-proof cap or seal. (Primary container)
b. Volume cannot exceed 50 ml or 50 grams.
c. Place primary container inside a secondary watertight/leak-proof container. Several primary containers may be enclosed in a single secondary container, if the total volume does not exceed 50 ml.
d. The space between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the at least twice the volume to contain of any liquid within the container (s) in case of breakage or leakage.
e. Place the primary and secondary container and absorbent material into an approved box. If you do not have a box, the BioSafety Office may have one. Do not seal up the box until the BioSafety has inspected the package.

50 to 1000 ml volume or 1000grams

a. Use a strong, tight inner container with a secure watertight/leak-proof cap or seal. (Primary container)
b. Volume can not exceed 1000 ml or 1000 grams.
c. Place primary container inside a secondary watertight/leak-proof container. Several primary containers may be enclosed in a single secondary container, if the total volume does not exceed 50 ml.
d. The space between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the at least twice the volume to contain of any liquid

within the container (s) in case of breakage or leakage. In addition, a shock absorbent material at least twice to that of the absorbent material must be added between the primary and secondary containers.

e. The maximum amount, which may be enclosed within a single shipping container/drum, shall not exceed 4000 ml or 4000 grams. (Four separate primary and secondary containers in a single shipping drum.)
f. Place the primary and secondary container and absorbent material into an approved box. If you do not have a box, the BioSafety Office may have one. Do not seal up the box until the BioSafety has inspected the package.

3. Dry ice – If dry ice is used, it must be placed outside the secondary container(s). If dry ice is used, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

4. Transfer the box to the Authorized Investigator after the BioSafety Office has given approval.

5. 3. The BioSafety Office will fill out the "New BSO Log Number" before transfer.

6. 4. Users must record any transfers on the Inventory Form.

It is the responsibility of the Investigator transferring the material to confirm that no contamination hazard is present.

PROCEDURE FOR PACKAGE CHECK-IN

****NO ONE SHOULD USE BIOHAZARDOUS? INFECTIOUS MATERIAL UNLESS HE OR SHE HAS BEEN APPROPRIATELY TRAINED.****

1. Examine address label to verify that the package belongs to the UPR Medical Science Campus and to the appropriate laboratory.

2. If the packaged, crushed, or has been opened contact the Bio Safety Office. Do not move or touch the package.

3. Open package and verify contents with requisition.

4. Deface radiation labels, UN number, and "Biohazardous Material" wording and signs.

5. Completely fill out the survey page. Complete one inventory sheet for each vial unless two or more vials contain the same material.
NOTE: sometimes the vials actually contain more material than ordered.

6. The inventory sheets (or equivalent) must be used to record laboratory usage. One copy of the inventory sheet must be returned to the BioSafety Office when the material is completely disposed or transferred to another BSO Log number.

7. Correct your total inventory records to acknowledge receipt of new shipment.

CAUTION: ALWAYS OPEN CONTAINERS UNDER A HOOD. CONTENTS ARE OCCASIONALLY UNDER PRESSURE. WEAR PROTECTIVE GLOVES, LAB COAT, AND FACE PROTECTION.

COMPLETE ALL BLANKS ON THE SURVEY FORMS.

Biohazardous/infectious Receipt and Disposal Record

PO #: _____ Approved by: _____ BSO Log #: _____
PI: _____ Date Ordered: ___/___/___ Amount: _____
Biohazardous/infectious material: _____ Vendor: _____

Surveyor: _____ Date Rec: ____/____/____
Biohazardous/infectious material Rec: _____ Amount: _____

If the package is damaged/stained or if contamination is suspected, do not move the package and avoid spreading the contamination. Call the BioSafety Office.

Condition of Package: _____ OK
_____ Damaged/Stained (Notify BioSafety Office)

Comparison of the packing slip and vial contents:
_____ Agrees _____ Does not agree

Disposition of Empty Package and shipping material
Regular Trash _____ No labels or wording
_____ No labels but wording defaced
_____ Labels & wording defaced
or
_____ Incineration
or
_____ Autoclaved

Note: Wording consists of the UN number, "Biohazardous Materials" or signs that may be printed on the outside of the package.

Biohazardous/Infectious Material Disposal Record

Disposal Date	Disposal Method				Amount still in possession)	Initials	Comments
	Incineration	Autoclaved	Transfer	Other*(1 or 2)			

* Other methods of disposal: #1 ____________________ #2 ____________________

T – Transfer to BSO Log # ____________

Return to the BSO when material has been completely disposed to remaining activity has been transferred to another sheet.

EMERGENCY PROCEDURES

All accidents involving hazardous/infectious material must be immediately reported to the BioSafety Office. Minor contamination that occurs in laboratories and is easily decontaminated should be recorded on the lab survey form. Minor contamination in not considered a spill. If the accident is serious in nature and occurs at night, contact Security Service or the operator to contact BioSafety personnel.

BIOHAZARDOUS/INFECTIOUS SPILLS

Spills of biohazardous/infectious materials, no matter how minor, must be cleaned up immediately. It is the responsibility of the person causing a spill to clean it up. The BioSafety Office will provide guidance, waste containers, and assistance to all individuals for all contamination incidents, but the persons involved in the incident should carry out the actual decontamination procedure.

Decontamination efforts should always be conducted in a manner, which minimizes the aerosols/exposure to workers.

For most spills, ordinary detergents and water applied with disposable cleaning materials will be adequate. Commercially available decontamination solutions are usually strong detergents to which complexing agents or surfactants have been added. They may need to be diluted before use.

Use the following guidelines in decontamination efforts:

1. ***Notify individuals in the immediate work area of the spill so they can avoid contamination.***

2. ***Call the BioSafety Office for assistance if you have doubts about how to proceed.***

3. ***Use appropriate protective clothing: gloves, lab coat, protective goggles, shoe covers, and a face mask if conditions are dusty.***

4. ***If there is personnel decontamination take care of this first then decontaminate the work area.***

- Flush the skin with soap and water - DO NOT ABRADE SKIN.

- Remove any decontaminated clothing and store in a biohazard plastic bag.

5. ***Use disposable materials for cleaning: paper towels, kimwipes, and biohazard plastic bags.***

6. *Dampen dry spills with water (e.g., by application of a dampened paper towel, being careful not to spread contamination). Absorb wet spills immediately with paper towels or kimwipes.*

7. *Work from the least contaminated area at the perimeter of the spill to the most contaminated area. Do not increase the contaminated area any more than necessary.*

8. *Place contaminated items in approved biohazard waste containers.*

10. *Use time, distance, and shielding strategies to minimize dose. Use long-handled tools for spills of energetic beta or gamma emitters. Avoid hand contact.*

11. *Do not use equipment which was contaminated or which was used in the decontamination effort until it has been checked.*

15. *Complete the Incident Report and return it to the BioSafety Office.*

Appendix G, Incident Report

INCIDENT REPORT

Prepared By:_____ Date:_____

Please describe the following:

Date and time of Incident:

Place of occurrence:

Personnel involved:

Incident (Include causes for the incident and any laboratory safety precautions taken or avoided. Use extra paper if needed.):

Is there any way to stop this type of incident from happening, or make its effects less severe?

Please return to: BioSafety Office

Date received:_____ By:_____

HOOD SURVEY

Building _____ Room Number _____
Principal Investigator _____

Annually, determine the class of the fume hood. With the sash a height of 15 inches, measure the face velocity at three points into the plane as shown below, then calculate the average velocity. Then label the sash height on the fume hood to indicate/verify the classification.

A	B	C

Sash Ht at 15"

Date	Surveyor	Reading at A	Reading at B	Reading at C	Avg Face Velocity

Class A: 125 to 150 fpm - I-125 (>1 mCi) & P-32 (>10 mCi)
Class B: 120 fpm - Extremely toxic, hazardous material or Carcinogens. (Working with chemicals or radiation)
Class C: 100 fpm - Storage of toxic or hazardous material. (Working with chemicals, radiation or biological safety levels I, II, and III)
Class D: 80 fpm - Storage of non-hazardous material.

NOTE: Airflow cannot exceed 150 fpm
Comments:

SOP for Security to the ARC

ACCESS CODES
The Animal Research Center will provide access codes to personnel that have approved authorization for Animal Care and Usage. Before assigning an access code, the Director of Research will review the qualifications of the personnel, facility, the animals in the research, and research proposal. The director will also review and approve the qualifications of those personnel who work under the authorized user before access codes are assigned.

ENTRANCE TO THE Facility
All main doors to the facility will be locked at all times. All rooms inside the ARC and rooms containing animals are locked at all times.

HAZARDOUS MATERIALS
Rooms that contain Hazardous material such as radioactive materials, biohazardous material, or chemical carcinogens will be posted and only authorized personnel will be allowed in those rooms.

Research Personnel
General Instructions for the use of Biohazardous/Infectious materials in Animals

LOCATION
All animals containing hazardous are to be housed separately from all other animals. Preferably located in separate rooms, if possible.

POSTING
All animal's cages and rooms will be posted with biohazard signs or the door will be posted if the room is used solely for biohazardous/infectious animals. The Door sign will be entitled "Biohazard Material".

The instruction sheet from the Waste Policy will be posted on the door (See BioSafety Manual).

SURVEYS
Appropriate radiation surveys should be made before the beginning and at the end of each experiment.

WASTE
Principal investigators and/or technicians will be responsible for handling all bedding and waste products associated with their experiments. All waste will be packaged according to the BioSafety Manual. All bedding will be stored in a biohazardous container before disposal. The principal investigator and technicians will be responsible for making sure that animal cages are free of

contamination before the cages are given to animal caretakers. Housekeeping duties may be assigned to the principal investigator or technicians if deemed necessary by the BSO.

Animal Caretakers
General Instructions for the use of Biohazardous/Infectious materials in Animals

Animal Caretakers will wear gloves and lab coats around animals that contain hazardous materials.

Animal caretakers will be only responsible for feeding and watering of the animals. All trash that is swept up in the room will be collected and hold for disposal by the principal investigator or technicians.

Animal caretakers will be to a medical surveillance program base on the Biohazardous/Infectious material that is present in the room.

Appendix J, Termination of Biohazardous/Infectious Material and/or Relocation

---

Authorized User: _____

Date: _____/_____/_____

Termination of Biohazardous/Infectious Material Usage

☐ No material have ever been procured or possessed by the Authorized User.

Or

☐ All activities authorized by the University have ceased and all materials procured and/or possessed by the Authorized User cited above have been disposed of in the following manner.

☐ Biohazardous/Infectious materials were transferred to the Radiation Safety Office.

Transferred to:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Date of transfer:	
Recipient's signature:	

☐ Biohazardous/Infectious materials were transferred to another University.

Name and address:	
Phone Number:	
Biohazardous/Infectious material:	

Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Biohazardous/Infectious material:	
Date of transfer:	

Relocation of Biohazardous/Infectious Material

Bldg and room #:	
Date of transfer:	
BSO approval (signature):	
Date of completion:	

Survey (Close-out survey)

A survey was conducted by the Authorized User to confirm the absence of biohazardous/infectious materials and to determine whether any contamination remains on the premises.

☐ The results are attached.

☐ The results were forwarded to the BioSafety Office.
Date: _____/_____/_____

Future mailing address:	
Phone #:	

CERTIFICATION: I certify that all the information contained in this form is true and that all procedures dealing with the use of radioisotopes or ionizing radiation sources will be performed in accordance with current federal regulations and the Radiation Safety Manual. Furthermore, I understand that failure to comply with the above requirements could result in penalty of perjury by the Nuclear Regulatory Commission.

_____
APPLICANT Date

_____
BIOSAFETY OFFICER Date

_____
BSC CHAIRMAN Date

APPENDIX I

BioSafety Training

It is essential that an employee or student at the Medical Science Campus who use biohazardous/infectious materials be adequately educated and trained in the basic biosafety principles and any special principles regarding the particular material/equipment he or she will be using. It is also required by federal regulations that the education and training be documented in the BioSafety Office.

Personnel who require training are those that use, handle, have access to restricted areas, or are exposed to biohazardous/infectious sources. Only those personnel that are trained are allowed in a biohazard area. Individuals that do not handle biohazardous/infectious materials must be trained not to handle, move, or work within the area of the laboratory that biohazardous/infectious materials are handled and/or stored. These individuals are required to follow the regulatory requirements out lined in the BioSafety Manual. Individuals that enter a laboratory must be trained or must be under direct supervision of a trained individual.

Biohazardous/Infectious worker:
Available resources for training shall include:

1. Annual or special biosafety seminars sponsored by the BioSafety Office.
2. Individual instruction and training given by the Authorized User.
3. Written material provided by the BioSafety Office or Authorized User.

Non- Biohazardous/Infectious worker:

1. Areas of the laboratory that are off limits.
2. Not to handle items that are either marked or located in a refrigerator marked "Caution Biohazardous Materials" or within a biohazard work area (laboratory work bench).
3. Security of the Laboratory.
4. No eating, drinking, smoking, applying cosmetics, or storing food within the laboratory.
5. Eating utensil such as coffee mugs silverware, plates, etc should not be located in laboratories.

The form located at the end of this Appendix is provided to assist the Authorized User (AU) in documenting the training that an individual receives under his or her supervision. If the AU is unable to provide the training, a senior laboratory technician may provide the necessary training. A letter from the AU must be sent to the BioSafety Office authorizing the individual to provide training in their place to the laboratory personnel.

Minimum training requirements:

1. BioSafety Manual. The chapters and appendixes that apply to the duties of the individual.

    a. Authorized users of biohazardous materials.
        i. Chapters 1, 2, 3, 4 (appropriate BSL), 5 and related appendixes.
        ii. Medical surveillance program

    b. Individuals working under an Authorized User.
        i. Chapters 2, 3, 4 (appropriate BSL) and related appendixes.

    c. Animal Care personnel.
        i. Chapters 2, 3, 4 (appropriate BSL) and related appendixes.

Alternatively, the Authorized User may substitute similar training material (of their own). If you do this, the material must cover all the topics that the individual will be responsible while performing their duties. However, this training does not relieve the Authorized User from the responsibility of the individuals working in his or her laboratory.

Please make sufficient copies of the "BioSafety Training Program" form and give one to each employee and student who is currently working or will work with biohazardous/infectious materials.

Each employee is required to have annual refresher training. Please have each employee fill out the "BioSafety Training Program" form and return it upon request from the BioSafety Office.

Please return the original form signed by the employee or student and the Authorized User to the BioSafety Office.

BioSafety Training Program

Biohazardous/Infectious Worker

Namc(Last):	(First):	(MI):

Training Date: _____/_____/_____ (MM/DD/YR)

The following material:

1. Biosafety Manual. The chapters and appendixes that apply to the duties of the individual.
   i. Chapters 2, 3, 4 (appropriate BSL) and related appendixes.

2. Additional Information:

Were given as part of his/her training in the safe use of biohazardous/infectious materials. Instructions were to read the material and then come to his/her supervisor or the BioSafety Office with any questions.

_____

Signature of Authorized User or Supervisor

I rcccivcd and rcad thc matcrial abovc and I undcrstand it. Thc Supcrvisor and/or BioSafety Office have answered all of my questions.

_____

Signaturc of Employcc or Studcnt

Date: _____/_____/_____ (MM/DD/YR)

BioSafety Training Program
Non- Biohazardous/Infectious Worker

Name(Last):	(First):	(MI):

Training Date: _____/_____/_____ (MM/DD/YR)

The following material:

1. Biosafety Manual. The chapters and appendixes that apply to laboratory/personal safety.
   ii. Chapters 2, 3, 4 (appropriate BSL) and related appendixes.

2. Non- Biohazardous/Infectious worker:
   a. Areas of the laboratory that are off limits.
   b. Not to handle items that are either marked or located in a refrigerator marked "Caution Biohazardous Materials" or within a biohazard work area (laboratory work bench).
   c. Security of the Laboratory.
   d. No eating, drinking, smoking, applying cosmetics, or storing food within the laboratory.
   e. Eating utensil such as coffee mugs silverware, plates, etc should not be located in laboratories.

3. Additional Information:

Were given as part of his/her training, in the safe use of biohazardous/infectious materials. Instructions were to read the material and then come to his/her supervisor or the BioSafety Office with any questions.

_____
Signature of Authorized User or Supervisor

I received and read the material above and I understand it. The Supervisor and/or BioSafety Office have answered all of my questions.

_____
Signature of Employee or Student

Date: _____/_____/_____ (MM/DD/YR)

Appendix L, Equipment Release Form

APPENDIX J

This form must accompany all equipment whether it is sent off for repair or relocated to another area located within the Medical Science Campus. Laboratories may not be renovated or reoccupied until the BioSafety Office has confirmed that the area is adequately cleaned.

Instrument	Brand Name	Serial No.

Could the equipment produce or harbor any of the following:

	Yes	No
Biohazardous/Infectious substances?		
Potentially infectious biological materials?		
Potentially harmful chemicals or gases?		
In its present condition, are there any physical hazards associated with the equipment?		

If all of the above answers are "no", skip the following table. If not, list all hazardous substances, which have come in contact with the equipment.

Chem./Substance Name	Chemical Symbol	Precautions needed with substance, e.g., Personal Protective Equipment Required	Action if Spillage or Human Contact

Reason for the Repair/Shipment:

Laboratory Signature: _____ Date: _____

Safety Officer Signature: _____ Date: _____

Appendix M Disinfectants and Decontamination

Appendix K Decontamination agents

For the safety of employees and the environment, it is very important that work surfaces and materials be properly cleaned when a spill occurs and at the conclusion of a work period. There is specific terminology to indicate the level of cleaning to be achieved. Certain agents only work against certain microorganisms, so it is crucial that the appropriate agent be used for the application. Designated areas can become contaminated with residues over a period of time and use. Contamination typically results from spills, splashes, failed containers, uncontrolled chemical reactions, storage of incompatible chemicals next to each other and simply using the areas for their intended purposes.

Terminology

- Decontamination-destruction or removal of microorganisms to some lower level, but not necessarily zero.
- Sanitization- reduction of microbial load on an inanimate surface to an acceptable level.
- Disinfection- chemical or physical treatment that destroys most resistant vegetative microbes or viruses, but not the spores, on inanimate objects.
- Sterilization- complete destruction of all viable organisms.

Types of Disinfectants/Sterilants

Formaldehyde gas is used to decontaminate biosafety cabinets, HEPA filters, and the BL3 facilities.

Most other disinfectants/sterilants are liquids. Below is a listing of the compounds and some of their properties.

DISINFECTANTS

Compound	Example	Effective Against:				Used On:			Comments
		Bact.	Virus	Spore	TB	Skin	Instr	Env	
Alcohols	Ethanol, isopropanol	Good	Mod	No	Good	X	X		
Chlorine	Sodium hypochlorite (bleach)	Good	Good	Mod.	Good		X	X	Corrosive to metal, bleach fabric
Glutaral-dehyde	Cidex	Good	Good	Good	Good		X		
Iodine	Betadine, Wescodyne	Good	Good	Mod.	Good	X		X	May be corrosive

Phenol	Wexcide, Vesphene, Amphyl spray	Good	Mod.	No	Good		X	X	
Quaternary Ammonium	3M Quat Cleaner, Triad Cleaner	Good	Mod.	No	No		X	X	

Notes:

Bact. = Bacteria

Instr. = Instruments

Mod. = Moderate action

TB = Tuberculosis

Env. = Environmental surfaces

Appendix N, Spill Protocols

Appendix L, Spills

Decontamination Information: It is important to make sure the appropriate disinfectant is used for the work being performed. The instructions on the agent must be followed. Dilution, shelf life, and contact time are all vital to assuring an effective kill. Care must be used to ensure mixing of incompatible materials does not occur.

Spill in a Biological Cabinet

A spill confined to the interior of a biological safety cabinet generally presents little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once while the cabinet ventilation system continues to prevent the escape of the contaminants from the cabinets.

- Maintain cabinet ventilation.
- Warn others in the laboratory.
- Notify the principal investigator.
- Wear protective gloves, a lab coat, and eye protection during the procedure.
- Spray or wipe walls, work surfaces, and equipment with appropriate disinfectant. A disinfectant with detergent has the advantage of detergent activity that will help clean the surfaces by removing both dirt and microorganisms.
- Use sufficient disinfectant to ensure that grain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container.
- Observe the recommended contact time for the disinfectant.
- Place the used disinfectant, gloves, and wiping materials into an autoclavable container and autoclave them.

This procedure will not disinfect the filters, fans, air ducts, and other interior parts of the cabinet. If the entire interior of the cabinet needs to be disinfected, contact the BioSafety Office.

Spill in the Open Laboratory

For a small spill of biological material in the open laboratory, take the following action:

- Warn others in the laboratory.
- Notify the principal investigator.
- Wear protective gloves, a lab coat, and eye protection during the procedure.
- Decontaminate with an appropriate disinfectant.
- Autoclave wastes as described above.
- If clothing is contaminated, carefully remove it, folding the contaminated area inward. Place the clothing into an autoclavable bag.
- Wash arms, face, and hands.

Spill in a Centrifuge

Spills in centrifuges have the potential for generating large volumes of aerosols. When the operator becomes aware that a spill has occurred, the following action should be taken.

- Turn off the centrifuge and allow time for the aerosols to settle.
- Warn others in the laboratory.
- Notify the principal investigator.
- Wear protective gloves, a lab coat, and eye protection during the procedure.
- Decontaminate with an appropriate disinfectant. Place contaminated equipment in a leakproof bag and move it to a biological safety cabinet, if possible, for decontamination.

BSL2 Spills

Small spills: Wipe up spills with a disinfectant-soaked paper towel and clean the surface with a suitable disinfectant.

Larger spills within a BSC

1. Cabinet must run during cleanup to contain aerosols and HEPA-filter exhaust air.
2. Don appropriate personal protective gear before initiating cleanup.
3. Ensure the drain valve under the cabinet is closed.
4. Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is *not* recommended. Large quantities may create the risk of fire or explosive hazard.
5. If the spill is contained on a bench diaper, remove the contaminated bench diaper and discard as infectious waste.
6. If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time then remove the contaminated towels and discard as infectious waste.
7. Wipe down the interior of the cabinet and any splatter on items within the cabinet with a disinfectant-soaked towel.
8. Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
9. Place items designated as *contaminated used sharps* in a sharps container *using tongs/forceps*. Place other contaminated disposable materials used in the cleanup process in an infectious waste bag. Process as infectious waste.
10. Place contaminated re-usable items in biohazard bags or autoclavable pans with lids. Sterilize, preferably by autoclaving, then clean for re-use.
11. If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required.
12. Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
13. Absorb spilled fluid-disinfectant from work surface with paper towels and discard in biohazard bag.
14. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands whenever gloves are removed.

15. Notify PI or supervisor to determine whether formaldehyde decontamination of the cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.
16. Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

Large spills inside the laboratory (If a spill occurs in a BSL2 facility, outside the BSC, notify other individuals in the laboratory to evacuate.)

1. Exit the laboratory, closing the door behind you.
2. Remove any contaminated clothing and place it in an autoclave bag.
3. Wash all exposed skin.
4. Place signs on door(s) to the laboratory warning individuals who may want to enter that a spill occurred and access is denied.
5. Allow aerosols to settle for 30 minutes before re-entering the laboratory.
6. Assemble supplies (disinfectant, sharps containers, towels, tongs, autoclave bags, etc.) before entering the laboratory.
7. Don appropriate personal protective equipment (i.e. disposable gown, protective eyewear, gloves, shoe coverings and respiratory protection if needed).
8. Clean up spill with a suitable disinfectant as follows:
9. Surround spill area with disinfectant or diking material that is soaked in disinfectant.
    - Place paper towels soaked in a disinfectant over the entire spill area.
    - Allow 20-minute contact time with the disinfectant to ensure adequate germicidal action.
    - Wipe down non-autoclavable materials with germicidal disinfectant.
    - Place items designated as *contaminated used sharps* in a sharps container. Place other disposable materials used in the cleanup process in an infectious waste bag. Process as infectious waste.
    - Place contaminated re-usable items in biohazard bags or autoclavable pans with lids. Sterilize, preferably by autoclaving, then clean for re-use.
10. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving.
11. Wash hands whenever gloves are removed.
12. Notify PI or supervisor and the BioSafety Office.

Large spills inside a centrifuge. The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. All opening of centrifuges must be performed slowly. If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. DO NOT OPEN THE CENTRIFUGE. If breakage is discovered after the machine has stopped, re-close the lid immediately and allow the unit to be at rest for 30 minutes. Have all personnel leave the lab and call BioSafety Office for guidance on how to proceed.

BSL3 Spills

Larger spills within a BSC

1. Cabinet must run during cleanup to contain aerosols and HEPA-filter exhaust air.

2. Don appropriate personal protective gear before initiating cleanup (disposable back-closing gown, double gloves).
3. Initiate clean-up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is *not* recommended. Large quantities may create risk of fire.
4. If the spill is small and contained on a bench diaper, remove the contaminated bench diaper, and discard as infectious waste.
5. If the spill is small and on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time then remove the contaminated towels and discard as infectious waste.
6. Wipe down the interior of the cabinet and any splatter on items within the cabinet with a disinfectant-soaked towel.
7. Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
8. Place items designated as *contaminated used sharps* in a sharps container *using tongs/forceps*. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
9. Place contaminated re-usable items in biohazard bags or autoclavable pans with lids. Sterilize, preferably by autoclaving, then clean for re-use.
10. If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required.
11. Ensure the drain valve under the cabinet is closed.
12. Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
13. Absorb spilled fluid-disinfectant from work surface with paper towels and discard in biohazard bag.
14. Remove protective clothing used during cleanup and it place in a biohazard bag for autoclaving. Wash hands after removing gloves.
15. Notify PI or supervisor and DOHS. Consult with DOHS to determine whether formaldehyde decontamination of the cabinet and filters is necessary.
16. Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

Spills inside the laboratory

1. Notify other individuals in the laboratory to evacuate the laboratory immediately.
2. Hold your breath and exit the laboratory to the anteroom.
3. Remove contaminated clothing (place into autoclave bag). Wash hands after gloves are removed.
4. Wash all exposed skin with germicidal soap. If eyes were splashed, flush at eyewash station for 15 minutes then contact DOHS.
5. Notify PI or supervisor and DOHS. DOHS will consult with the PI to determine the appropriate method of decontamination and spill cleanup (personnel spill response or formaldehyde decontamination of the entire facility).
6. Place a sign on the door to the BL3 lab, to warn individuals of the spill and advise them keep out of the lab.
7. If personnel spill response is required, do the following:
   - Allow aerosols to settle for a minimum of 30 minutes before re-entering the laboratory.

- Assemble supplies (disinfectant, sharps containers, towels, tongs, autoclave bags and protective gear [disposable Tyvek suit/back-closing gown, protective eyewear, gloves, shoe coverings, respiratory protection], etc.) before initiating spill cleanup.
- Don appropriate PPE. Double gloving is recommended.

8. Clean up spill with a suitable disinfectant as follows:
   - -Surround spill area with disinfectant or diking material that is soaked in disinfectant.
   - -Place paper towels soaked in a disinfectant over the entire spill area.
   - -Allow a minimum 20 minute contact time with the disinfectant to ensure adequate germicidal action.
   - -Wipe down non-autoclavable materials with germicidal disinfectant, allowing 20 minute contact time.
   - -Place items designated as *contaminated used sharps* in a sharps container *using tongs/forceps*. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
   - -Place contaminated autoclavable re-usable items in biohazard bags or autoclavable pans with lids. Sterilize, preferably by autoclaving, then clean for re-use.
   - -Repeat decontamination of spill area (floor and work surfaces) after contaminated materials are removed.
9. Remove outer gloves before exiting laboratory to the anteroom.
10. Remove protective clothing used during cleanup in the following order: shoe coverings, gown/suit, respiratory protection, and gloves last. If reusable, wipe down respirator with disinfectant. Place disposable PPE in a biohazard bag for autoclaving.
11. Wash hands with germicidal soap after gloves are removed; shower recommended.
12. Spills inside a centrifuge
13. The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. All opening of centrifuges must be performed slowly. If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. DO NOT OPEN THE CENTRIFUGE. If breakage is discovered after the machine has stopped, re-close the lid immediately and allow the unit to be at rest for 30 minutes. Have all personnel leave the lab and call BioSafety Office for guidance on how to proceed.

Appendix O, Autoclave Guidelines

Appendix M, Autoclave

Autoclaves

Elements Required for Effective Autoclave Use - Autoclaves must be used properly to effectively decontaminate potentially biohazardous materials. The following elements all contribute to autoclave effectiveness.

Temperature: Adequate chamber temperature is at least 121°C (250°F).

Time: Adequate autoclaving time is a *minimum* of 30 minutes, measured *after* the temperature of the material being sterilized reaches 121°C and 15 psi pressure. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load.

Contact: Steam saturation of the load is essential for effective decontamination. Air pockets or insufficient steam supply will prevent adequate contact. To ensure adequate steam contact, leave autoclave bags partially open during autoclaving to allow steam to penetrate into the bag. Add a small amount of water inside the bag to help ensure heat transfer to the items being decontaminated (do not add water if it will cause biohazardous materials to splash out of the bag).

Containers: Use leak-proof containers for items to be autoclaved. Place plastic bags inside a secondary container in the autoclave in case liquids leak out. Plastic or stainless steel containers are appropriate secondary containers. Make sure plastic bags and pans are *autoclavable*, to avoid having to clean up melted plastic.

Indicators: Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C, but cannot measure the length of time spent at 121°C. Biological indicators (such as *Bacillus stearothermophilus* spore strips) and certain chemical indicators (such as Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill microorganisms.

Use a chemical indicator in every load to monitor the effectiveness of individual autoclave runs (temperature only).

Once a month, use either a biological indicator (such as *Bacillus stearothermophilus* spore strips) or a chemical indicator that measures both time and temperature (such as Sterigage). Bury the indicator in the center of the load to validate adequate steam penetration. Keep a log book to record the results.

Autoclave Safety - Autoclaves are classified as pressure vessels, and must be inspected at least annually. Repairs to all autoclaves on campus are done by the vendor or the supplier.

Because an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Prevent injuries when using the autoclave by observing the following rules:

- Wear heat resistant gloves, eye protection and a lab coat, especially when unloading the autoclave.
- Prevent steam burns and shattered glassware by making sure that the pressure in the autoclave chamber is near zero before opening the door at the end of a cycle. Slowly crack open the autoclave door and allow the steam to escape gradually.
- Allow items to cool for 10 minutes before removing them from the autoclave.
- Never put sealed containers in an autoclave. They can explode. Large bottles with narrow necks may also explode if filled too full of liquid.
- Never put solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials in an autoclave. Call EH&S at 294-5359 if you have questions about proper disposal of these materials.

Inspect your autoclave components regularly. If you find a problem, notify your area mechanic. Do not operate an autoclave until it has been properly repaired.

UNIVERSITY OF PUERTO RICO
MEDICAL SCIENCE CAMPUS

Biosafety Manual

José R. Carlo, MD, FAAN
Chancellor

2007