

OLD DOMINION UNIVERSITY

Biosafety Procedure Manual

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Office of Research
Environmental Health and Safety Office**

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I. ADMINISTRATION

A. General Policy

It is the responsibility of each member of the Old Dominion University community to ensure the proper use, handling, and disposal of any biohazardous material, including recombinant DNA (rDNA). Principal Investigators bear primary responsibility for ensuring that their research complies with all applicable federal and state regulatory standards.

This Manual is intended to assist Principal Investigators in compliance. Although this Manual may incorporate information from government references, it should be considered a companion to and not a substitute for the actual regulations or guidelines.

This Manual is intended to be fully consistent with applicable government references, such as the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) or the US Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* (CDC-NIH Standards). Accordingly, the applicable government references shall take precedence over inconsistencies between this Manual and the government references.

In the event of a regulatory or statutory change, then this procedure should be construed to conform to that change. A Principal Investigator or any other concerned person should bring any change to the attention of the Institutional Biosafety Committee (IBC) and the Office of Research.

All Principal Investigators shall be familiar with the laws applicable to that research. Failure to follow the procedures in this Manual is cause for administrative action and suspension or cancellation of any research approved by the IBC.

B. Scope

This Manual applies to University research, all University employees and students, and all those using University facilities. Except as described below, all Principal Investigators will apply to the IBC for approval prior to the submission of a proposal for funding or the commencement of research.

1. IBC approval is not required for experiments needing Biosafety Level 1 (Risk Group 1) or exempt activities under the NIH Guidelines. Only the Biological Safety Officer (BSO) may classify research as not requiring IBC approval. Nevertheless, any researcher working with human blood, clinical specimens, or other potentially infectious materials must still comply with any applicable guidelines, such as the University's *Bloodborne Pathogen Exposure Control Plan* (<http://www.odu.edu/af/ehs/Bloodborne%20Pathogens%20ECP.pdf>)
2. With respect to research involving rDNA, the scope of this Manual includes the NIH Guidelines (see Appendix D). By way of convenience, certain portions of the NIH Guidelines are repeated here, with instructions for implementation:

- a. Experiments described in Section III-F of the NIH Guidelines are exempt from registration with the IBC.
- b. Experiments described under Section III-E of the NIH Guidelines require IBC notification no later than simultaneous with initiation of the experiment. Only the BSO may classify research as falling into this category. It is the responsibility of the Principal Investigator to ensure simultaneous notification. Principal Investigators may desire to submit their notification to the IBC in advance of the experiment.
- c. Experiments described under Section III-D of the NIH Guidelines shall be reviewed and approved by the IBC prior to the commencement.
- d. Experiments using DNA that are exempt under Appendix C-II of the NIH Guidelines.

The IBC will review research proposals in order to assure the University that the work as described will be performed safely and lawfully, including the appropriate biosafety level according to Section IV of this manual. Some activities may require the approval of the Director of NIH or other party outside the University, which must be coordinated with the Office of Research. Non-exempt activities must be reviewed at least annually. Any substantial change, including location or personnel, must be approved beforehand by the IBC or the BSO, as applicable.

3. There shall be *no* research requiring Biosafety Level 3 or 4.

C. Primary Investigator Responsibilities

1. Introduction

The term Principal Investigator refers to the faculty member in charge of a research project. Principal Investigators bear the primary responsibility for compliance with all applicable laws, regulations, and guidelines. A subordinate role on a research project does not excuse non-compliance.

2. Basic Responsibilities

Principal Investigators have the following responsibilities:

- a. To initiate or modify no research which requires BSO review or IBC approval until that research or the proposed modification thereof has been approved and has met all other requirements of the all applicable references.
- b. To determine whether experiments are covered by any statute, regulation, or guideline, and to comply with the applicable requirements before, during, and after the experiment.

- c. To instruct and train laboratory staff and self in the practices and techniques required to ensure safety and proper emergency response and notification procedures in the event of an accident or injury. The Principal Investigator must also familiarize his/her staff with the symptoms of exposure and other pertinent information about the biohazardous agent used in the experiment *before* allowing lab personnel to work with the agent.
- d. To supervise the laboratory staff diligently to ensure that all required safety practices and techniques are employed.
- e. To report any significant incidents (including failure of safety controls), violations of the CDC-NIH Standards, NIH Guidelines, or any significant research-related accidents and illnesses to the BSO immediately.
- f. To adhere to University procedures for handling accidental spills and personnel contamination.
- g. To inform research staff of the reasons and provisions for any precautionary medical practices, such as immunization or serum collection and to identify the need for health surveillance of individual research personnel and, with the BSO, the need for a health surveillance program for the overall project.
- h. To provide personal protective equipment to all research staff based on the experimental procedures used in the lab.
- i. To make a copy of all applicable guidelines, such as the CDC-NIH Standards, the NIH Guidelines, and other laboratory safety procedures available to the research staff.
- j. To maintain written documentation for all training activities, including instruction in laboratory safety procedures for all research staff personnel.
- k. To correct conditions that may result in the release of human etiologic agents.
- l. To refrain from activities with agents in Risk Group 3 and 4.
- m. To ensure that any research proposal budget includes funding for all safety, administrative, transportation, and waste disposal costs associated with the activity.

These responsibilities are exemplary and should not be construed as exhaustive. Clearly, any applicable guidance could impose particular procedures and safety practices beyond those listed here. For example, licensed clinical laboratories may be subject to guidelines

imposed by the appropriate licensing boards of the Commonwealth of Virginia. In the event of any questions, Principal Investigators shall contact the BSO and/or the Office of Research.

3. Applications

Principal Investigators shall prepare the following paperwork:

- a. For sponsored research proposals, the Principal Investigator shall complete a Proposal Transmittal Form taking care to choose all correct special review categories. The form can be found online at: <http://www.researchfoundation.odu.edu/forms.htm>
- b. Principal Investigators conducting rDNA non-exempt research shall complete the *Application for Review/Approval for Research Involving the Use of Recombinant DNA (rDNA)* (see Appendix A). Principal Investigators shall submit the application to the IBC in care of the Office of Research, Attention: BSO, no later than three weeks prior to the proposal deadline. The BSO will forward copies to members of the IBC, and the Environmental Health and Safety Office.
- c. Principal Investigators who believe their research is exempt from review shall submit the *Principal Investigator's Statement of Exemption* (see Appendix A) to the IBC in care of the Office of Research, Attention: BSO. In the event that the exempt status of the research is questionable, the BSO may submit the information to the IBC for review.

D. Biological Safety Officer (BSO)

1. Introduction

The BSO shall be the Office of Research, Research Compliance Coordinator (RCC). In the absence of the RCC, the Director of Environmental Health and Safety shall serve as the BSO. The BSO shall be generally responsible for facilitating and managing the biosafety program.

2. Basic Responsibilities

Principal Investigators bear the primary responsibility for compliance with all applicable laws, regulations, and guidelines. In general, the BSO shall assist Principal Investigators and the University with such compliance. The BSO shall have the following responsibilities:

- a. Review and approval of research protocols involving the use of Biosafety Level 2 agents, excluding non-exempt rDNA activities under the NIH Guidelines.

- b. Conducting periodic inspections to ensure laboratory standards are rigorously followed.
- c. Reporting to the IBC and the University's administration any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the BSO becomes aware.
- d. Developing laboratory specific emergency plans for handling accidental spills and personnel contamination, and investigating laboratory accidents involving rDNA research.
- e. Providing advice on laboratory security.
- f. Providing technical advice to Principal Investigators and the IBC on research safety procedures for the purpose of assuring that the use of human etiologic agents conforms to the University policy and applicable governmental regulations.
- g. In support of Principal Investigators, determine the necessity for health surveillance of rDNA research personnel, and conduct, if appropriate, a health surveillance program for the project.

E. Institutional Biosafety Committee (IBC)

1. Introduction

The primary role of the IBC is to review research and to assure that the University complies with applicable guidelines. The IBC reports to the University President via the Vice President for Research.

2. Jurisdiction

In accordance with this Manual, the IBC reviews all teaching and research projects involving non-exempt use of rDNA under NIH Guidelines. A form entitled *Application for Review/Approval for Research Involving the Use of Recombinant DNA (rDNA)* (see Appendix A) must be submitted to the IBC for review and approval. Through these reviews, the IBC assures the University that the proposed activities and related facilities comply with applicable University policies and external guidelines.

3. Composition

- a. The Vice President for Research shall appoint at least seven (7) members to form the IBC. One of the seven members shall serve as Chair.
- b. The BSO shall be a permanent member of the Committee: however he/she may not serve as Chair.
- c. At least two members shall not be affiliated with the institution (apart from their membership on the IBC) and shall represent the interests of the

surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community).

- d. In order to ensure the competence necessary to review and approve rDNA activities, it is recommended that the IBC:
 - (1) Include persons with expertise in rDNA technology, biological safety, and physical containment.
 - (2) Include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable laws, standards of professional conduct and practice, community attitudes, and environmental protection.
 - (3) Include at least one member representing the laboratory technical staff.
- e. If plant-related experiments require review, then the IBC shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles. Experiments utilizing Appendix P of the NIH Guidelines, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, require IBC approval prior to commencing with the experiment(s). Otherwise, the IBC shall not approve any plant-related experiments until the composition of the IBC is amended.
- f. If animal-related experiments require review, then the IBC shall include at least one individual with expertise in animal containment principles. Experiments utilizing Appendix Q of the NIH Guidelines, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, require IBC approval prior to commencing with the experiment(s). Otherwise, the IBC shall not approve any animal related experiments until the composition of the IBC is amended.
- g. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

4. Scope of Review

IBC approval must be obtained from each institution at which non-exempt rDNA research is conducted. The IBC shall review proposals and shall give proper consideration to the following:

- a. The IBC will review each application in order to assure the University that the work, as described, will be performed in accordance with the appropriate guidelines.
 - b. The IBC review should consider in depth experimental methods, laboratory procedures, and facilities to assure compliance in:
 - (1) Containment levels.
 - (2) Procedures, practices, training, and expertise of personnel, medical surveillance, data reporting, and incident reporting.
 - c. The adequacy of emergency plans for accidental spills and personal contamination.
 - d. In the event of a proposal for rDNA research involving human subjects, the IBC shall present a memorandum to the Vice President for Research showing that:
 - (1) The IBC has adequate expertise and training (using ad hoc consultants as deemed necessary).
 - (2) All aspects of Appendix M of the NIH Guidelines, *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)*, have been appropriately addressed by the Principal Investigator prior to submission to NIH/OBA.
5. General
- a. The IBC shall meet as necessary and prudent. The IBC should meet at least once a year. Official meetings shall not begin until at least a quorum of members is present, including the Chair, BSO and one community member.
 - b. The IBC is responsible for advising the University on all matters related to biosafety, for reviewing and approving proposed uses of human etiologic agents, and for advising and guiding the Office of Research in carrying out the Biosafety Program.
 - c. The IBC is collaterally responsible for review of any addendum to the *Biosafety Policies and Procedure Manual* for conformance with biosafety standards from the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), and other applicable regulations.

- d. The IBC Chair is responsible for preparation of an annual report to the Vice President for Research and the Research Compliance Coordinator (RCC). The RCC shall edit and forward the report to NIH/OBA. The annual report shall include the following:
 - (1) A roster of all IBC members clearly indicating the Chair, contact person, BSO, plant expert (if applicable), animal expert (if applicable), human gene therapy expertise or ad hoc consultant (if applicable).
 - (2) Biographical sketches of all IBC members (including community members).

F. College Deans

1. Introduction

The Dean of the College is responsible for ensuring that research conducted in his/ her College complies with federal and state laws and guidelines. However, these responsibilities in no way diminish the primary responsibilities of the Principal Investigators.

2. Basic Responsibilities

The Dean shall have the following responsibilities:

- a. Ensure that their Principal Investigators are reasonably trained and equipped so as to be capable of compliance with this Procedure. The Dean shall remain reasonably familiar with the substance of the research in the College that is subject to this Procedure.
- b. Each year prior to July 1, shall nominate no fewer than four faculty members to the Vice President of Research to become members of the IBC. The Dean's nominees shall include faculty members who are qualified in plant and animal containment research.
- c. In addition, the Dean shall nominate two members who are not affiliated with the University (apart from their membership on the IBC) and who represent the interest of the surrounding community.

G. Vice President for Research

1. Introduction

The Old Dominion University Vice President for Research serves as the administrator responsible for oversight of the IBC review process. The Vice President for Research is the institutional officer responsible for ensuring institutional compliance with regulations and statutes.

2. Basic Responsibilities
 - a. The Vice President for Research is responsible for the overall administration of the Biosafety Program.
 - b. The Vice President for Research is responsible for ensuring constructive communication among administrators, Deans, Principal Investigators, and others as a means to achieving safe research.
 - c. The Vice President for Research is responsible for periodic review of federal and state regulations or guidelines and along with the IBC, amending this manual.
 - d. The Vice President for Research, with the assistance of the Deans, shall appoint the members of the IBC in accordance with this manual.
 - e. For all research subject to federal guidelines, the Vice President for Research shall report promptly to the NIH, or other sponsoring federal entity within 30 days of:
 - (1) Any research related accidents and illnesses.
 - (2) Any significant problems, or violations of the NIH Guidelines.
 - f. At a minimum, the Vice President for Research shall maintain research records for at least three years after completion of the research activity.
 - g. The Vice President for Research shall make or have made reports required by applicable guidelines

II. REVIEW PROCEDURES

A. **Application: Application for Review/Approval for Research Involving the Use of Recombinant DNA (rDNA)**

Prior to the conduct of research involving NIH non-exempt rDNA, Principal Investigators must submit the form *Application for Review/Approval for Research Involving the Use of Recombinant DNA (rDNA)* (see Appendix A). These applications are available from the Environmental Health and Safety Office and the Office of Research. Completed applications must be sent to the IBC in care of the Office of Research, attention BSO.

An application may only be approved for one year and may be renewed for a total approval of up to five years, if reviewed annually. After five years, if work on the originally approved application is still continuing, the Principal Investigator will be asked to submit a new application to the IBC for approval. Researchers should use the Recombinant DNA Research Protocol Renewal/Amendment Form for annual renewal or amendments (See Appendix B).

B. **Biosafety Level 2 Safety Review (NIH Exempt Research)**

Prior to research involving Biosafety Level 2 agents, Principal Investigators must submit a summary of their research plans to the BSO for approval. Information that must be included in the summary is found on the form *Biosafety Level 2 Safety Review* (see Appendix C). To avoid delays in approval, Principal Investigators are encouraged to address each item listed on the form.

Send this information to the Office of Research, Attention: BSO. The BSO will review the information and consult with the Principal Investigator if there are any questions or concerns. The Principal Investigator will receive confirmation to proceed with the experiment(s) after all concerns are addressed.

III. PRINCIPLES OF BIOSAFETY

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where the agents are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

The three elements of containment include laboratory practice and technique, safety equipment, and facility design. *Both good microbiological technique and the use of appropriate safety equipment provide primary containment.* The use of vaccines may provide an increased level of personal protection. *Secondary containment is provided by a combination of facility design and operational practices.* A risk assessment of the work that will be undertaken with a specific agent will determine the appropriate combination of the elements.

A. Laboratory Practice and Technique

Persons working with infectious agents and/or potentially infectious materials must be aware of potential hazards, and must be trained and proficient in standard microbiological practices and techniques, and the practices and techniques required for handling such material safely. Only those persons who are trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents may direct laboratory activities.

Principal Investigators are responsible for developing and adopting standard operating procedures that identify the potential hazards that will or may be encountered when working with infectious agents in their area of concern. These standard operating procedures must specify practices and procedures designed to minimize or eliminate risks.

Principal Investigators are also responsible for advising personnel working in their facility of the procedures and policies established for minimizing the potential for exposure to infectious agents. This can be accomplished by requiring personnel to read and become familiar the practices and procedures outlined in the facility's standard operating procedures.

Standard laboratory practices may not be sufficient to control the hazard associated with a particular agent or laboratory procedure; therefore, additional measures must be employed. The Principal Investigator is responsible for selecting any additional safety measures required to minimize risk of exposure to facility personnel.

B. Safety Equipment (Primary Barriers)

Safety equipment includes biological safety cabinets, enclosed containers, and other *engineering controls* designed to remove or minimize exposures to hazardous biological materials.

The Biological Safety Cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many laboratory procedures. As with any other piece of laboratory equipment, personnel must be trained in the proper use of BSC's. Of particular note are those activities that may disrupt the inward directional airflow through the work opening of (Class I and II) cabinets. Strict adherence to recommended practices for the use

of BSCs and proper placement in the laboratory are important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself. All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system

There are three (3) types of BSC's:

1. A Class I BSC is an open-fronted, negative pressure ventilated cabinet with a minimum inward face velocity of 75 linear feet per minute at the work opening. The exhaust air is filtered with a HEPA filter. Class I BSC's must be tested and certified in situ at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter.
2. A Class II BSC is an open-fronted, vertical laminar flow ventilated cabinet with an average inward face velocity of 75 linear feet per minute at the work opening. The cabinet is equipped with a HEPA filter that filters air recirculated within the cabinet. HEPA filters also filter the exhaust air. Class II BSC's must be tested and certified in situ at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Class II BSCs are subclassified into two types (A and B) based on construction, airflow velocities and patterns, and exhaust systems:
 - a. Type A cabinets are suitable for work with microbiological research in the absence of volatile or toxic chemicals and radionuclides, since air is recirculated within the work area. Type A cabinets may be exhausted through HEPA filters into the laboratory, or to the outside via a "thimble" connection to the exhaust.
 - b. Type B cabinets are hard-ducted to the exhaust system, and contain negative pressure plena. These features, plus an increased face velocity of 100 linear fpm, allow work to be done with toxic chemicals or radionuclides.

Both Class I and II BSC's should be located away from traffic patterns and doors so as to minimize the disruption of air flow into the cabinet.

3. A Class III BSC is totally enclosed and ventilated and operates under negative pressure. Class III BSC's are gas-tight and offer the highest degree of personnel and environmental protection from infectious aerosols, as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require BioSafety Level 3 or 4 containment.
 - a. All operations in the work area of the cabinet are performed through attached rubber gloves.

- b. Supply air is HEPA-filtered, and the cabinet exhaust air is filtered by two HEPA filters in series, or HEPA filtration followed by incineration, before discharged outside of the facility.

The Class III cabinet must be connected to a double-doored autoclave and chemical dunk tanks to sterilize or disinfect all materials exiting the cabinet, and to allow supplies to enter the cabinet. Several Class III cabinets are typically set up as an interconnected system.

Table 1 summarizes the characteristics of each classification of biosafety cabinet:

Table 1 Characteristics of Biosafety Cabinets				
Type	Face Velocity (linear ft/min)	Radionuclides or Toxic Chemicals	BioSafety Level	Protects Product(s) Inside?
Class I	75	No	2 and 3	No
Class II/Type A	75	No	2 and 3	Yes
Class II/Type B1	100	Yes	2 and 3	Yes
Class II/Type B2	100	Yes	2 and 3	Yes
Class 3	N/A	Yes	3 and 4	Yes

Other examples of primary barriers include centrifuge cups (i.e., enclosed containers designed to prevent aerosols from being released during centrifugation), and items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with BSC's and other devices that contain the agents, animals, or materials as they are being manipulated.

In certain situations it is impractical to work in BSC's. In these cases personal protective equipment may form the primary barrier between personnel and the infectious materials.

C. Facility Design (Secondary Barriers)

The design of the facility is important in providing a barrier to protect persons working inside and outside of the laboratory within the facility, and to protect persons or animals in the community from infectious agents that might be accidentally released from the facility. The

Administration is responsible for providing facilities that meet the applicable criteria recommended for the biosafety level of the agents used in the facility.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Design features such as specialized ventilation systems to assure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules for isolation of the laboratory may be indicated depending on the agents used in the facility. Refer to Section III of this manual for specific recommendations.

D. Universal Precautions

All blood or other potentially infectious material (OPIM) described in the University's *Bloodborne Pathogen Exposure Control Plan* shall be handled as if contaminated by a bloodborne pathogen. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials. Universal precautions as described in the *Bloodborne Pathogen Exposure Control Plan* shall always be used in such circumstances.

IV. RISK GROUPS FOR BIOSAFETY LEVELS

The following descriptions of infectious agents shall be used to determine the level of protection needed when working with a particular microorganism:

A. Risk Group 1

Risk Group 1 agents are not known to cause disease in healthy adult humans. For work with Risk Group 1 agents, and for standard rDNA experiments, Biosafety Level 1 recommendations should be used.

B. Risk Group 2

Risk Group 2 agents may cause disease in humans but the disease process is rarely fatal and there are standard preventative and therapeutic interventions available. Biosafety Level 2 recommendations should be used when working with a Risk Group 2 microorganism.

C. Risk Group 3

Risk Group 3 agents can cause serious or fatal disease in humans as the result of exposure by inhalation. Preventative and therapeutic interventions may be available. Biosafety Level 3 recommendations should be used when working with a Risk Group 3 microorganism.

D. Risk Group 4

Risk Group 4 agents are likely to cause serious illness or lethal disease for which preventative or therapeutic interventions are not usually available. Biosafety Level 4 recommendations should be used when working with a Risk Group 4 microorganism. Specific agents and their assigned Risk Group can be found in Appendix E of this manual.

V. LABORATORY BIOSAFETY LEVEL CRITERIA AND REQUIREMENTS

Biosafety level designations are based on the degree of protection required to protect employees working with the agent(s), the environment and the community. Four designations are recognized: Biosafety Level 1, Biosafety Level 2, Biosafety Level 3, and Biosafety Level 4. A summary of requirements for each biosafety level is found in Table 2.

Table 2 Summary of Requirements for Biosafety Levels ¹				
Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy humans	Standard microbiological practices	None	Open bench/Sink required
2	Associated with human disease via auto-inoculation, ingestion, and mucous membrane exposure	Biosafety Level 1 practices plus: a. Limited access b. Biohazard warning sign c. Sharps precautions d. Biosafety manual	a. Class 1 or 2 biosafety cabinets or other physical containment when manipulating agents b. PPE such as gloves, lab coats and face protection	Biosafety Level 1 plus autoclave available for decontaminating cultures, etc.
3	Indigenous or exotic agents with potential for aerosol transmission. Agents may cause serious or lethal health effects	Biosafety Level 2 practices plus: a. Controlled access b. Decontamination of all waste c. Decon. of lab clothing before laundering d. Baseline serum taken	a. Class 1 or 2 biosafety cabinets or other physical containment when manipulating agents b. PPE such as gloves, lab coats and face protection c. Respiratory protection as needed	Biosafety Level 2 plus: a. Physical separation from access corridors b. Self-closing double door access c. Exhausted air not recirculated d. Negative airflow into lab
4	Dangerous/exotic agents with a high risk of life-threatening disease, aerosol transmitted infections or agents with unknown risk	Biosafety Level 3 practices plus: a. Clothing change before entering b. Shower before exiting c. All material decontaminated before leaving the facility	All manipulations conducted in Class 3 biosafety cabinets, or Class 1 or 2 biosafety cabinets in combination with air-supplied positive pressure suits	Biosafety Level 3 plus: a. Separate building or isolated zone b. Dedicated supply/exhaust vacuum and decon. systems c. Other

¹From *Biosafety in Microbiological and Biomedical Laboratories*

A. Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. For work with Biosafety Level 1 agents:

1. The laboratory is not necessarily separated from the general traffic patterns in the building.
2. Work is generally conducted on open bench tops using standard microbiological practices.
3. Special containment equipment or facility design is neither required nor generally used.
4. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Standard operating procedures, special practices, safety equipment and facilities for laboratories working with Biosafety Level 1 agents include:

1. Limited or restricted access to the facility at the discretion of the Principal Investigator when experiments or work with cultures and specimens are in progress.
2. Hand washing after glove removal, and before leaving the laboratory after viable materials are handled.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in Biosafety Level 1 work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. The use of mechanical pipetting devices (mouth pipetting is prohibited).
5. The institution of policies for the safe handling of sharps.
6. Procedures that are performed carefully to minimize the creation of splashes or aerosols.
7. Decontamination of work surfaces at least once a day and after any spill of viable material.
8. Decontamination of all cultures, stocks, and other regulated wastes before disposal by an approved decontamination method such as autoclaving. Materials that will be decontaminated outside of the immediate laboratory:

- a. Shall be placed in a durable, leakproof container and closed for transport.
 - b. Shall be packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
9. Posting biohazard warning signs at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and phone number of the investigator.
 10. Institution of an insect and rodent control program.

No special practices are indicated for work with Biosafety Level 1 agents, and *primary barriers* such as special containment devices and equipment such as biological safety cabinets are generally not required for manipulation of Biosafety Level 1 agents; however:

1. The use of laboratory coats, gowns, or uniforms is recommended to prevent contamination or soiling of street clothes.
2. Gloves should be worn especially if the skin on the hands is broken or if a rash is present.
3. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

Laboratory facilities; i.e., secondary barriers, should have:

1. Doors for access control.
2. At least 1 sink for handwashing.
3. Work and floor surfaces that can be easily cleaned:
 - a. Carpets and rugs in laboratories are not appropriate for BioSafety Level 1 facilities.
 - b. Benches and other work surfaces should be impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the surfaces and equipment.
 - c. Spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Laboratory furniture should be capable of supporting anticipated loading and uses.
5. Laboratory windows that open to the exterior (if any) should be fitted with fly screens.

B. Biosafety Level 2

Biosafety Level 2 is applicable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BioSafety Level 1 in that:

1. Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists.
2. Access to the laboratory is limited when work is being conducted.
3. Extreme precautions are taken with contaminated sharp items.
4. Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Standard operating procedures, special practices safety equipment and facilities for laboratories working with Biosafety Level 2 agents include:

1. Limited or restricted access to the facility at the discretion of the Principal Investigator when experiments or work with cultures and specimens are in progress.
2. Hand washing after glove removal, and before leaving the laboratory after viable materials are handled.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in BioSafety Level 1 work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. The use of mechanical pipetting devices (mouth pipetting is prohibited).
5. The institution of policies for the safe handling of sharps.
6. Procedures that are performed carefully to minimize the creation of splashes or aerosols.
7. Decontamination of work surfaces at least once a day and after any spill of viable material.
8. Decontamination of all cultures, stocks, and other regulated wastes by an approved decontamination method such as autoclaving before disposal. Materials that will be decontaminated outside of the immediate laboratory:
 - a. Shall be placed in a durable, leakproof container and closed for transport.

- b. Shall be packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
9. Posting biohazard warning signs at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and phone number of the investigator.
10. Institution of an insect and rodent control program.

Unlike Biosafety Level 1, work with agents designated as Biosafety Level 2 requires special practices. Special practices for work with Biosafety Level 2 agents:

1. Access to the laboratory is limited or restricted by the Principal Investigator when work with infectious agents is in progress. In general:
 - a. Persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences such as individuals who are immunocompromised or immunosuppressed and may be at increased risk of acquiring infections are not allowed in the laboratory or animal rooms.
 - b. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room in which Biosafety Level 2 agents are present.
2. The Principal Investigator shall establish policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when Biosafety Level 2 agents are in use. Appropriate information to be posted on the warning sign includes:
 - a. The agent(s) being used.
 - b. The Biosafety Level.
 - c. Any required immunizations.
 - d. The investigator's name and telephone number.
 - e. Any personal protective equipment that must be worn in the laboratory and any procedures required for exiting the laboratory.
4. Laboratory personnel must receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. When appropriate, a baseline serum sample for laboratory and other at-risk personnel shall be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. A biosafety manual must be prepared and adopted. Personnel must be advised of special hazards and are required to read and to follow instructions on practices and procedures.
7. Laboratory personnel shall receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel shall receive annual updates, or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken when handling contaminated sharps, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative.
 - b. Plasticware should be substituted for glassware whenever possible.
 - c. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) should be used for injection or aspiration of infectious materials.
 - d. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - e. Puncture-resistant containers must be used for non-re-useable sharps disposal.
 - f. Re-useable sharps must be placed in a hard-walled container when transporting them to a processing area for decontamination (e.g., autoclaving).
 - g. Syringes, which re-sheath the needle, needle-less systems, and other safe devices, should be used when appropriate.
 - h. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

- i. Containers of contaminated needles, sharp equipment, and broken glass must be decontaminated before disposal, according to any local, state, or federal regulations.
9. Cultures, tissues, or specimens of body fluids must be placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces must be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials.
11. Contaminated equipment must be decontaminated according to local, state, or federal regulations before it is sent for repair or maintenance or packaged for removal and transport from the facility.
12. Spills and accidents, which result in overt exposures to infectious materials, must be reported immediately to the Principal Investigator. Medical evaluation, surveillance, and treatment are provided as appropriate. The Environmental Health and Safety Office maintains written records of such incidents and follow up medical surveillance and treatment.
13. Animals not involved in the work being performed are not permitted in the Biosafety Level 2 laboratory.

The following safety equipment (*primary barriers*) is required in Biosafety Level 2 laboratories:

1. Properly maintained biological safety cabinets (BSC's), preferably Class II, or other appropriate personal protective equipment or physical containment devices. Biological safety cabinets and/or physical containment devices must be used when:
 - a. Performing a procedure that has the potential for creating infectious aerosols or splashes. Such procedures may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such agents may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used and the rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (e.g., goggles, mask, faceshield or other splatter guards) must be used to protect against splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use only must be worn while in the laboratory:
 - a. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas.
 - b. All protective clothing is either disposed of in the laboratory or laundered by the institution.
 - c. Personnel must never take protective clothing home.
4. Gloves must be worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment.
 - a. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs.
 - b. Gloves must be disposed of when contaminated, removed when work with infectious materials is completed.
 - c. Gloves must never be worn outside the laboratory.
 - d. Disposable gloves must never be washed or re-used.

The following *secondary barriers* must be present in a Biosafety Level 2 laboratory:

1. Doors for access control.
2. At least 1 sink for handwashing.
3. Work and floor surfaces that can be easily cleaned:
 - a. Carpets and rugs in laboratories are not appropriate for Biosafety Level 2 facilities.
 - b. Benches and other work surfaces should be impervious to water and be resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the surfaces and equipment.
 - c. Spaces between benches, cabinets, and equipment are accessible for cleaning.

4. Laboratory furniture should be capable of supporting anticipated loading and uses.
5. Laboratory windows that open to the exterior (if any) should be fitted with fly screens.
6. A method for disposal (e.g., autoclave, chemical disinfectant, incinerator, or other approved decontamination system) of regulated medical waste must be available.
7. An eyewash facility must be readily available.

C. Biosafety Level 3

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

D. Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Research requiring Biosafety Level 4 is currently prohibited at the University.

VI. VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA & REQUIREMENTS

If experimental animals are used, the University shall provide facilities and staff and establish practices that reasonably assure appropriate levels of environmental quality, safety, and care. As a general principle, the biosafety level (facilities, practices, and operational requirements) that is recommended for working with infectious agents in vivo and in vitro is comparable. Ideally, facilities for laboratory animals used for studies of infectious or noninfectious disease should be physically separate from other activities.

There are four escalating combinations of practices, safety equipment, and facilities for experiments on animals infected with agents that produce, or may produce, human infection. These four combinations are designated Animal Biosafety Level (ABSL) 1, Animal Biosafety Level (ABSL) 2, Animal Biosafety Level (ABSL) 3, and Animal Biosafety Level (ABSL) 4. A summary of requirements for each animal biosafety level is found in Table 3.

Table 3 Animal Biosafety Level Requirements ¹			
Biosafety Level	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Standard animal care and management	None	Basic
2	Animal Biosafety Level 1 plus: a. Lab coats and gloves b. Decontamination of all infectious waste and animal cages before washing c. Limited access d. Hazard warning sign(s)	Partial containment equipment and/or personal protective devices when working with infected animals or agents that produce aerosols	Basic
3	Animal Biosafety Level 2 plus: a. Special lab. clothing b. Controlled access	Partial containment equipment and/or personal protective devices when working with all infected animals and agents	Containment
4	Animal Biosafety Level 3 plus: a. Entrance through clothes change room b. Shower upon exit c. All wastes decontaminated before removal from facility	Class 3 biosafety cabinets or partial containment plus full body, air supplied positive pressure suit used for all activities involving infected animals and agents	Maximum containment

¹ From *Biosafety in Microbiological and Biomedical Laboratories*

A. Animal Biosafety Level 1

Standard operating procedures, special practices, and safety equipment for Animal Biosafety Level 1 facilities include:

1. Limited or restricted access to the facility at the discretion of the Principal Investigator or animal facility director when experiments or work with cultures and specimens are in progress.
2. Doors to animal rooms must open inward, are self-closing and are kept closed when experimental animals are present.

3. Hand washing after handling cultures and animals, after glove removal and before leaving the animal facility.
4. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in Animal Biosafety Level 1 facility. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
5. Procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Decontamination of work surfaces after use and after any spill of viable material.
7. Institution of an insect and rodent control program.
8. All wastes from the animal room, including animal carcasses, must be disposed of as regulated medical waste.
9. Carcasses and waste transported from the animal room must be transported in leakproof, covered containers.

The following special practices should be used in an Animal Biosafety Level 1 facility:

1. The Principal Investigator or animal facility director should limit access to the animal room while work is in progress to personnel who have been advised of the potential hazard(s). Generally, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room.
2. The Principal Investigator or animal facility director must establish policies and standard operating procedures so that:
 - a. Only those persons who have been advised of the potential hazard(s) may enter the animal room.
 - b. Only those persons who meet any specific requirements (e.g., immunization) may enter the animal room.
3. Bedding materials from animal cages must be removed in such a manner as to minimize the creation of aerosols.
4. Cages must be washed manually or in a cage washer. Temperature of final rinse water in a mechanical washer should be 180 degrees F.

5. Laboratory coats, gowns, or uniforms should be worn in the animal facility. Laboratory coats worn in the animal facility not be worn in other areas.
6. A biosafety manual must be prepared and adopted.

Special containment equipment (i.e., primary barriers) is not required for animals infected with agents assigned to Animal Biosafety Level 1.

Animal facilities, i.e., secondary barriers, should have:

1. At least 1 sink for handwashing.
2. Work and floor surfaces that are can be easily cleaned.
3. Animal room windows that open to the exterior (if any) should be fitted with fly screens.
4. Exhaust air from an Animal Biosafety Level 1 animal room must be is discharged to the outside without being recirculated to other rooms. It is recommended (but not required) that the direction of airflow in the animal facility is inward (i.e., negative pressure with respect to the adjacent areas).

B. Animal Biosafety Level 2

Standard operating procedures, special practices, and safety equipment for Animal Biosafety Level 2 facilities include:

1. Limited or restricted access to the facility at the discretion of the Principal Investigator or animal facility director when experiments or work with cultures and specimens are in progress.
2. Doors to animal rooms must open inward, are self-closing and are kept closed when experimental animals are present.
3. Hand washing after handling cultures and animals, after glove removal and before leaving the animal facility.
4. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in Animal Biosafety Level 2 facility. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
5. Procedures that are performed carefully to minimize the creation of splashes or aerosols.
6. Decontamination of work surfaces use and after any spill of viable material.

7. Institution of an insect and rodent control program.
8. All wastes from the animal room, including animal carcasses, must be disposed of as regulated medical waste.
9. Infected carcasses and waste transported from the animal room must be transported in leakproof, covered containers.

The following special practices should be used in an Animal Biosafety Level 2 facility:

1. Access to the animal room is limited or restricted by the Principal Investigator when work with infectious agents is in progress. In general:
 - a. Persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences such as individuals who are immunocompromised or immunosuppressed and may be at increased risk of acquiring infections are not allowed in the animal rooms.
 - b. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the animal room in which Animal Biosafety Level 2 agents are present.
2. The Principal Investigator shall establish policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the animal room.
3. A biohazard sign must be posted on the entrance to the animal room when infectious agents are in use. Appropriate information to be posted on the warning sign includes:
 - a. The agent(s) being used.
 - b. The biosafety level.
 - c. Any required immunizations or special entry requirements.
 - d. The animal facility supervisor's name and telephone number.
 - e. Any personal protective equipment that must be worn in the animal room and any procedures required for exiting the animal room or facility.
4. Laboratory personnel must receive appropriate immunizations or tests for the agents handled or potentially present in the animal room.
5. When appropriate, a baseline serum sample for animal care and other at-risk personnel shall be collected and stored. Additional serum specimens may be

collected periodically, depending on the agents handled or the function of the facility.

6. A biosafety manual must be prepared and adopted. Personnel must be advised of special hazards and are required to read and to follow instructions on practices and procedures.
7. Laboratory personnel shall receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken when handling any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the animal room for use only when there is no alternative.
 - b. Plasticware should be substituted for glassware whenever possible.
 - c. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) should be used for injection or aspiration of infectious materials.
 - d. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - e. Puncture-resistant containers must be used for non-re-useable sharps disposal.
 - f. Re-useable sharps must be placed in a hard-walled container when transporting them to a processing area for decontamination (e.g., autoclaving).
 - g. Syringes, which re-sheath the needle, needle-less systems, and other safe devices, should be used when appropriate.
 - h. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.
 - i. Containers of contaminated needles, sharp equipment, and broken glass must be disposed of as regulated medical waste.

9. Cultures, tissues, or specimens of body fluids must be placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Equipment and work surfaces must be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials.
11. Cages must be appropriately decontaminated, preferably by autoclaving, before they are cleaned and washed.
12. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for removal and transport from the facility.
13. Spills and accidents, which result in overt exposures to infectious materials, must be reported immediately to the Principal Investigator. Medical evaluation, surveillance, and treatment are provided as appropriate. The Environmental Health and Safety Office maintains written records of such incidents and follow up medical surveillance and treatment.
14. Animals not involved in the work being performed are not permitted in the Animal Biosafety Level 2 room.
15. A biosafety manual must be prepared and adopted.

The following safety equipment (*primary barriers*) is required in Animal Biosafety Level 2 rooms:

1. Properly maintained Biological Safety Cabinets (BSC's), preferably Class II, or other appropriate personal protective equipment or physical containment devices. Biological safety cabinets and/or physical containment devices must be used when:
 - a. Performing a procedure that has the potential for creating infectious aerosols or splashes. Such procedures may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - b. When high concentrations or large volumes of infectious agents are used. Such agents may be centrifuged in the open room if sealed rotor heads or centrifuge safety cups are used and the rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (e.g., goggles, mask, faceshield or other splatter guards) and all personnel entering animal rooms housing non-human primates must wear respiratory protection.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for animal room use only must be worn while in the animal room:
 - a. Protective clothing must be removed and left in the animal room before leaving for other areas.
 - b. All protective clothing is either disposed of in the animal room or laundered by the institution.
 - c. Personnel must never take protective clothing home.
4. Gloves must be worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment.
 - a. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs.
 - b. Gloves must be disposed of when contaminated, removed when work with infectious materials is completed.
 - c. Gloves must never be worn outside the animal room.
 - d. Disposable gloves must never be washed or re-used.

The following *secondary barriers* must be present in Animal Biosafety Level 2 rooms:

1. At least 1 sink for handwashing.
2. Work and floor surfaces that are can be easily cleaned and maintained:
 - a. Bench and other work surfaces should be impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the surfaces and equipment.
 - b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
3. Animal room windows that open to the exterior (if any) should be fitted with fly screens.
4. A method for decontamination (e.g., autoclave, chemical disinfectant, incinerator, or other approved decontamination system) of infectious or regulated medical

wastes must be available.

5. An eyewash facility must be readily available.
6. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
7. Exhaust air from an Animal Biosafety Level 2 animal room must be discharged to the outside without being recirculated to other rooms. It is recommended (but not required) that the direction of airflow in the animal facility is inward (i.e., negative pressure with respect to the adjacent areas).

C. Animal Biosafety Level 3

Research requiring Animal Biosafety Level 3 is currently prohibited at the University.

D. Animal Biosafety Level 4

Research requiring Animal Biosafety Level 4 is currently prohibited at the University.

VII. DISINFECTANS AND STERILIZATION

A. Disinfectants

A *disinfectant* is a chemical that is used to kill growing forms of a microorganism. A disinfectant will not necessarily kill the resistant spore forms of bacteria and may either be a chemical or a physical process. Typical disinfectants include:

1. Chlorine compounds.
2. Iodine compounds.
3. Phenolics.
4. Ethyl and isopropyl alcohol.
5. Glutraldehyde.

Choosing the most effective disinfectant for a particular type of microorganism is a function of the microorganism's sensitivity to that agent. For most applications, chlorine compounds, e.g., a 10% solution of Chlorox in water will be effective in disinfecting surfaces and is recommended. Table 4 summarizes the uses and requirements for various disinfectants.

Table 4 Summary of Uses and Requirements of Various Disinfectants

		Phenolics	Chlorine Compounds	Ethyl Alcohol	Isopropyl Alcohol	Iodophor	Gluter-aldehyde
Requirements	Dilution	1 to 5%	5000 ppm ²	70 to 85%	70 to 85%	25-1600 ppm	2%
	Viruses w/envelope ¹	10 min.	10 min.	10 min.	10 min.	10 min.	10 min.
	Broad spectrum ¹	NE	30 min.	NE	NE	30 min.	30 min.
Inactivates	Bacteria	+	+	+	+	+	+
	Viruses w/envelope	+	+	+	+	+	+
	Viruses w/out envelope	variable	+	variable	variable	+	+
	Bacterial spores	-	+	-	-	+	+
Applications	Work surfaces	+	+	+	+	+	-
	Glassware	+	+	+	+	+	-
	Large area decon.	-	-	-	-	-	-
	Liquid for discharge	-	+	-	-	-	-
	Penetrating decon.	-	-	-	-	-	+

NE = not effective ¹ Contact time ² Available chlorine (for Chlorox this corresponds to solution that 1:10 Chlorox to water)

B. Sterilization

Sterilization destroys all forms of infectious microorganisms, including disinfectant resistant bacterial spores. Sterilization can be accomplished using certain chemicals such as ethylene oxide and gluteraldehyde, or physically by exposing the microorganisms to ultraviolet light (non-ionizing radiation), ionizing radiation, heat and/or steam.

Of the means of sterilization, steam sterilization in an autoclave is the most readily available means to deactivate infectious and regulated medical waste, and sterilize instruments and equipment. Refer to the University's *"Regulated Medical Waste Management Guidelines"* (<http://www.odu.edu/af/ehs/programs.html>) for specific requirements regarding identifying regulated medical waste streams and autoclaving regulated medical waste.

VIII. EMERGENCY PROCEDURES

When a spill of infectious materials occurs the chosen course of action should minimize the potential hazard to those persons working in the laboratory or animal room, and to those persons in close proximity to the spill, and minimize impact on the environment. A generic set of procedures may not be sufficient to anticipate all types of spills; however, the following general emergency procedures should be followed in case of a spill:

1. To the extent possible and prudent, contain the spill.
2. Notify everyone in the affected area that a spill has occurred.
3. Vacate all persons in the affected area.
4. Contact the Environmental Health and Safety Office (757-683-4495) during normal working hours) and Public Safety (757-683-4000) outside of normal working hours and on weekends.
5. Determine the extent of possible exposure(s) and treat affected persons to the extent of the available resources.
6. Assess the extent of the spill noting any additional equipment that might be needed before re-entry into the facility.
7. Re-enter and decontaminate the affected area.

Following any spill involving infectious material, the Environmental Health and Safety Office is responsible for determining the need for any follow-up medical attention or monitoring as well as any corrective action(s) that must be taken in order to minimize the risk for further incidents.

A. Spills Inside Containment

The effects of spills inside containment e.g., inside biosafety cabinets are minimal so long as the containment is functioning properly. This is due to the fact that the material, even if it is aerosolized, is still contained (the risk of inhalation of the material is minimal) and the area affected is relatively small. When a spill occurs inside containment:

1. Don gloves and face protection.
2. Wipe or spray the walls, work surfaces and any affected equipment with a disinfectant appropriate for the infectious agent being used (see Section V.) A suitable disinfectant for most applications is a 1:10, (Chlorox:water) bleach solution.
3. Waste from disinfecting the containment (i.e., gloves, and absorbent paper) must be handled as regulated medical waste.

B. Laboratory Spills

Unlike spills that occur inside containment, spills that occur in open areas, e.g., bench tops, floors, etc. have a much greater hazard potential because laboratory/animal room personnel may be exposed to aerosolized infectious agents. If a spill occurs, it is essential that persons in the laboratory/animal room vacate the area without inhaling any airborne material.

When a spill occurs outside of containment:

1. Hold your breath and exit the area.
2. Warn others that a spill has occurred. Prevent any entry into the area.
3. Remove all clothing that is contaminated or suspected of being contaminated, taking care to avoid contact with other clothing, etc.
4. Contact the Environmental Health and Safety Office (or Public Safety).
5. Wait at least 30 minutes before reentering to disinfect the area(s).
6. Don personal protective equipment, i.e., gloves, shoe covers, outerwear and a respirator.
7. Apply a 1:10, (Chlorox:water) bleach solution to the affected area(s). Allow at least 15 minutes of contact time.
8. Remove the material/bleach solution with absorbent paper. Dispose of the waste (including gloves and any contaminated clothing) as regulated medical waste.

IX. TRANSPORTATION AND SHIPMENT OF INFECTIOUS AGENTS AND RECOMBINANT DNA

For information concerning the importation, exportation and receipt of etiological agents, consult 42 CFR, Section 71.156, *Health Service Foreign Quarantine Regulations*. Permits authorizing the importation or receipt of regulated materials and specifying conditions under which the agent or vector is shipped, handled, and used are issued by the Centers for Disease Control and Prevention (Attention: Biosafety Branch Office of Health and Safety, Mail Stop F-05 1600 Clifton Road N.E. Atlanta, Georgia 30333 Telephone: (404) 639-3883 Fax: (404) 639-2294)

Persons needing to report leaking damaged packages of etiologic agents may call 1-800-232-0124.

**APPENDIX A:
Application for the Use of Recombinant DNA in Research**

Directions for Completing "Application for the Use of Recombinant DNA in Research"

Submission and approval of "Application for the Use of Recombinant DNA in Research" by the IBC is required for experiments described in the NIH Guidelines (April 2002), Sections III A through E. Additional approval by other agency(s) is also indicated for experiments described in the NIH Guidelines (April 2002), Sections III A through C.

1. Exemptions

The rDNA experiments listed below are exempt from NIH Guidelines (Section III F) and therefore do not require IBC approval (or completion of "Application for the Use of Recombinant DNA in Research"):

- A. Experiments using DNA that is not in an organism or virus. [Section III, F-1 of the NIH Guidelines]
- B. Experiments using DNA segments from a single nonchromosomal or viral DNA source. [Section III, F-2 of the NIH Guidelines]
- C. Experiments using DNA entirely from a prokaryotic host propagated only in that host. [Section III, F-3 of the NIH Guidelines]
- D. Experiments using DNA entirely from an eukaryotic host propagated only in that host. [Section III, F-4 of the NIH Guidelines]
- E. Experiments (exclusively) using DNA segments from different species that exchange DNA by known physiologic processes [Section III, F-5 of the NIH Guidelines].
- F. Experiments using DNA that are exempt under Appendix C-II of the NIH Guidelines

If the experiments that are proposed are exempt according to the NIH Guidelines, do not complete "Application for the Use of Recombinant DNA in Research," instead complete the "Principal Investigator's Statement of Exemption" included in this packet and return it to the Office of Research, attention BSO.

2. Information required for proposed experimental protocol (number 9 of the application):

In order for the IBC to thoroughly evaluate the proposed experiment(s), the Principal Investigator must provide the Committee with the following information:

- A. Sources of DNA (species, organ or tissue, etc.).
- B. Nature of the inserted DNA sequence.
- C. Describe hosts and vectors, and the host/vector combination(s) that will be used.
- D. Will a deliberate attempt be made to obtain expression of a foreign gene? If so, what protein will be produce?

- E. A risk assessment which addresses the following:
 - a. Virulence.
 - b. Pathogenicity.
 - c. Infectious dose.
 - d. Agent/environmental stability.
 - e. Route of spread.
 - f. Communicability.
 - g. Quantity, i.e., volume(s)/concentration(s) of the culture(s).
 - h. Availability of effective prophylaxis.
 - i. Gene product effects (e.g., toxicity, physiological activity, and allergenicity).
- F. Biosafety level specified in the NIH Recombinant DNA Guidelines.
- G. Procedures for storage, transport (both within and outside Old Dominion University), and disposal.
- H. Provisions for laboratory security including access control.
- I. Microbiological techniques that will be used (e.g., mechanical pipettes, gloves, lab coats, etc.). Safety controls that are available for use when performing the experiment(s).
- J. Provisions for medical intervention (if necessary), either preventative or therapeutic available to laboratory personnel.
- K. A description of containment equipment (e.g., biosafety cabinets, centrifuge caps etc.) that will be used when manipulating the agent(s).
- L. Procedures for disinfecting surfaces and equipment, and sterilizing viable cultures of the agent(s) before disposal.
- M. Emergency procedures that will be taken in case of spills and personal contamination.
- N. Instruction and training for laboratory personnel.

Principal investigators should address each item such that there is enough detail for the IBC to decide whether the safety controls that will be employed comply with NIH Guidelines and are adequate to protect the safety of the employees and others and protect the environment. The IBC will review the information and consult with the Principal Investigator if there are any questions or concerns. The Principal Investigator will receive confirmation to proceed with the experiment(s) after all concerns are addressed.

IBC No.: _____

Date: _____

**Old Dominion University
Institutional Biosafety Committee
Application for the Use of Recombinant DNA in Research**

1. Principal Investigator: _____
Department: _____
Bldg.: _____ Room No.: _____
Phone No.: _____ Fax No.: _____
E-Mail: _____
2. Project Title* _____
3. Funding Source/ODURF No.: _____
4. Co-Investigator(s):
Investigator: _____ Phone: _____
Investigator: _____ Phone: _____
Investigator: _____ Phone: _____
5. Project Site(s):
(List each site where activities/procedures will be performed)
 - A. Investigator: _____ Site: _____
Activity/Procedure: _____
 - B. Investigator: _____ Site: _____
Activity/Procedure: _____
 - C. Investigator: _____ Site: _____
Activity/Procedure: _____
6. Laboratory Personnel: List laboratory personnel who will be working with the agents, and other persons who have a reasonable opportunity to frequent the laboratory. Specify the average amount of time per week the person(s) will be working with the agents:

7. Additional Committee Reviews Required (place X on line):

___ Radiation Safety Committee
Use Committee

___ Institutional Review Board

___ Institutional Animal Care &

8. Federally Mandated Category of Review: Check the boxes that describe all of the proposed uses of rDNA. Refer to the NIH Guidelines for a more detailed description of each category.

- A. Experiments that require IBC approval, RAC (Recombinant DNA Advisory Committee), and NIH approval prior to initiation (Major Actions).
- Deliberate transfer of a drug resistant trait to microorganisms not known to acquire this trait naturally. [Section III, A of the NIH Guidelines]
- B. Experiments that require IBC and NIH/ORDA (Office of Recombinant DNA Activities) approval before initiation.
- Cloning of Toxic molecules with LD 50 of less than 100 nanogram per kg of body weight. [Section III, B of the NIH Guidelines]
- C. Experiments that require IBC and Institutional Review Board approval and NIH/ORDA registration prior to initiation.
- Deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA into one or more human subjects. [Section III, C of the NIH Guidelines]
- D. Experiments that require IBC approval before initiation
- Using risk group 2,3, or 4 restricted agents as Host-Vector Systems [Section III, D-1 of the NIH Guidelines]
 - Cloning DNA for risk group 2, 3, or 4 restricted agents into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. [Section III, D-2 of the NIH Guidelines]
 - Using infectious or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems. [Section III, D-3 of the NIH Guidelines]
 - Using whole animals. [Section III, D-4 of the NIH Guidelines]
 - Using whole plants. [see Sections III, D-5 E-2 of the NIH Guidelines]
 - Using more than 10 liters of culture. [Section III, D-6 of the NIH Guidelines]
- E. Experiments that require IBC notice simultaneous with initiation
- Forming rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus. [Section III, E-1 of the NIH Guidelines]
 - Using whole plants. [see Sections III, D-5 and E-2 of the NIH Guidelines]
 - Experiments involving transgenic rodents. [Section III, E-3 of the NIH Guidelines]

9. Using the Directions for Completing "Application for the Use of Recombinant DNA in Research as a guide, attach a description of the proposed experiments. Include all information as outlined in the directions.

10. Principal Investigator's Certification

I attest that the information contained in this application is accurate and complete. I agree to comply with the requirements pertaining to shipment, handling, transfer, and disposal of biohazardous agents, and rDNA. I am familiar with and agree to abide by the provisions of the current NIH Guidelines and other specific granting agency instructions pertaining to the proposed project.

I further attest that all my research personnel are familiar with and understand the potential biohazards, proposed precautions, and appropriate emergency procedures, and that the practices and techniques required to ensure safety will be followed. I agree to accept responsibility for training of all laboratory workers involved in the project.

I will submit written reports, as required, to the IBC (through the Office of Research) concerning:

- A. Any accident that results in inoculation, ingestion, and inhalation of biohazardous agents or any incident causing serious exposure of personnel or danger of environmental contamination.
- B. Any problems pertaining to operation and implementation of biological and physical containment safety procedures or equipment or facility failure.
- C. Any new information bearing on the Guidelines such as technical information relating to hazards and safety procedures or innovations.

I will not carry out the work described in this application until it has been filed and/or approved by the IBC or until the requirements of all sponsoring agency(s) have been met.

Signature: _____

Date: _____

Principal Investigator's Statement of Exemption

The following is a list of exempt activities for the use of rDNA under the NIH Guidelines (April 2002), Section III F. For more details, reference the NIH Guidelines. Check the appropriate box corresponding to the type of experiment(s) that you propose to conduct:

- Experiments using DNA that is not in an organism or virus. [Section III, F-1 of the NIH Guidelines]
- Experiments using DNA segments from a single nonchromosomal or viral DNA source. [Section III, F-2 of the NIH Guidelines]
- Experiments using DNA entirely from a prokaryotic host propagated only in that host. [Section III, F-3 of the NIH Guidelines]
- Experiments using DNA entirely from an eukaryotic host propagated only in that host. [Section III, F-4 of the NIH Guidelines]
- Experiments (exclusively) using DNA segments from different species that exchange DNA by known physiologic processes. [Section III, F-5 of the NIH Guidelines].
- Experiments using DNA that are exempt under Appendix C-II of the NIH Guidelines.

Submit this statement to the IBC (in care of the Environmental Health and Safety Office, attn. BSO) prior to initiating the experiment(s).

By submitting this statement to the IBC, I confirm that the project for which I am proposing to undertake is exempt from NIH Guidelines and that I acknowledge if non-exempt agents will be used, I will submit "Application for the Use of Recombinant DNA in Research" prior to acquisition/use of those agents.

Signature: _____

Date: _____

<p><i>For IBC use only</i></p> <p>Approval:</p> <p>IBC Chair _____</p> <p>BSO _____</p> <p>Member _____</p> <p>Member _____</p> <p>Date of Approval: _____</p>
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**APPENDIX B:
Old Dominion University Institutional Biosafety Committee
Recombinant DNA Research Protocol Renewal/Amendment Form**

1. Principal Investigator:			
2. Department:			
3. Application Type:	<input type="checkbox"/> Amendment	<input type="checkbox"/> Renewal	
4. Project Title:		IBC No.:	
5. Office: Bldg:		Room No.:	
6. Lab(s): Bldg:		Room No.:	
7. Phone No.:		Fax No.:	
8. Etiologic Agents - Are there any etiologic agents to be added or deleted from the current authorization that was approved by the Institutional Biosafety Committee? If yes, complete the items below that apply, if "no" go to item 9. <div style="text-align: center;"><input type="checkbox"/> Yes <input type="checkbox"/> No</div>			
Etiologic Agent(s) to be added to current authorization:			
Etiologic Agent:		Biosafety Level:	<input type="checkbox"/> 1 <input type="checkbox"/> 2
Etiologic Agent:		Biosafety Level:	<input type="checkbox"/> 1 <input type="checkbox"/> 2
Etiologic Agent(s) to be removed from current authorization:			
Etiologic Agent:		Biosafety Level:	<input type="checkbox"/> 1 <input type="checkbox"/> 2
Etiologic Agent:		Biosafety Level:	<input type="checkbox"/> 1 <input type="checkbox"/> 2
9. Experimental Use - Are there any changes in the current authorization that was approved by the Institutional Biosafety Committee. If "yes," attach a description of the proposed experimental protocol if different from the currently authorized protocol, if "no" go to item 10. <div style="text-align: center;"><input type="checkbox"/> Yes <input type="checkbox"/> No</div>			
10. Personnel - List all personnel who will be working with the agents under your current authorization:			
Name:			
Name:			
Name:			
Certification The signature below affirms that this application accurately reflects any changes in the applicant's current authorization to conduct research involving recombinant DNA technology. Furthermore, the applicant has read and will comply with the policies and procedures of Old Dominion University's Institutional Biosafety Committee.			
Signature:		Date:	

<p>For IBC use only Approval: IBC Chair _____ BSO _____ Member _____ Member _____ Date of Approval: _____</p>
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**APPENDIX C:
Biosafety Level 2 Safety Review**

Principal Investigators planning to use viable Risk Level 2 agents (see Appendix C of the *Biosafety Policies and Procedures Manual* and CDC's *Biosafety in Microbiological and Biomedical Laboratories*) must submit the following information to the University's Biosafety Officer prior to using those agents:

- A. The specific agent(s) that will be used in the experiment(s).
- B. The location where the experiments will be conducted.
- C. Provisions for laboratory security including access control.
- D. A brief description of the experimental protocol including:
 - 1. Microbiological techniques that will be used.
 - 2. Volume(s)/concentration(s) of the culture(s).
 - 3. Safety controls (e.g., mechanical pipettes, gloves, lab coats, etc.) that are available for use in the experiment(s).
- E. Provisions for medical intervention, either preventative or therapeutic available to laboratory personnel.
- F. A description of containment equipment (e.g., biosafety cabinets, centrifuge caps etc.) that will be used when manipulating the agent(s).
- G. Procedures for disinfecting surfaces and equipment, and sterilizing viable cultures of the agent(s) before disposal.
- H. Waste disposal procedures.
- I. Emergency procedures that will be taken in case of spills and personal contamination.

Send this information via campus mail to the Office of Research, Attention: Biological Safety Officer. The Biological Safety Officer will review the information and consult with the Principal Investigator if there are any questions or concerns. The Principal Investigator will receive confirmation to proceed with the experiment(s) after all concerns are addressed.

(11/04)

APPENDIX D:
NIH Guidelines for Research Involving Recombinant DNA Molecules
(NIH Guidelines, April 2002) Sections I-V.

http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc7261577

APPENDIX E:
NIH Guidelines (April 2002)
"Classification of Human Etiological Agents on the Basis of Hazard"
http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm