TUSKEGEE UNIVERSITY

Division of Research and Sponsored Programs
Office of Grantsmanship and Compliance
BioSafety Committee

BioSafety Manual

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I. TUSKEGEE UNIVERSITY HEALTH AND SAFETY POLICY

Tuskegee University is committed to providing a safe and healthy work and study environment for its faculty, staff, and students. All research activities involving biological agents by faculty, staff or students of Tuskegee University are under the review and approval jurisdiction of the Biosafety Committee.

Under the governmental statutes and regulations, all employees of the University, faculty, staff, and students have an obligation to work in compliance with the Federal laws and regulations through using protective equipment and/or wearing protective clothing that the University requires when working with biohazards. Investigators and supervisors have an obligation to ensure that employees are advised of the existence of any potential danger to their health and safety at their worksite. Faculty, staff and students have an obligation to report any safety issue immediately to their supervisor. New faculty, staff or students must be trained appropriately to work with biohazardous material within ninety days of hiring.

II. BIOSAFETY PROGRAM

The Tuskegee University Biosafety Program is designed to protect workers and the public from hazards associated with potential exposure to infectious agents. The program includes a number of components such as containment (engineered controls), administrative controls, medical surveillance, and immunization (when appropriate). Overall control of the program is through the Biosafety Committee with administrative and technical support being provided by the Assistant Director, Office of Grantsmanship and Compliance.

III. TUSKEGEE UNIVERSITY BIOSAFETY COMMITTEE

The Tuskegee University Biosafety Committee is charged with ensuring that all proposed research activities within Tuskegee University involving chemical and potentially infectious biological agents are conducted in a safe manner and in conformity with federal mandated standards. The term "infectious biological agents" include biological agents of a chemical nature, any animal and human tissue, or fluids, viruses, bacteria, fungi, parasites and other organisms/genetic systems that, by virtue of their replication properties, are potentially harmful to humans and/or other living systems.

A. The Biosafety Committee has, the following responsibilities:

1. To review Tuskegee University's policies and procedures related to the use of biohazards as necessary whenever there are changes in institutional, governmental or funding agency guidelines that impact the work of this committee. The committee will use the guidelines of EPA, OSHA, DHHS, and CDC as the primary references for developing institutional policies and procedures.
2. To provide faculty, staff, and students with basic biohazard training for all personnel who work with infectious biological agents.
3. Review all proposals that involve the use of biohazard material. Approve and require modifications in or withhold approval of proposals and ongoing activities involving biohazardous materials.
4. To advise the Vice-President, Research and Sponsored Programs on the needs of the University community for biosafety facilities, policies and programs;

5. To assist in the preparation of all reports related to Biohazard research required by the institution, funding agencies or other governmental entities.
6. To report promptly to the Vice President, Research and Sponsored Programs, for transmittal to the Provost significant events related to violation of Federal statutes and regulations.
7. To represent the University externally on matters relating to Biosafety.
8. To submit, on behalf of the Committee, by June 30 of each year, an annual report to the Vice President, Research and Sponsored Programs on the activities of the Chair and the Committee, for transmittal to the Provost.

IV. BIOSAFETY COMMITTEE STRUCTURE

A. Chair

The Chair is appointed by the Provost for a 2 year (renewable) term. The Chair shall be a faculty with extensive experience in working with biohazardous agents.

B. Vice-Chair

The Vice-Chair is appointed by the Provost, on the recommendation of the Chair for a 2 year (renewable) term. The Vice-Chair shall act in the absence of the Chair.

C. Members

Members are appointed by the Provost on the recommendation of the Deans and Department Heads for each school on campus. The 2 year term is renewable. They are selected based upon their experience in working with hazardous agents. Their function is to assist the Chair in all matters relating to Biosafety within the University. Individual members may also be appointed as Local Biosafety Coordinators, with authority over specific geographical regions of the University. Responsibilities of the Local Biosafety Coordinators include ascertaining that all teaching and research programs within their jurisdiction meet the required Biosafety standards of the University. The Committee shall also contain at least one member whose work is not associated with the university and is a community person.

D. Ex Officio Members

Ex officio members include the Assistant Director, Grantsmanship and Compliance, and the Vice President, Research and Sponsored Programs and Provost whose responsibilities include the implementation and maintenance of Biosafety standards and regulations.

E. Associate Members

The principal function of Associate Members is to provide a source of expertise that can be called upon when appropriate and under specific circumstances. They may be appointed from within or outside of the University community, and are selected by the Chair on the basis of their special expertise in matters relevant to biosafety. They may attend, and participate in, but not vote at committee meetings.

F. Meetings

The Committee shall meet once a month. The Committee shall keep minutes of meetings in the Office of Granstmanship and Compliance.

G. Proposal Review

The Committee shall review proposals as received. Initial review shall be done by the chair or a designate. Final approval of any proposal shall be by three members. A minimum of two, but not exceeding 4 weeks should be allowed for review and approval of each request. All proposals/requests should be submitted through the Office of Grantsmanship and Compliance.

V. PREFACE

The National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), and other Federal agencies have developed comprehensive guidelines on which the Tuskegee University Biosafety Manual is based. These guidelines can be found from individual websites or can be obtained directly from the agencies. In addition, the Tuskegee University Research website and Office of Grantsmanship and Compliance, maintains copies of these and other documents that can provide essential reference for all interested in biohazard regulations.

A. Scope of the Guidelines

Tuskegee University Biosafety Manual is directed primarily to those who work in laboratories in which human pathogens are handled. The University's Biosafety Committee has adopted the risk group classifications of NIH and CDC for viruses, bacteria, fungi, parasites and other biohazardous material, in conjunction with the acceptance of the new levels of physical containment required for work with microorganisms, parasites, recombinant DNA and animal cells. The range of laboratory activities affected remains unchanged in that the new standards and practices described here apply to both laboratory research and teaching carried out within the University.

These guidelines are part of the Tuskegee University Office of Grantsmanship Compliance and Safety program and apply to the handling of all known or suspected hazardous organisms.

Within these guidelines, a biohazard is defined as a replicating organism (fungus, bacteria, virus, etc.) that, alone or in combination with its products (hormones, toxins, genetic material. etc.), poses a hazard to humans, animals, plants, or other biological Organisms.

B. Goals

These guidelines are intended to allow the handling of suspected or known hazardous organisms with proper concern for the health of laboratory workers and the environment.

This guide incorporates biological safety concepts and standards published in several federal documents, including those of the National Institutes of Health (NTH) of the PHS, the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), the United States Department of Agricultural (USDA), the Department of Transportation (DOT), Environmental Protection Agency (EPA), Occupational Safety & Health Administration (OSHA), and Alabama Department of Environmental Management (ADEM). We have also incorporated portions of guidelines on handling cells from the Medical Research Council of Canada and recommendations from staff members from several recognized institutions that culture animal and plant cells. By using these sources, we have attempted to incorporate the collective professional judgment from each of several institutions with a history of compliance in handling biological materials. They will be reevaluated and changed as the state of the art evolves. Updates to these regulations can be found on the World Wide Web at sites maintained by NIH and CDC, including www.nih.gov/od/orda.

VI. PROGRAM ORGANIZATION AND RESPONSIBILITIES

All Tuskegee University faculty, staff and students are responsible for safe laboratory operations. Specific organizational responsibilities and authorities have been established for the use of known and suspected biohazards.

A. Management Responsibility and Authority

The Vice President for Research and Sponsored Programs is responsible for standards to provide a safe work place for employees and prevent damage to the surrounding community. This responsibility is delegated jointly to the Office of Grantsmanship and Compliance Staff and other committees including Radiation Safety, the Biosafety Committee and each department/division head.

Line management responsibility is carried out in part through specific line organizations but is shared with other staff management as follows:

i Principal Investigator/Laboratory Supervisor

The Principal Investigator (PI) or laboratory supervisor shall be the faculty or staff member responsible for the conduct of specific investigations for teaching or research that involves biohazards. The PI is responsible for safe practices including the implementation of Tuskegee University policies and procedures, training of employees, assuring compliance with safe practices (including requiring the use of proper protective clothing and equipment) and assuring special practices unique to specific activities. The Principal Investigator is also responsible for defining the nature of biohazards for his program and recommending containment classifications. He is responsible to consult with the Office of Grantsmanship and Compliance (OGC), and the Biosafety Committee (BSC) when questions arise.

These procedures provide employees a safe workplace. To ensure that employees are properly protected, the Principal Investigator may require employees to have written medical approval before permitting entry to the laboratory. These employees include among others, persons with illness or who are taking medication that would reasonably be expected to lower the person's resistance to biohazards. Further, the Principal Investigator may refuse an employee, who has an infection or illness diagnosed by a medical doctor or an appropriate health official, entry to a laboratory where that infection or illness poses an unreasonable risk to the employee's health and/or to the experiment's integrity.

ii. Individual Responsibility

Each employee is responsible for being knowledgeable of the risks associated with any biohazard he/she handles or that is being used in the immediate work area. He/she is also responsible for being aware of those personal illnesses or infections (viral, bacterial, yeast or fungal) which may adversely affect the integrity of any work or system in his area.

In the interest of the employees, personal safety and health and the integrity of the system or work, special precautions may be required of the employee. Employees working in programs involving potential biohazards or wishing to enter such work areas must report to OGC any illness, infection or symptoms thereof or use of prescription medication of all types and must then secure approval before entering the work area. Special attention is directed to the need for the employee to report any use of medications that may adversely affect the employee's immune system (including cortisone and cortisone-like medications) and chemotherapy agents. It is essential also that any medication being taken to combat viral, bacterial, fungal or yeast infection be reported. Such an employee may, at his/her discretion, confer first with his/her PI, who then will consult with the OGC on a case by case basis before allowing entry to a work area.

Since the nature of the biohazard may vary from program to program, the principal investigator together with OGC may waive, in writing the reporting requirements for special situations. The employee is further responsible for reporting any accidental exposure to biohazards or spills.

B. Resources

1. The Office of Grantsmanship and Compliance (OGC) and the BioSafety Committee provide a range of services and consultation, including a review of new programs or program changes that alter the nature of a biohazard risk. Guidance in the design of laboratories and the selection and testing of biological safety cabinets, operation and effectiveness of HVAC systems, safe handling practices, personal protection equipment and other safety equipment will be provided. In addition, the Physical Plant and Consultants will, upon the request of OGC and BSC, periodically test biological cabinets to assure compliance with federal standards and determine compliance with this Safety-Guide. Special references relative to the safe handling of potential biohazards can be found on the websites, from OGC and the BSC.

2. Outside consultants will be utilized as necessary especially for training purposes.

VII. CRITERIA CLASSIFICATION OF INFECTIVE PATHOGENS BY RISK GROUP

Biohazard materials and organisms include all infectious agents or biologically derived infectious materials that present either a risk or a potential risk to the health or humans or animals, either directly through infections or indirectly through damage to the environment. It must be understood that the risk lists included in this manual may be incomplete and that many agents may have undergone taxonomic name changes. Thus, before assuming that an unlisted agent can be assigned to risk group, individuals working with the agent must verify its identity and characteristics. Work with an agent should be considered to pose an increased risk if manipulations required include sonication or blending, or the use of other procedures likely to produce unusually large amounts of aerosols or infectious droplets. In general, such manipulations should be carried out one containment level higher than that which is normally used.

Judgments of the inherent risks of a pathogen are made on the basis of such factor as 1) pathogenicity of the organism, 2) mode of transmission and host range, 3) availability of effective preventive measures (e.g., vaccines), 4) availability of effective treatment (e.g., antibiotics), and other factors (e.g., quantity of the agent, effects on other species, economic environmental effects, and agent is indigenous to the US). Organisms are assigned to risk groups based on the risk factors and levels of containment. Risk group assignments presume ordinary circumstances in the research laboratory, or growth in small volumes for experimental, diagnostic, or teaching purposes.

Potentially hazardous biological materials and agents include:

1. Human, animal, and plant pathogens:
    - Viruses, including oncogenic and effective viruses, (includes viral vectors)
    - *Rickettsiae*
    - *Chlamydiae*
    - Bacteria, including those with drug resistance plasmids
    - Fungi
    - Parasites
    - Undefined or other infectious agents, such as prions
    - Toxins (bacterial, fungal, plant)
        1. All human blood, blood components and products, tissues, and body fluids.
        2. Cultured cells (all human and non-human primates) and potentially infectious agents these cells may contain
        3. Infected animals and animal tissues
        4. Non-human primates and any tissues derived there from (can transmit Herpes B Virus)

5. Sheep and any tissues derived there from (can transmit *Coxiella burnetii*, the causative agent of Q fever)

NIH Guidelines on Recombinant DNA (April 2002)

1. Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans
2. Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive ort therapeutic interventions are often available.
3. Risk Group 3 (RG3) agents are associated with serious or lethal disease for which preventive or therapeutic interventions may be available.
4. Risk Group 4 (RG4) agents are likely to cause serious or lethal disease for which preventive or therapeutic interventions not usually available.

CDC.NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition 1999)

1. BIOSAFETY LEVEL 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult human and of minimal potential hazard to laboratory personnel and the environment.
2. BIOSAFETY LEVEL 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.
3. BIOSAFETY LEVEL 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.
4. BIOSAFETY LEVEL 4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol transmission laboratory infections and life-threatening disease.

A. Biosafety Level 1 (BSL-1)

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

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

Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.

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 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. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. Policies for the safe handling of sharps are instituted.

6. All procedures are performed carefully to minimize the creation of splashes or aerosols.

7. Work surfaces are decontaminated at least once a day and after any spill of viable material.

8. All cultures, stocks, and other regulated wastes are recontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.

9. A biohazard sign must be posted 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. An insect and rodent control program is in effect:

Special Practices None

Safety Equipment (Primary Barriers)

1. Special containment devices or equipment such as a biological safety cabinet is generally not required for manipulations of agents assigned to Biosafety Level 1.

2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.

4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.

2. Each laboratory contains a sink for hand washing.

3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

B. Biosafety Level 2 (BSL-2)

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

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

Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

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, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. Policies for the safe handling of sharps are instituted.

6. All procedures are performed carefully to minimize the creation of splashes or aerosols.

7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.

8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

9. An insect and rodent control program is in effect.

Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. When appropriate, considering the agent(s) handled baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

8. A high degree of precaution must always be taken with 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 laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.

    b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

    c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

    d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. Animals not involved in the work being performed are not permitted in the lab.

Safety Equipment (Primary Barriers)

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

   a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.

   b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

Laboratory Facilities (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).

2. Consider locating new laboratories away from public areas.

3. Each laboratory contains a sink for hand washing. Foot, knee, or automatically operated sinks are recommended.

4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.

8. An eyewash station is readily available.

9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

C. Biosafety Level 3 (BSL –3)

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

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

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

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

Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

2. Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. Policies for the safe handling of sharps are instituted.

6. All procedures are performed carefully to minimize the creation of aerosols.

7. Work surfaces are decontaminated at least once a day and after any spill of viable material.

8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.

9. An insect and rodent control program is in effect.

Special Practices

1. Laboratory doors are kept closed when experiments are in progress.

2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.

3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.

4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.

6. Baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.

7. A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

8. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.

9. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.

10. A high degree of precaution must always be taken with 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 laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.

    b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

    c. Syringes which re-sheathe the needle, needleless systems, and other safe devices are used when appropriate.

    d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.

11. All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.

12. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.

    a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.

    b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.

13. Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

14. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.

15. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

16. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Safety Equipment (Primary Barriers)

1. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.

2. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.

3. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.

4. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs, etc., are conducted in a Class II or Class III biological safety cabinet.

5. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.

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

Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable. A clothes change room may be included in the passageway.

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

3. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

6. All windows in the laboratory are closed and sealed.

7. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.

8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.

9. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.

11. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.

12. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).

13. An eyewash station is readily available inside the laboratory.

14. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

D. Biosafety Level 4 (BSL – 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. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents. Access to the laboratory is strictly controlled by the laboratory director. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted.

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

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

Standard Microbiological Practices

1. Access to the laboratory is limited by the laboratory director when experiments are in progress.

2. Policies for safe handling of sharps are instituted.

3. All procedures are performed carefully to minimize the creation of aerosols.

4. Work surfaces are decontaminated at least once a day and after any spill of viable material.

5. All waste is decontaminated before disposal by an approved method such as autoclaving.

6. An insect and rodent control program is in effect.

Special Practices

1. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women are not allowed in the laboratory or animal rooms.

   The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazard control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook, signed by all personnel, indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

2. When infectious materials or infected animals are present in the laboratory or animal rooms, hazard warning signs, incorporating the universal biohazard symbol, are posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

3. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 4, all personnel demonstrate a high proficiency in standard microbiological practices and techniques and in the special practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in these unique safe microbiological practices and techniques.

4. Laboratory personnel receive available immunizations for the agents handed or potentially present in the laboratory.

5. Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.

6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.

8. Personnel enter and leave the laboratory only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the laboratory. Personnel use the airlocks to enter or leave the laboratory only in an emergency.

9. Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the laboratory. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.

10. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

11. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

a. Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.

b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes that re-sheath the needle, needleless systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass must be decontaminated before disposal, according to any local, state, or federal regulations.

12. Biological materials to be removed from the Class III cabinet or from the Biosafety Level 4 laboratory in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

13. No materials, except biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the laboratory. Equipment or material that might be

damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

14. Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Equipment is decontaminated before it is sent for repair or maintenance.

15. Spills of infectious materials are contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with concentrated infectious material. A spill procedure is developed and posted within the laboratory.

16. A system is established for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

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

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

Laboratory Facility (Secondary Barriers) there are two models for Biosafety Level 4 laboratories: (A) the Cabinet Laboratory where all handling of the agent is performed in a Class III Biological Safety Cabinet, and (B) the Suit Laboratory where personnel wear a protective suit. Biosafety Level-4 laboratories may be based on either model or a combination of both models in the same facility. If a combination is used, each type must meet all the requirements identified for that type.

(A) Cabinet Laboratory

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through a minimum of two doors prior to entering the room(s) containing the Class III biological safety cabinet (cabinet room). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the cabinet room. A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom for decontamination is provided at the containment barrier for passage of those materials, supplies, or equipment that are not brought into the cabinet room through the change room.

2. Daily inspections of all containment parameters (e.g., directional airflow) and life support systems are completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

3. Walls, floors, and ceilings of the cabinet room and inner change room are constructed to form a sealed internal shell which facilitates fumigation and is resistant to entry and exit of animals and insects. Floors are integrally sealed and coved. The internal surfaces of this shell are resistant to liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Openings around doors into the cabinet room and inner change room are minimized and are capable of being sealed to facilitate decontamination. Any drains in the cabinet room floor are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters and protection against vermin.

4. Bench tops have seamless or sealed surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

5. Laboratory furniture is of simple open construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

6. A hands-free or automatically operated hand washing sink is provided near the door of the cabinet room(s) and the outer and inner change rooms.

7. If there is a central vacuum system, it does not serve areas outside the cabinet room. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the cabinet room are protected by devices that prevent backflow.

8. If water fountains are provided, they are automatically or foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is isolated from the distribution system supplying water to the laboratory areas and is equipped with a backflow preventer.

9. Access doors to the laboratory are self-closing and lockable.

10. Any windows are breakage-resistant and sealed.

11. Double-door autoclaves are provided for decontaminating materials passing out of both the Class III biological safety cabinet(s) and the cabinet room(s). Autoclaves that open outside of the containment barrier must be sealed to the wall of the containment barrier. The autoclave doors are automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

12. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s).

13. Liquid effluents from the dirty-side inner change room (including toilets) and cabinet room sinks, floor drains (if used), autoclave chambers, and other sources within the cabinet room are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and clean-side toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.

14. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate any system malfunction. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the cabinet room is provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and the HVAC control system is designed to prevent sustained positive pressurization of the laboratory. The Class III cabinet should be directly connected to the exhaust system. If the Class III cabinet is connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinet.

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

16. The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.

17. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, and computer).

(B) Suit Laboratory

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through the changing and decontamination areas prior to entering the room(s) where work is done with BSL-4 agents (suit area). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the suit area. A specially designed suit area is maintained in the facility to provide personnel protection equivalent to that provided by Class III biological safety cabinets. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system protected by HEPA filtration. The life support system includes redundant breathing air compressors, alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. An automatically starting emergency power source is provided at a minimum for the exhaust system, life support systems, alarms, lighting, entry and exit controls, and BSCs. The air pressure within the suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area, chemical shower, and airlocks, are sealed.

2. A daily inspection of all containment parameters (e.g., directional airflow, chemical showers) and life support systems is completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

3. A double-doored autoclave is provided at the containment barrier for decontaminating waste materials to be removed from the suit area. The autoclave door, which opens to the area external to the suit area, is sealed to the outer wall of the suit area and is automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle. A dunk tank, fumigation chamber, or ventilated airlock for decontamination is provided for passage of materials, supplies, or equipment that is not brought into the suit area through the change room. These devices can be also used for the safe removal of materials, supplies, or equipment from the laboratory that cannot be decontaminated in the autoclave.

4. Walls, floors, and ceilings of the suit area are constructed to form a sealed internal shell, which facilitates fumigation and is animal and insect prohibitive. The internal surfaces of this

shell are resistant to liquids and chemicals, facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floor of the suit area contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters.

5. Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area.

6. Bench tops have seamless surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

7. Laboratory furniture is of simple open construction capable of supporting anticipated loading and uses. Non-porous materials are preferable. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

8. A hands-free or automatically operated hand washing sink is provided in the suit area(s); hand washing sinks in the outer and inner change rooms should be considered based on the risk assessment.

9. If there is a central vacuum system, it does not serve areas outside the suit area. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the suit area are protected by devices that prevent backflow.

10. Access doors to the laboratory are self-closing and lockable. Inner and outer doors to the chemical shower and inner and outer doors to airlocks are interlocked to prevent both doors from being opened simultaneously.

11. Any windows are breakage-resistant and are sealed.

12. Liquid effluents from sinks, floor drains (if used), autoclave chambers and other sources within the containment barrier are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.

13. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. Redundant supply fans are recommended. Redundant exhaust fans are required. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the suit area must be provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and an HVAC control system is installed to prevent positive pressurization of the laboratory.

14. The supply air to the suit area, decontamination shower, and decontamination airlock is protected by passage through a HEPA filter. The general room exhausts air from the suit area, decontamination shower and decontamination airlock is treated by a passage through two HEPA filters in series prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA filters are located as near as

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

15. The positioning of the supply and exhaust points should be such that dead air space in the suit room is minimized.

16. The treated exhaust air from Class II biological safety cabinets, located in a facility where workers wear a positive pressure suit, may be discharged into the room environment or to the outside through the facility air exhaust system. If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system.

17. The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.

18. Appropriate communication systems should be provided between the laboratory and the outside.

VIII. Summary of Recommended Biosafety Levels for Infectious Agents

IX *Risk Groups: Bacteria*

X. *Risk Groups: Viruses*

The classifications of biological agents primarily reflect the judgments made on their inherent risks. In addition to the severity of disease or other risks caused by a pathogen, the conditions under which an infectious agent is used also concern laboratory safety. Large volumes and high concentrations of agent in growth media evidently pose greater risks than smears of the same agent on a microscope slide. Other unusual manipulations may also increase the hazard.

CLASS I or Risk Group 1 (low individual and community risk)

A microorganism or virus that is unlikely to cause disease in healthy workers or animals.

CLASS 2 or Risk Group 2 (moderate individual risk, limited community risk)

A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures

rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

CLASS 3 or Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

CLASS 4 or Risk Group 4 (high individual risk, high community risk)

A pathogen that usually produces very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

CLASS 5
Organisms forbidden in the U.S. by law.

Specific definitions: (See Appendix)

XI. RECOMBINANT DNA AND GENETIC MANIPULATION

Strain or mutant selection and genetic crosses have been used for many years to obtain genetically altered viruses, microorganisms, animals and plants. These methods have been supplemented by newer and more powerful ones, the best known of which are the molecular techniques used to create recombinant forms of DNA. For the purposes of these guidelines, recombinant DNA includes:

- DNA molecules produced outside living cells by joining natural or synthetic segments to DNA molecules capable of replication in living cells;
- DNA molecules produced in living cells by joining enriched or natural segments to intracellular DNA; and
- DNA molecules resulting from replication of such recombinant molecules.

With the introduction of recombinant DNA technology in the 1970's it became possible to transfer genes between unrelated organisms and species. Initial concerns about the possible risks inherent in the production of altered organisms by this technology led several countries, including Canada, to develop stringent biosafety guidelines. Experience soon showed that those fears were not justified, and by 1980 many of the containment requirements of 1975-1977 had been removed. For most research based on recombinant DNA technology there is only the remotest possibility of creating a new hazard, because the source of the DNA being transferred, the vector and the host are all innocuous or have low risk characteristics. However, some recombinant DNA manipulations could result in organisms with a significantly elevated level of risk.

Since it is not realistic to try to define in advance all of the possible genetically engineered organisms which might be created or used in the laboratory, or to attempt a comprehensive assignment of risk categories, the following criteria and considerations will be used to assign containment levels for recombinant DNA work.

1. If neither the DNA, vector (if used), or host employed in the genetic manipulation possesses characteristics that could be considered hazardous, and no hazard can be reasonably foreseen in the combination, then no restrictions beyond the requirements of containment Level 1 are needed.

2. If one or more of the components (DNA, vector, or host) involved in the genetic manipulation is known to pose a hazard, then, in general, determination of the containment level will start at the level appropriate for the most hazardous component. Whether the containment level might be reduced or increased will be determined by the particular gene transferred, its expression in the host organism, the biological containment afforded by the host-vector system, envisaged interactions between the gene being transferred and the host-vector system, and other relevant factors. In the case of animal virus vectors, including retroviruses, these factors include the likelihood of production of replication-competent viruses and the nature of the helper cells used.
3. Characteristics of viruses and cells which contribute to their pathogenicity can be altered by mutation; the containment level required may be reduced if it is known that the mutant DNA and/or vector are defective in disease-causing or replication characteristics.
4. In any work with genes coding for hazardous products, host-vector systems of limited ability to survive outside the laboratory (i.e., offering biological containment) should be used; their use may reduce the level of physical containment required.

XII. IMPORTATION AND TRANSPORTATION OF INFECTIOUS SUBSTANCES

A. IMPORTATION

Permits are required for the importation of all biological/biohazard materials into U.S. regardless of whether they infect humans, animals or plants. The importation of infectious agents which are predominantly pathogenic to animals is regulated by the USDA. In addition, certain agents listed in this document require permits issued by either local, state or federal agencies. Please check with the Office of Grantsmanship and Compliance to determine if an agent you want transported requires a permit.

B. TRANSPORTATION

The efficient transfer of infectious substances requires good coordination between the sender, carrier, and receiver to ensure safe and prompt transport and arrival in proper condition. It is important that the sender make advance arrangements with the carrier to ensure that specimens will be accepted and promptly processed. In addition, the sender must prepare the appropriate dispatch documents according to state and federal Departments of Transportation Regulations. The sender should also forward all transportation data to the receiver. No infectious substances should be dispatched before advance arrangements have been made between the sender, the carrier, and the receiver, or before the receiver has confirmed with national authorities that the substance can be imported legally and that no delay will be incurred in the delivery of the consignment to its destination.

XIII. USE OF ANIMAL CELLS IN CULTURE AND HANDLING OF BODY FLUIDS

Biological hazards that could be associated with the handling of tissues and cells, or of body fluids, arise from the possible presence in such materials of viruses or other infectious agents. Although the physical containment levels referred to below will usually apply, factors such as the particular source of the material, times of culturing (if any) and the types of manipulations involved could influence the containment level required.

A. ANIMAL CELLS

1. Animal tissues and primary cell cultures

   The following containment requirements apply to tissues and primary cell cultures from human, non-human primate and non-primate animal sources when handled in the laboratory or used for animal passage.

   - Human and non-human primate material: Level 2 containment
   - Non-primate animal material: Level 1 containment

2. Established cell lines

   Human or other animal cell lines known not to be infected with any infectious agents may be handled under Level 1 containment. Cultures known or suspected to be infected with any of the infectious agents covered by these guidelines must be handled at the containment level assigned to the agent.

B. BODY FLUIDS

The need for precautionary measures extends also to situations in which blood, saliva, urine and other body fluids or feces from humans must be handled. The precautions required may be more stringent when the specimens are used for culturing purposes, but, as a minimum, their handling should be consistent with Level 2 containment. Reduction of the containment level may be acceptable if potential hazards associated with the material can be expected to be diminished because of dilution, or use of chemical or other treatments.

Blood or blood fractions and other fluid specimens of human or other animal origin that are known or suspected to contain any of the infectious agents covered by these guidelines must be handled at the containment level to which the agent is assigned.

C. RISK LEVELS ASSOCIATED WITH THE USE OF LABORATORY ANIMALS

The use of experimental animals and insects poses special problems. Animals can harbour infectious organisms which are acquired naturally. These infections can give rise to a chronic carrier state, or the agent might persist in a latent non-infective form which can be reactivated periodically or as a result of certain stimuli. If the possibility that such an agent may be excreted by an animal during the course of an experiment cannot be excluded, all those animals should be kept at a containment level appropriate to the risk.

Animals may also be deliberately inoculated with viruses or organisms in each of the four risk groups or with viable materials (i.e., transformed cells) suspected of containing these infectious agents. Under these circumstances, the animals should be kept at the containment level appropriate to the risk of the agent, recognizing that, in some cases, *in vivo* work may increase that risk.

In all situations, it is the responsibility of the principal investigator and the biosafety committee, in consultation with the animal care authorities, to determine the risk levels inherent in the proposed research.

D. ANIMAL DISEASES

The distribution and use of any pathogen which may cause infectious or contagious disease in animals is also covered by regulations in this manual. This control also applies to materials of animal origin which contain potential pathogens. In practical terms, this means that approval must be obtained for the importation of every animal pathogen. It may also establish, for any animal pathogen, the conditions under which work will be carried out and restrict distribution of the organism to a certain specified person or laboratory. Any recombinant organism could fall into a restrictive category and it may be necessary to prove to appropriate authorities that its use or release into the environment would not constitute a risk to any animal. Thus, for any organism, it will be necessary to consider not only the risk to human health, but also the level of containment needed to prevent its escape into the environment where it might infect some indigenous animal species.

XIV. TUSKEGEE UNIVERSITY PHYSICAL CONTAINMENT CLASSIFICATION

The safe handling of a potentially biohazardous organism requires physical containment levels including laboratory design, equipment and practices, appropriate to the suspected or known hazard. Scientists handling organisms such as fungi, bacteria, viruses and mutant cells have grouped these organisms into classes (NIH refers to these as Risk Groups) according to their perceived or known potential hazard to animals. Four general levels of containment have evolved that reflect the minimum precautions judged by the scientific community in academia, industry and government agencies as necessary to assure an acceptable level of risk. These hazard class/containment level correlations for animal pathogens are shown in Table 1.

Three levels of physical containment, designated BLI, BL2, and BL3, provide the basis for the handling of organisms at TU. A fourth level, designated BL4, requires extreme precaution. Research requiring a BL4 facility will not be permitted at TU.

TABLE I

Hazard Class Containment Level Correlations
for Animal Pathogens

TU[a]	NIH	CDC	NCI	Cell/Tissue
BLI	RG1	1	-	BL1
BL2	RG2	2	Low	BL2
BL3	RG3	3	Moderate	BL3
BL4	RG4	4	High	BL4

[a]Tuskegee University, classification, based primarily on Reference 1.
Detailed laboratory design, equipment and practice criteria are provided in Appendix II.
Classification details for bacterial, fungal and viral agents are provided in Appendix II.

The Tuskegee University classification levels correspond closely with the BL1, BL2, BL3, and BL4 designations of NIH in their guidelines for recombinant DNA research (1). The correlations of containment level with the CDC classification of fungi, bacteria and viruses (2) and the National Cancer Institute (NCI) classification of oncogenic viruses (3.4) that were also established by NIH (1). The containment levels specified for mutant human cells are essentially those designated by the Medical Research Council (Canada) and the U. S. Department of Health (5,6). Information on the identification and transmission of organisms has been compiled by NIH (7).

Please note that the BLI level constitutes a minimum standard for handling any known or suspected biohazard at Tuskegee University.

Research proposals involving biohazards including recombinant DNA shall be submitted to the Biosafety Committee. If Class 1, Class 2 or Class 3 pathogens will be used, the appropriate form shall be submitted to the OGC.

Information for project approval should be submitted to the appropriate office by the PI or project supervisor. It is the responsibility of the PI that the project is reviewed and that all appropriate plans and procedures are on record for the specific project or experiment. Proposals that require review, have to be submitted to the committee at least five weeks prior to date of submission to the granting agency.

The research plan (or safety information provided OGC by the PI) should address pertinent sections contained within the referenced documents with respect to protection of employees, community and environment. If, as a result of the review, specific procedures are required which appear to conflict with procedures in the referenced documents, the specific procedures developed by the appropriate office or council are to dominate.

Any change in a program that alters the nature and/or magnitude of the potential risk requires a subsequent safety audit of procedures.

The Biosafety Committee has final responsibility for the determination of the appropriate constraints for all operations with biohazardous materials.

Four levels of containment (1-4), appropriate for the four risk groups of infectious agents, are described. These levels of containment are regarded as appropriate for most laboratory scale uses of the listed agents. A higher level of containment will be required for specific manipulations, if they appreciably increase the possibility of infection.

A. CONTAINMENT LEVEL 1

This level applies to the basic laboratory for the handling of Risk Group 1 agents. Level 1 requires no special design features beyond those suitable for a well designed and functional laboratory. Containment cabinets are not required. Work may be done on an open bench top and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

B. PHYSICAL REQUIREMENTS

- A room separated from public areas by a door is required. There are no particular restrictions on locating the facility near public or heavily traveled corridors; however, doors should remain closed.
- Coatings on walls, ceilings, furniture, and floors should be cleanable. Windows that can be opened should not be near working areas or containment equipment and should be equipped with fly screens.
- There are no special air handling requirements beyond those concerned with proper functioning of the biological safety cabinets, if used, and those required by building codes.
- Hand-washing facilities must be provided, preferably near the point of exit to public areas.
- Separate hanging areas should be provided for street clothing and laboratory coats.
- Eye wash stations may be required by local statute.

C. OPERATIONAL REQUIREMENTS

Basic safety practices as described above in must be followed.
In addition, where chemical disinfection procedures are practiced, effective concentrations and contact times must be employed. Chemical disinfectants used to decontaminate materials to be removed from the laboratory must be replaced regularly.

D. CONTAINMENT LEVEL 2

Containment Level 2 is suitable for work with agents in Risk Group 2. In addition to the requirements of containment Level 1, the following are required:

E. PHYSICAL REQUIREMENTS

- The laboratory should be located away from public areas, general offices, and patient care areas.
- A biohazard sign with appropriate information must be posted on the entrance to the laboratory.
- Laboratory furnishings should be constructed with special attention to the use of impervious and readily cleanable work surfaces.
- Coat hooks must be provided for laboratory coats near the exit.
- An autoclave must be available in or near the laboratory.
- Laboratory doors should be self-closing.

F. OPERATIONAL REQUIREMENTS

Class I or II biological safety cabinets are required for all manipulations involving the agent which may create an aerosol. The biological safety cabinet must have been tested and certified

within the previous 12 months according to accepted. Inspection and retesting is mandatory if the cabinet is relocated. Moves of a minor nature may be exempt if the move is supervised by the testing technologist to ensure that the equipment has not been subjected to undue stress. At the time certification is carried out, the testing technologist should ascertain that the users are familiar with the containment capability of the equipment under various operating conditions and familiarize such individuals with precautions to be taken in its use.

Air from these cabinets may be recirculated to the room only after passage through a HEPA filter. Centrifugation must be carried out using closed containers or safety heads or sealed cups. These should be opened only in the biological safety cabinet described above.

Animals or insects which have been experimentally infected must remain in the laboratory or appropriate animal containment facility. An emergency plan for handling spills of infectious materials must be developed and be ready for use whenever needed. Vacuum lines used for work involving the agent must be protected from contamination by HEPA filters or equivalent equipment. A laboratory coat to be worn only in the laboratory area is required. Coats that fasten on the front are permissible. These coats shall not be worn outside the containment laboratory. Special care should be taken to avoid contamination of the skin with infectious materials; gloves should be worn when handling infected animals or when skin may be exposed to infectious materials.

Contaminated glassware must not leave the facility; decontamination must be carried out using procedures demonstrated to be effective. If there is no autoclave or incinerator in the laboratory, contaminated materials must be disinfected chemically or double bagged and transported to the autoclave or incinerator in durable, leak proof containers which are closed and wiped on the outside with disinfectant before leaving the laboratory.

Service personnel and cleaning staff that enter the facility must be informed of the hazards that might be encountered. Cleaning staff should clean only the floors. The laboratory personnel have the responsibility for rendering the facility safe for routine cleaning. Periodic intensive cleaning must be done at regular intervals. Cleaning and maintenance staff may receive appropriate immunization and medical surveillance if appropriate.

G. CONTAINMENT LEVEL 3

Containment Level 3 is suitable for work with agents in Risk Group 3. The operational requirements for the Level 3 laboratory are substantially greater than those for Levels 1 and 2 and the laboratory staff must receive specific training in the safe handling and manipulation of the agents used in this laboratory.

A Level 3 containment laboratory requires specialized design and construction. Those responsible for biosafety in an institution should maintain close control and seek expert advice for all phases of design, construction, commissioning, operation and maintenance, and annual testing. In addition to the requirements of containment Levels 1 and 2, the following are required:

H. PHYSICAL REQUIREMENTS

The laboratory must be located away from general work areas and have controlled access from other areas. This is accomplished by entry through a lockable changing room with self-closing doors. A body shower should be provided within the containment perimeter.

The laboratory must be held at a negative pressure relative to the surrounding areas at all times such that a directional airflow is created by air ingressing through all entry and exit areas. The air discharged from the laboratory cannot be recirculated back into either the air supply system of the laboratory itself or into the building or adjacent buildings. Provided there is a dedicated sealed exhaust system, air may be exhausted from the laboratory to the exterior of the building without HEPA filtration. The sealed exhaust system may draw part of the air from an adjacent Level 2 area, if it serves a support function for the area reserved for Level 3 work. At the discharge point the exhausted air must be dispersed away from air intakes or populated areas. However, when the air is not exhausted by means of a dedicated exhaust system, it must be passed through a HEPA filter before discharging into the main building exhaust air ventilation system. This exhaust HEPA filter housing must be designed to allow *in situ* decontamination and must pass annual testing and certification by aerosol challenge and scan techniques. A control system must be provided to ensure that the Level 3 laboratory does not become positively pressurized relative to the surrounding area. When the supply air is not provided by a dedicated system, back draft dampers or HEPA filters must be installed in the supply system.

Biological safety cabinets must be installed in a manner which does not interfere with the air balance of the cabinet or room. Thimble unit connections are recommended. The laboratory must have a dedicated hand-washing sink with foot, knee or automatic controls, located near the exit.

The laboratory should have an autoclave located within the work zone. Where this is not possible, contaminated materials may be autoclaved or incinerated outside the laboratory if they are chemically disinfected first or double bagged then transported to the autoclave or incinerator in a properly identified closed, unbreakable and leak proof container. Laboratory furnishings should be kept to a minimum, be readily cleanable, and resistant to chemical disinfectants.

All penetrations for services in the floors, walls, and ceiling of the laboratory must be sealed. The air exhaust system should be provided with a manual damper that may be closed as required to permit gas decontamination. Water supplied to the laboratory must be provided with back flow preventers. Sink and floor drains from this suite should be piped separately to the main building drain and be appropriately labeled. Floor drains are not generally recommended. Infectious materials must never be placed in sinks or floor drains.

In animal care facilities for small animals, the disposal of wastes will not differ from other contaminated laboratory materials. Large animals producing quantities of infectious wastes require special facilities which must be individually approved. Portable vacuum pumps must be fitted with in-line HEPA filters or equivalent equipment. Laboratory windows must be sealed and unbreakable.

I. ANIMAL HANDLING

The use of animals in the laboratory requires precautions to minimize unwanted infection of the colony, assure containment of any potentially biohazardous organisms. and minimize the possibility of worker infection or injury. The handling of animals in the labs is subject to USDA regulations (10) and the NIH "Guide for the Care and Use of Laboratory Animals". See information provided by the Animal Care and Use Committee for information regarding animal experiments. The Animal Care and Use Committee may request review of the proposal by the Biosafety Committee or the OGC.

The interstate shipment, receipt and handling of plant pests (insects, nematodes, bacteria, viruses and so forth) are regulated by the Alabama Department of Agriculture and the USDA as authorized by The Federal Plant Pest Act of 1957. Th principal investigator in charge of projects using plant pests is responsible for being aware of all regulations and assuming compliance.

Summary of Containment requirements

A. BL1

1. Special Laboratory Design
   Special laboratory design is not required at the BL1 level

2. Containment equipment
   Special containment equipment is not required at the BL1 level.

3. Laboratory Practices
   a. Laboratory doors shall be kept closed except as needed for access
   b. The laboratory shall be kept neat and clean.
   c. Work surfaces shall be decontaminated daily and immediately following spills of organisms.

   d. All wastes with a known or suspected biohazard shall decontaminated before disposal. Other contaminated materials s as clothing, glassware, animal cages, and laboratory equipment shall be decontaminated before washing, reuse, or disposal. Routine waste, cages, and the like related to the handling of healthy animals need not be decontaminated for safety reasons before disposal or reuse.

   e. Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container which is closed before removal from the laboratory.

   f. The use of laboratory gowns, coats, or uniforms is discretionary the laboratory supervisor.

   g. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

   h. Persons shall wash their hands after handling organisms and when they leave the laboratory.

   i. Eating, drinking, smoking, and storage of foods are not permitted in the work area. The office area is not considered to be in the work area for BL1 level containment.

   j. Care shall be taken in the conduct of all procedures to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculating loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

   k. Use of hypodermic needles and syringes shall be avoided when alternative methods are available.

   1. Insect and rodent control shall be maintained.

B. BL2 - Includes BLI standards (above) plus the following:

1. Special laboratory design

An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms are used.

2. Containment Equipment

Biological safety cabinets shall be used for all equipment and manipulations that produce aerosols--e.g. pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, vortexing, centrifuging—where these procedures involve organisms, except where equipment design provides for containment of aerosols. CLASS I horizontal flow cabinets are not acceptable for employee protection.

3. Laboratory Practices

a) Work surfaces shall be decontaminated following the completion of the experimental activity.
b) Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.
c) The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring BL2 containment are in progress. Freezers and refrigerators used to store organism shall also be posted with the universal biohazard sign.
d) The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch-room or outside of the building in which the laboratory is located.
e) Animals not related to the experiment shall not be permitted in the laboratory.
f) Experiments of lesser biohazard potential can be carried on concurrently in carefully demarcated areas of the same laboratory.

C. BL3 - Included BL2 standard plus the following:

1. Special Laboratory Design

a. The laboratory shall be separated from areas which are open to unrestricted traffic flow by a controlled access area. A controlled access area is anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas which are open to unrestricted traffic flow.
b. The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed.
c. Windows in the laboratory shall be sealed.
d. Laboratory doors shall be self-closing.
e. A double-door autoclave for sterilization of wastes and contaminated materials shall be available in the controlled laboratory area.
f. A forced exhaust ventilation system shall be provided. This system shall be balanced so that the direction of airflow is from the controlled access area into the laboratory environment. The exhaust air shall not be re-circulated to any

other areas of the building. Re-circulation of air within the laboratory room however, may be provided. The exhaust air from the laboratory shall be discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The exhaust air from the laboratory shall be passed through a HEPA filter or otherwise decontaminated before discharge to the outdoors.

g. The treated exhaust air from Class II biological safety cabinets may be discharged either directly to the laboratory or to the out doors. The treated exhaust air from a class III cabinet shall be discharged directly to the outdoors. If the treated exhaust air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet or building exhaust air system.

2. Containment Equipment

Laboratory animals held in a BL3 area shall be housed in partial containment caging systems such as Horsfall units, open cages placed in ventilated enclosures, solid-wall-and-bottom cages covered by filter bonnets, equipped with ultraviolet radiation lamps and reflectors. Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

3. Laboratory Practices

a) Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall comply with all required entry and exit procedures.
b) The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring BL-3 containment are in progress.
c) Laboratory clothing that protects street clothing (i.e., long-sleeve solid-front or, wrap-around gowns, no-button or slipover jackets etc.) shall be worn in the laboratory. Front-button laboratory coats unsuitable. Laboratory coats are shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.
d) Raincoats, overcoats, topcoats, coals, hats, caps and such street outerwear shall not be kept in the laboratory.
e) Impermeable gloves shall be worn when handling materials requiring BL-3 containment. They shall be removed aseptically immediately after the handling procedure and stored in an autoclave bag until decontaminated.
f) Plants not related to the experiment shall not be permitted in the laboratory.
g) Vacuum outlets shall be protected by filters and liquid disinfectant traps.
h) If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 physical containment they shall be conducted in accordance with all BL3 laboratory practices.

XV. FACILITY MONITORING, CERTIFICATION AND MAINTENANCE

All facilities and equipment used to provide containment are to be tested before program initiation and periodically thereafter by the Biosafety Committee, OGC or Principal Investigator. Such monitoring will include checks of air balance, biohazard cabinet exhaust filters, sterilizers, and centrifuges.

The Biosafety Committee and OGC will use NIH guidelines to certify performance of facilities and equipment. Occasionally, a third party test may be arranged if reviews by the committee and OGC are conflicting.

No maintenance or testing of facilities or equipment other than routine repair involving no potential biohazard is allowed without approval by the Committee and OGC. Decontamination of all equipment by laboratory personnel is required before any maintenance activities will be allowed. The NIH Safety Monograph provides guidance on decontamination procedures.

XVI. SHIPPING

All potential biohazardous materials are to be packaged, labeled, and shipped in accordance with appropriate federal regulations of the PHS (14), The Department of Transportation (15) and the USDA (16).

XVII. DISPOSAL

The disposal of infectious waste generated by Tuskegee University research laboratories must follow regulations established by the Alabama Department of Agriculture as stipulated in OGC Guidelines. These regulations permit investigators to use chemical sterilization, steam sterilization, or incineration for disposal of infectious waste. Due to the complexity of the autoclave regulations, which are detailed below, many laboratories may have difficulty complying with them.

A. Incineration

The ORC will provide a container in which infectious wastes may be placed. The waste will be thermally destroyed in an approved incinerator. To arrange to have a container delivered, please call OGC at 724-4474. A schedule for pickup and replacement of containers can be arranged or replacements can be called for as needed.

B. Steam sterilization

The following regulations governing steam sterilization will apply to investigators who choose not to incinerate or use chemical sterilization.

1. The vacuum autoclave must be equipped with automatic, continuous, time and temperature recording chases and be operated according to the manufacturer's instructions.

2. An initial test using four (4) simulated waste charges representing the maximum amount of waste shall be used. Each type of waste must be included in each charge.

    a. Three approved test strips, such as Bacillus stearothermophilus, must be placed inside containers of simulated waste. The test strips shall be recovered, cultured and analyzed after the test run.

b. Any positive reading constitutes a failure of the unit and requires re-testing.

3. Each sterilizer must be tested weekly by placing one (1) approved test strip inside a waste container prior to sterilization. The test strip shall be recovered, cultured and analyzed.

   a. A positive reading indicates a failure. The autoclave may not be used until repairs are made and four (4) re-tests are conducted successfully.

4. A log containing the weight and number- of containers of waste treated in each until repairs are made and four (4) re-tests are conducted successfully.

   a. Results of a weekly test shall be recorded on the log.

   b. Logs and recorder charts shall be forwarded to the ORC each month.

   c. Logs and charts will be maintained for three years as required by NIH.

5. Sterilized sharps must be placed in rigid, impervious, and puncture resistant containers and sealed before disposal.

Any investigator planning to use steam sterilization in a vacuum autoclave shall contact the ORC for testing and record keeping.

C. Chemical Sterilization

1. A suitable chemical sterilization method may be used in the laboratory to render the agent non-infectious. A reprint that describes the method and the effectiveness of the treatment must be on file in the laboratory and available for inspection by the Biosafety Committee and OGC or inspectors from the responsible agencies.

   a. A record that describes -the treatment utilized for each batch including details of the reagent(s) used, length of treatment, quantity of reagents used, quantity of infectious agents, etc. shall be maintained in a lab book.

D. Sanitary Sewer System Disposal

TU policy concerning classification of infectious waste and disposal in the sanitary sewer system is as follows:

1. All human blood, blood products, tissues and body fluids shall be considered as contaminated (example with HIV or HBV). Appropriate personal protective equipment shall be used, e.g., safety glasses, goggles or face shield, gloves, lab coat or protective clothing, mechanical pipetting devices, biosafety hood, etc.

2. All human blood, blood products, tissues and body fluids known or suspected to be contaminated with infectious agents shall be disposed of by incineration, chemical sterilization or approved steam sterilization in a tested vacuum autoclave with automatic continuous time and temperature recording. Blood and body fluids may be placed directly into a sanitary sewer.

E. Pathogens Not Infectious To Humans

Investigators that use pathogens that are not infectious to humans, but which may be infectious to plants or animals if released into the environment shall follow the procedures outlined below:

1. Materials may be containerized for incineration. Contact the OGC as described above.

2. Materials may be sterilized in a vacuum autoclave that is operated in accordance with the provisions described for human pathogens.

3. A suitable sterilization method may be used in the laboratory to render the agent non-infectious. A reprint that describes the method and the effectiveness of the treatment must be on file in the laboratory and available for inspection by the Committee or OGC or inspectors from the responsible agencies.

    a. A record that describes the treatment utilized for each batch including details of the reagent(s) used, length of treatment, quantity of reagents used, quantity of infectious agents, etc. shall be maintained in a lab book.

Other regulations prohibit the release of pathogens with sufficient virulence and quantity so that exposure of a susceptible host to the waste could result in an infectious disease. Additional information concerning the disposal of infectious waste not covered by this section may be obtained from the OGC at 727-8985.

XVIII. FDA Good Laboratory Practices

FDA has promulgated final regulations covering what it considers good laboratory practices for obtaining data in non-clinical laboratory studies in support of FDA Approval applications (9). Compliance with this document is not required for safety purposes at TU. The document is referenced for the information of the user of this guide for possible utilization in individual programs.

XIX. RECORD KEEPING

The TU ORC and Biosafety Committee will maintain records, as appropriate, of all actions including copies of guidelines, program approvals, emergency plans, results of airflow monitoring or equipment testing, physical examinations, and investigation of accidents.

XX. EMERGENCY ACTIONS AND REPORTING

Each protocol shall have an emergency plan approved by the appropriate office and known to all occupants for dealing with accidental exposures or spills. The plan is to cover sources of potential exposure, decontamination of persons, decontamination of the facility and necessary reporting.

All exposures or accidental spills that may result in exposure to organisms requiring BL2 or BL3 containment are to be reported immediately to the Biosafety Committee chair and confirmed in writing to the OGC.

XXI. REFERENCES (Copies of references are available from the web or ORC)

1. U.S. Department of Health, Education and Welfare, "Guidelines for Research Involving Recombinant DNA Molecules". Federal Register 43, 60107 (December 22, 1978); also FR 43, 60080 (December 22, 1978).

2. "Classification of Etiological Agents on the Basis of Hazard", Center for Disease Control, Public Health Service, 4th Edition, July 1974.

3. "National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses", Office of Research Safety, National Cancer Institute, DHEW Publication No. (NIH) 75-790, October, 1974.

4. "National Institutes of Health Biohazards Safety Guide", U. S. Department of Health, Education, and Welfare, Public Health Service, 1974, pp. TV 16 through 18.

5. "Human Genetic Mutant Cell Repository Minimum Safety Guidelines Recommended for Working with Lymphoid and Virus Transformed Human Cell Lines", as contained in U-S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health publication entitled "-ne Human Genetic Mutant Cell Repository", 4th Edition, October 1977.

6. "Guidelines for the Handling of Recombinant DNA Molecules and A-nimal Vir-uses and Cells", Medical Research Council (Canada), February, 1977.

7. "National Institutes of Health Blohazards Safety Guide", U. S. Department of Health Education and Welfare, Public Health Service. 1974.

8. "Laboratory Safety Monograph - A Supplement to the NIH Guidelines for Recombinant DNA Research", U.S. Department of Health. Education and Welfare, Public Health Service, National Institutes of Health, July, 1978.

9. "Biosafety in Microbiological and Biomedical Laboratories", Centers for Disease Control/National Institutes of Health, 2nd Edition, May, 1988.

10. U. S. Department of Health, Education and Welfare, Food and Drug Administration,"Non-clinical Laboratory Studies, Good Laboratory Practice Regulations', Federal Register 43, pg. 59985 (December 22, 1978).
11. U. S. Code of Federal Regulations, Title 9.
12. "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DREW Publication No. (NIH) 72-73, Revised 1972.
13. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

14. U.S. Code of Federal Regulations, Title 42, section 72.25.

15. U.S. Code of Federal Regulations, Title 49, Section 173386 - 173.388.

16. U.S. Code of Federal Regulations, Title 7.

Risk Groups Bacteria

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in the text

Risk Group: Viruses

BL indicates the Biosafety Level; RG indicates the Risk Group

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
1	Acinetobacter		was Herellea vaginicola, A. calcoaceticus ssp.anitratus		2
2	Acinetobacter	lwoffi	formerly Mima polymorpha		
3	Actinobacillus	actinomycetemcomiana	genus Hemophilus		2 implied
4	Actinobacillus	spp	EC-Haemophilus actinomycetem comitans		2
5	Actinomadura	madurae			
6	Actinomadura	pelletieri			
7	Actinomyces	bovis			
8	Actinomyces	gerencseriae			
9	Actinomyces	israelii			
10	Actinomyces	naeslundii			
11	Actinomyces	pyogenes	was Corynebacterium		2
12	Actinomyces	spp			
13	Aeromonas	hydrophilia			2
14	Aeromonas	punctata			
15	Aeromonas	spp.			
16	Afpia spp				
17	Amycolata	autotrophica			2
18	Arachnia	propionica	was Propionibacterium propionicum		
19	Arcanobacterium	haemolyticum	was Corynebacterium		2
20	Archanobacterium	equi	was Corynebacterium		
21	Arizona	hinshawii	all serotypes		2
22	Bacillus	anthracis		2/3	2
23	Bacillus	cereus			
24	Bacillus	thuringiensis			
25	Bacteroides	fragilis			
26	Bacteroides	spp			
27	Bartonella	bacilliformis			
28	Bartonella	elizabethae			
29	Bartonella	spp.			see species listed
30	Bartonella	henselae	was genus Rochalimaea		2
31	Bartonella	quintana	was genus Rochalimaea	2	2
32	Bartonella	vinsonii	was genus Rochalimaea	2	2
33	Bordetella	all spp		See 1 sp	2
34	Bordetella	bronchiseptica			2 implied
35	Bordetella	parapertussis			2 implied
36	Bordetella	pertussis		2	2

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
37	Borrelia	burgdorferi			2
38	Borrelia	duttoni			
39	Borrelia	recurrentis			2
40	Borrelia	spp			see species listed
41	Borrelia	vincenti			
42	Brucella	abortus		3	3
43	Brucella	canis		3	3
44	Brucella	melitensis		3 (USDA restricted)	3
45	Brucella	ovis			3 implied
46	Brucella	spp	except B. ovis	3	3
47	Brucella	suis		3	3
48	Burkholderia	spp	Australia, EU still as Pseudomonas spp.	see sp	2, unless listed at 3
49	Burkholderia	mallei	was Pseudomonas		3
50	Burkholderia	pseudomallei	was Pseudomonas	2/3	3
51	Calymmatobacterium	granulomatis			
52	Campylobacter	coli		2	2
53	Campylobacter	fetus	subsp fetus	2 ssp fetus	2
54	Campylobacter	jejuni		2	2
55	Campylobacter	spp		2 implied	see spp
56	Campylobacter	sputorum			
57	Capnocytophaga	spp			
58	Cardiobacterum	hominis			
59	Chlamydia	pneumoniae			2
60	Chlamydia	psittaci	avian strains, 3	2	2
61	Chlamydia	spp	C. pneumoniae*, EU=2, Belgium=2	see 2 spp	see 3 spp
62	Chlamydia	trachomatis	lymphogranuloma venereum agent	2	2
63	Citrobacter	spp			
64	Clostridium	botulinum		2 V/3	2
65	Clostridium	chauvoei			2
66	Clostridium	difficile			
67	Clostridium	equi			
68	Clostridium	haemolyticum			2
69	Clostridium	histolyticum			2
70	Clostridium	novyi			2
71	Clostridium	perfringens			
72	Clostridium	septicum			2
73	Clostridium	sordelli			
74	Clostridium	spp			2
75	Clostridium	tetani		2 V	2

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
76	Corynebacterium	bovis			
77	Corynebacterium	diphtheriae		2 V	2
78	Corynebacterium	matruchotii			
79	Corynebacterium	minutissimum			
80	Corynebacterium	pseudotuberculosis			2
81	Corynebacterium	renale			2
82	Corynebacterium	spp			see 3 species listed
83	Corynebacterium	ulcerans			
84	Coxiella	burnetii		3	3
85	Dermatophilus	congolensis			2
86	Edwardsiella	tarda			2
87	Eikenella	corrodens			
88	Enterobacter	aerogenes/cloacae			
89	Enterobacter	spp.			
90	Enterococcus	spp	(Streptococcus faecalis)		
91	Erlichia	sennetsu	(Rickettsia)		
92	Erlichia	spp			
93	Erysipelothrix	rhusiopathiae	was spp insidiosa		2
94	Erysipelothrix	spp.			
95	Escherichia	coli	(pathogenic strains)		2
96	Escherichia	coli K12	genetically crippled		1
97	Flavobacterium	meningosepticum			
98	Flavobacterium	spp			
99	Fluoribacter	bozemanae	was Legionella		
100	Francisella	novocida			
101	Francisella	tularensis	Type A	3 V	3
102	Francisella	tularensis	Type B	2 V	
103	Fusobacterium	necrophorum			
104	Fusobacterium	spp			
105	Gardnerella	vaginalis			
106	Haemophilus	ducreyi			2
107	Haemophilus	influenzae			2
108	Haemophilus	spp			see 2 spp
109	Hartmanella	spp			
110	Helicobacter	pylori	was genus Campylobacter		2
111	Herellea	vaginicola	see Actinetobacter baumannii		
112	Kingella	kingae			
113	Klebsiella	oxytoca			1
114	Klebsiella	pneumoniae			2 implied
115	Klebsiella	spp			2

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
116	Lactobacillus	spp			1
117	Legionella	pneumophila		2	2
118	Legionella	spp		2	2
119	Legionella-like organisms			2	
120	Leptospira	interrogans	all serotypes (icterohemorragiae)	2	2
121	Listeria	ivanovii			2 implied
122	Listeria	monocytogenes			2 implied
123	Listeria	spp			2
124	Mima	polymorpha	Acinetobacter lwoffi		
125	Moraxella	spp			2
126	Morganella	morganii			
127	Mycobacterium	africanum			2 implied
128	Mycobacterium	asiaticum		2	2
129	Mycobacterium	avium-intracelluare	(avium in Canada)	2	2
1300	Mycobacterium	bovis	BCG is 2	3	3
131	Mycobacterium	chelonei	EU chelonae	2	2
132	Mycobacterium	fortuitum		2	2
133	Mycobacterium	kansasii		2	2
134	Mycobacterium	leprae		2	2
135	Mycobacterium	malmoense		2	2
136	Mycobacterium	marinum		2	2
137	Mycobacterium	microti			2 implied
138	Mycobacterium	paratuberculosis	Mycobacterium avium subsp. Paratuberculosis	2	2
139	Mycobacterium	scrofulaceum		2	2
140	Mycobacterium	simiae		2	2
141	Mycobacterium	spp	except. MDR M.tb complex	see spp	2 unless listed as 3
142	Mycobacterium	szulgai		2	2
143	Mycobacterium	tuberculosis	3, V,T for multiply resistant	3	3
144	Mycobacterium	ulcerans		2	2
145	Mycobacterium	xenopi		2	2
146	Mycoplasma	hominis			2 implied
147	Mycoplasma	mycoides			Restricted AP
148	Mycoplasma	pneumoniae			2 implied
149	Mycoplasma	agalactiae			Restricted AP
150	Mycoplasma	spp			2, except M. mycoides, M agalactiae
151	Neisseria	gonorrhoeae		2 (BSC)	2

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
152	Neisseria	meningitidis		2 (BSC)	2
153	Neisseria	spp		see species listed	see species listed
154	Nocardia	asteroides			2
155	Nocardia	brasiliensis			2
156	Nocardia	caviae			
157	Nocardia	farcinica			
158	Nocardia	nova			
159	Nocardia	spp			see species listed
160	Nocardia	transvalensis			2
161	Nocarida	otitidis-caviarum	EU-otitidiscaviarum		2
162	Pasteurella	haemolytica			2 implied
163	Pasteurella	multocida			3
164	Pasteurella	pneumotropica			
165	Pasteurella	spp	type B, "buffalo", other virulent strains		2, except those listed as 3
166	Peptostreptococcus	anaerobius			
167	Plesiomonas	shigelloides			
168	Porphyromonas	spp			
169	Prevotella	spp			
170	Proteus	mirabilis			
171	Proteus	penneri			
172	Proteus	spp.			
173	Proteus	vulgaris			
174	Providencia	alcalifaciens			
175	Providencia	rettgeri			
176	Providencia	spp			
177	Pseudomonas	aeruginosa			2
178	Pseudomonas	spp	except P. mallei, Burholderia maiiei		
179	Rhodococcus	equi			2
180	Rickettsia	(vole)			2
181	Rickettsia	akari		3	3
182	Rickettsia	australis		3	3
183	Rickettsia	canada		3	3
184	Rickettsia	conorii		3	3
185	Rickettsia	montana			
186	Rickettsia	mooseri	see R. typhi		
187	Rickettsia	parkeri			
188	Rickettsia	prowazekii		3	3
189	Rickettsia	rhipicephali			
190	Rickettsia	rickettsii		3	3

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
191	Rickettsia	sennetsu	see Bartonella		
192	Rickettsia	sibirica		3	3
193	Rickettsia	spp			3 (except vole, 2)
194	Rickettsia	tsutsugamushi		3	3
195	Rickettsia	typhi (mooseri)			3
196	Salmonella	arizonae			2
197	Salmonella	cholerasuis			2
198	Salmonella	enteritidis			2
199	Salmonella	gallinarum-pullorum			2
200	Salmonella	meleagridis			2
201	Salmonella	paratyphi,A,B,C	A, B, C		2
202	Salmonella	spp	all spp and serotypes/ serovars	see 1 species listed	2
203	Salmonella	typhi		2 V, 3	2
204	Salmonella	typhimurium			2
205	Serpulina	spp			
206	Serratia	marcescens			
207	Serretia	liquefaciens			
208	Shigella	boydii		2 implied	2
209	Shigella	dysenteriae	Type 1	2 implied	2
210	Shigella	flexneri		2	2
211	Shigella	sonnei		2 implied	2
212	Shigella	spp.		2	2
213	Sphaerophorus	necrophorus			2
214	Staphylococcus	aureus			2
215	Staphylococcus	epidermidis			
216	Streptobacillus	moniliformis			2
217	Streptobacillus	spp			see species listed
218	Streptococcus	agalactiae			2 implied
219	Streptococcus	pneumoniae			2
220	Streptococcus	pyogenes	Canada Lancefield Groups A-D, G		2
221	Streptococcus	spp.	see also Enterococcus spp		2
222	Streptococcus	suis			
223	Treponema	carateum			2
224	Treponema	pallidum		2	2
225	Treponema	pertenue			
226	Treponema	spp			see 2 species listed
227	Treponema	vincentii			

				BL	RG
ID	Genus	Species	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
228	Ureaplasma	urealyticum			
229	Vibrio	cholerae	including El Tor(EU, Belgium)	2	2
230	Vibrio	parahaemolyticus		2	2
231	Vibrio	spp		see 2 spp	see 3 species listed
232	Vibrio	vulnificus			2
233	Yersinia	enterocolitica			2
234	Yersinia	pestis		2 V, 3 aerosols and antibiotic resistant strains	3
235	Yersinia	pseudotuberculosis			
236	Yersinia	spp	except Y. pestis	see 1 spp	see 2 species listed
237					

BL indicates the Biosafety Level; RG indicates the Risk Group

				BL	RG
ID	Name	Viral Group	Comments	BMBL-93 CDC/NIH	NIH rDNA-97
2	Absettarov, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4
3	Acute haemorrhagic conjunctivitis virus (AHC)	Picornaviridae			
4	Adenovirus (human, all types)	Adenoviridae			2
5	Aino	X-Arboviruses			
6	Akabane	X-Arboviruses			
7	Alastrim	Poxviridae			Restricted
8	Aphthovirus	Picornaviridae			
9	Araguari	X-Arboviruses			
10	Astroviridae	Astroviridae	(feces of children and lambs)		
11	Avian leukosis virus (ALV)	Viral vector/ Animal retrovirus			1
12	Avian sarcoma virus	Viral vector/ Animal retrovirus			1

13	Baculovirus	Viral vector/ Animal virus			1
14	Barmah Forest	Togaviridae/ Alphavirus (Grp A Arbovirus)			
15	Batama	X-Arboviruses			
16	Batken	X-Arboviruses			
17	Bebaru virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2	2
18	Bhanja	X-Arboviruses			
19	Bimbo	X-Arboviruses			
20	Bloodborne hepatitis viruses not yet identified	Unclassified viruses		2 implied	2 implied
21	Bluetongue	X-Arboviruses			
22	Bobaya	X-Arboviruses			
23	Bobia	X-Arboviruses			
24	Bovine immunodeficiency virus (BIV)	Viral vector/ Animal retrovirus			
25	Bovine leukemia virus (BLV)	Viral vector/ Animal retrovirus			1
26	Bovine papilloma virus	Papovavirus/ Animal virus vector			1
27	Bovine spongiform encephalopathy (BSE)	Unconventional agents, prions			
28	Buenaventura	X-Arboviruses			
29	Buffalopox virus: 2 viruses (1a vaccinia variant)	Poxviridae		2 implied	2
30	Bunyamwera virus	Bunyaviridae/ Bunyavirus Group		2	2
31	Bunyavirus	Bunyaviridae/ Bunyavirus Group			
32	Cabassou	X-Arboviruses			
33	Cache valley	X-Arboviruses			2 see arbovirus table
34	California encephalitis virus	Bunyaviridae/ Bunyavirus Group		2	2 see arbovirus table
35	Camel pox virus	Poxviridae		2 implied	2
36	Cardiovirus	Picornaviridae			
37	Central European Tick-borne encephalitis virus, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4
38	Chick embryo lethal orphan CELO	Viral vector/ Animal virus			
39	Chikungunya virus	Togaviridae/ Alphavirus (Grp A Arbovirus)	2, high passage strains;vaccine	3 arbovirus table	3 arbovirus

			strain131/25		table
40	Chim	X-Arboviruses			
41	Cocal	X-Arboviruses			
42	Coltiviruses	Reoviridae			2 (incl. Colorado Tick Fever)
43	Congo Crimean haemorrhagic fever TBE	Bunyaviridae/ Nairoviruses		4	4 (Crimean -Congo)
44	Coronavirus	Coronaviridae			2
45	Cowpox virus	Poxviridae		2 V	2
46	Coxsackie	Picornoviridae/ Enterovirus			2 (Types A and B)
47	Creutzfeldt-Jacob disese	Unconventional agents/prion		2	3
48	Cytomegalovirus (CMV) Genus Lymphocryptovirus	Herpesviridae/ Betaherpesviridae		2	2
49	Dengue virus	Flaviviridae/ Flavivirus (Grp B Arbovirus)	Type 1-4	2	2 (Types 1-4), 3
50	Dhori	X-Arboviruses		3 arbovirus table	3 arbovirus table
51	Dog sarcoma	Viral vector/ Animal virus			
52	Dugbe	X-Arboviruses			
53	Eastern equine encephalomyelitis (EEE)	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 V	2 V
54	Ebola virus	Filoviridae		4	4
55	Echoviruses	Picornoviridae/ Enterovirus			2
56	Ectromelia (mousepox)	Orthopoxvirus			
57	Elephantpox virus (variant of cowpox)	Poxviridae			2
58	Entero	Picornoviridae/ Enterovirus			
59	Epstein-Barr virus (EBV)	Herpesviridae/ Gamma-herpesviridae		2	2
60	Everglade virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 arbovirus table	3 arbovirus table
61	Feline leukemia virus, FeLV	Viral vector/ Animal retrovirus			1
62	Feline sarcoma virus, FeSV	Viral vector/ Animal retrovirus			1
63	Flanders-Hart Park virus	Rhabdoviridae	from mosquitoes and	2 arbovirus	2

	(see Zinsser, pg 777)		birds, human sx	table	arbovirus table
64	Gammaherpes	Herpesviridae / Gamma-herpesvirinae			
65	Ganjam (USDA permit)	X-Arboviruses			
66	Garba	X-Arboviruses		3 arbovirus table	3 arbovirus table
67	Germiston	X-Arboviruses		3	3 see arbovirus table
68	Gerstmann-Straussler-Scheinker syndrome	Unconventional agents, prions		2 implied	3 implied
69	Getah	X-Arboviruses		3 arbovirus table	3 arbovirus table
70	Gibbon leukemia virus (GaLV)	Viral vector/ Animal retrovirus			1
71	Gordil	X-Arboviruses		3 arbovirus table	3 arbovirus table
72	Guanarito	Arenaviruses		4	4
73	Guaratuba	X-Arboviruses		2 arbovirus table	2 arbovirus table
74	Guinea pig herpes	Viral vector/ Animal virus			1
75	Hamster leukemia	Viral vector/ Animal virus			1
76	Hantaan (Korean haemorrhagic fever)	Bunyaviridae/ Hantaviruses	see also sin nombre virus	3	3
77	Hanzalova,TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4
78	Hart Park virus (see Zinsser, pg 777)	Rhabdoviridae		2 arbovirus table	2 arbovirus table
79	Hazara virus	Bunyaviridae/ Nairoviruses		2 arbovirus table	2 arbovirus table
80	Hepatitis A virus, human enterovirus type 72	Picornoviridae/ Hepatovirus		2 V	2
81	Hepatitis B virus	Hepadnaviridae		2 V	2
82	Hepatitis C	Togaviridae/ Pestivirus (Canada)	(hepatitis non-A,non-B)	2	2
83	Hepatitis D	Hepadnaviridae	Delta, only pathogenic	2	2

	(Delta) virus (b)		with HBV inf.		
84	Hepatitis E virus	Calciviridae		2	2
85	Herpes saimiri	Herpesviridae/ Rhadinovirus			
86	Herpes simplex viruses	Herpesviridae/ Alpha-herpesviridae	Type 1 and 2 (EC)	2	2 (types 1 and 2)
87	Herpesvirus ateles	Herpesviridae/ Rhadinovirus			1
88	Herpesvirus saimiri, Genus Rhadinovirus	Herpesviridae/ Animal virus vector			1
89	Herpesvirus simiae (B virus)	Herpesviridae/ Alpha-herpesviridae		3/4	4
90	Herpesvirus zoster (Varicella)	Herpesviridae/ Alpha-herpesviridae		2	2
91	Human B lympho-tropic virus	Herpesviridae			2 (Herpes types 6 and 7)
92	Human Immunodeficiency virus (HIV) Types 1 and 2 Oncornavirus C	Retroviridae/ Lentiviridae	1, 2 (AIDS causing)	2/2+/3	3 (Types 1 and 2)
93	Human T-cell lymphotropic viruses (HTLV)	Retroviridae/ Oncovirinae/ Genus Oncornavirus C	Types 1-3 (EC 1 and 2)	2/3	3 (Types 1 and 2)
94	Hypr,TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4
95	Ibaraki	X-Arboviruses		3 arbovirus table	3 arbovirus table
96	Influenza virus (vaccine strain)	Orthomyxoviridae	vaccine strains: A/PR8/34, A/WS/33	1	
97	Influenza virus, Types A-C	Orthomyxoviridae	Types A,B, and C (EC)	2	2 (types A,B, C)
98	Inhangapi	X-Arboviruses		3 arbovirus table	3 arbovirus table
99	Inini	X-Arboviruses		3 arbovirus table	3 arbovirus table
100	Israel Turkey Mening.	X-Arboviruses		3 arbovirus table	3 arbovirus table
101	Issyk-Kul	X-Arboviruses		3 arbovirus table	3 arbovirus table
102	Itaituba	X-Arboviruses		3 arbovirus table	3 arbovirus table

103	Japanese B encephalitis	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
104	Japanese encephalitis, Nakayama	Flaviviridae/ Flavivirus (Grp B Arbovirus)			
105	Junin virus	Arenaviruses		3V/4	4, 3 (v)
106	Kairi(x)	X-Arboviruses	i	3 arbovirus table	3 arbovirus table
107	Khasan, Koutango	X-Arboviruses		3 arbovirus table	3 arbovirus table
108	Kokobera	Flaviviridae/ Flavivirus (Grp B Arbovirus)			
109	Kumlinge,TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)	I	4	4
110	Kunjin	Flaviviridae/ Flavivirus (Grp B Arbovirus)		2 arbovirus table	2 arbovirus table
111	Kuru	Unconventional agents/prion		2	3
112	Kyasanur Forest, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4
113	Kyzylagach	X-Arboviruses		3 arbovirus table	3 arbovirus table
114	LaCrosse virus	X-Arboviruses		2 arbovirus table	2 arbovirus table
115	Langat virus	X-Arboviruses		2 arbovirus table	2 arbovirus table
116	Lassa fever virus	Arenaviruses		4	4
117	Lentivirinae , except HIV-1 and HI	Retroviridae			
118	Looping ill , TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
119	Lucke (frog) virus	Viral vector/ Animal virus			
120	Lymphocytic choriomeningitis (neurotropic) virus	Arenaviruses	neurotropic strains	2/3	3
121	Lymphocytic choriomeningitis virus	Arenaviruses	other viscerotrophic (Canada:lab adapted)	2	2
122	Machupo virus	Arenaviruses		4	4
123	Marburg virus	Filoviridae		4	4

124	Marek's disease virus	Herpesviridae/ Animal virus vector			1 (vector)
125	Mason-Pfizer monkey virus	Viral vector/ Animal retrovirus			1
126	Mayaro virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 arbovirus table	3 arbovirus table
127	Measles virus	Paramyxoviridae/ Morbillivirus			2
128	Middelburg	X-Arboviruses		3 arbovirus table	3 arbovirus table
129	Milker's node virus	Poxviridae	also Pseudocowpox virus	2 implied	2
130	Molluscum contagiosum virus	Poxviridae		2 implied	2
131	Monkeypox virus	Poxviridae/ Orthopoxvirus		2 V	3
132	Mopeia virus (and other Tacaribe viruses)	Arenaviruses		3 arbovirus table	3 arbovirus table
133	Morbillivirus,except Rinderpest	Paramyxoviridae/ Morbillivirus			
134	Mouse mammary tumor virus	Viral vector/Animal retrovirus			1
135	Mucambo virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 arbovirus table	3 arbovirus table
136	Mumps virus	Paramyxoviridae/ Paramyxovirus			2
137	Murine cytomegalovirus	Herpesviridae/ Animal virus vector			1
138	Murine leukemia virus	Viral vector/ Animal retrovirus			1
139	Murine sarcoma virus	Viral vector/ Animal retrovirus			1
140	Murray Valley encephalitis (Australia encephalitis)	Flaviviridae/ Flavivirus (Grp B Arbovirus)	(Australian encephalitis)	3 arbovirus table	3 arbovirus table
141	Nairobi Sheep Disease	Bunyaviridae/ Nairovirus	USDA Restricted	3 arbovirus table	Restricted
142	Nariva, Negishi	X-Arboviruses		3 arbovirus table	3 arbovirus table
143	Ndumu	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 arbovirus table	3 arbovirus table

144	New Minto, Nodamura, Northway	X-Arboviruses		3 arbovirus table	3 arbovirus table
145	Newcastle Disease virus	Paramyxoviridae/ Paramyxovirus			2
146	Norwalk virus	Calciviridae			2 implied
147	O'Nyong-Nyong virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 arbovirus table	2 arbovirus table
148	Omsk (hemorrhagic fever) TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)	hemorrhagic fever	4	4
149	Oncornavirus B	Retroviridae/ Oncovirinae			
150	Oncornavirus C, except HTLV I and II	Retroviridae/ Oncovirinae			
151	Orbiviruses	Reoviridae			2
152	Orf virus	Poxviridae/ Parapoxvirus		2 implied	2
153	Oropouche virus	Bunyaviridae/ Bunyavirus Group		3 arbovirus table	3 arbovirus table
154	Other bunyaviridae known to be pathogenic	Bunyaviridae			
155	Other calciviridae	Calciviridae			2
156	Other flaviviruses known to be pathogenic	Flavivirus			
157	Other hantaviruses	Bunyaviridae/ Hantaviruses	epidemic nephrosis virus		3
158	Other known alphaviruses	Togaviridae/ Alphavirus (Grp A Arbovirus)			
159	Other pathogenic orthopoxviruses not in RG 2 or 4	Poxviridae/ Orthopoxvirus			
160	Ouango, Oubangui	X-Arboviruses			
161	Papillomaviruses (human)	Papovaviridae			2
162	Parainfluenza virus Type 3, SF4 strain	Paramyxoviridae/ Paramyxovirus			
163	Parainfluenza viruses	Paramyxoviridae/ Paramyxovirus	Types 1 to 4		2 (Types 1-4)
164	Paramushir, Piry	X-Arboviruses		3 arbovirus table	3 arbovirus table
165	Paravaccinia virus	Poxviridae		2 implied	2
166	Parvovirus (human)	Parvoviridae	B19		2 (B19)
167	Polioviruses	Picornoviridae/ Enterovirus		2 V	2 (all types, wild and atten-uated)
168	Polyomavirus, BK and JC viruses	Papovaviridae			

169	Powassan	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
170	Prospect Hill virus	Bunyaviridae/ Hantaviruses		2 arbovirus table	2 arbovirus table
171	Pseudorabies virus	Herpesviridae/ Alphaherpesviridae			
172	Puumala virus	Bunyaviridae/ Hantaviruses		3 arbovirus table	3 arbovirus table
173	Rabbitpox virus (vaccinia variant)	Poxviridae		2	2
174	Rabies virus	Rhabdoviridae/ Lyssavirus	fixed virus is 2, street virus, 3	2 V /3	2/3
175	Rat leukemia virus	Viral vector/ Animal retrovirus			1
176	Razdan	X-Arboviruses		3 arbovirus table	3 arbovirus table
177	Reoviruses	Reoviridae			2
178	Respiratory syncytial virus	Paramyxoviridae/ Pneumovirus			2
179	Rhadinovirus, except H. ateles and H. saimiri	Herpesviridae/ Rhadinovirus			
180	Rhadinovirus, except H.ateles,H. saimiri	Herpesviridae			
181	Rhinovirus	Picornaviridae/ Rhinoviruses	Bovine, Equine, Human		2 (all types)
182	Rift Valley Fever, (Zinga virus)	Bunyaviridae/ Phleboviruses	2, vaccine strain MP-12; Grp C Bunyaviridae	3 Zinga	3 (MF12 vaccine strain,2)
183	Rochambeau	X-Arboviruses		3 arbovirus table	3 arbovirus table
184	Rocio	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
185	Ross River virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 arbovirus table	2 arbovirus table
186	Rotavirus (human)	Reoviridae			2f
187	Rubivirus (Rubella)	Togaviridae/ Rubivirus			2
188	Russian spring-summer encephalitis, TBE	Flaviviridae/ Flavivirus (Grp B Arbovirus)		4	4
189	Sabia	Arenaviruses			
190	Sagiyama	X-Arboviruses		3 arbovirus table	3 arbovirus table
191	Salanga, Santa Rosa, Saumarex Reef	X-Arboviruses		3 arbovirus table	3 arbovirus table
192	Sammarez Reef	Flaviviridae/ Flavivirus (Grp B Arbovirus)			
193	Sandfly fever virus	Bunyaviridae/ Phleboviruses		2 arbovirus table	2 arbovirus table

194	Scrapie	Unconventional agents prions:			
195	Semliki Forest virus	Togaviridae/ Alphavirus (Grp A Arbovirus)	most recombinant activities BL2	3 arbovirus table	3 arbovirus table
196	Sendai virus (murine parainfluenza virus type 1)	Paramyxoviruses/ Parainfluenza viruses			
197	Seoul virus	Bunyaviridae/ Hantaviruses		3 arbovirus table	3 arbovirus table
198	Sepik, Slovakia, Spondweni	X-Arboviruses		3 arbovirus table	3 arbovirus table
199	Shope papilloma virus	Papovavirus/ Animal virus vector			1
200	Simian immunodeficiency virus	Retroviridae/ Lentiviridae		2/2+/3	3
201	Simian sarcoma virus, SSV-1	Retroviridae			
202	Simian virus 40 (SV40)	Papovaviridae/ Animal virus vector			1
203	Sin nombre virus	Bunyaviridae/ Hantaviruses	hantavirus pulmonary syndrome		
204	Sindbis virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 arbovirus table	2 arbovirus table
205	St. Louis encephalitis	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
206	Subsclerosing pancencephalitis	Paramyxoviridae			
207	Tacaribe complex	Arenaviruses		2	2
208	Tamdy, Telok Forest, Tiacotalpan,	X-Arboviruses		3 arbovirus table	3 arbovirus table
209	Tanapox	Poxviridae	see Yabapox	2	2
210	Tensaw virus	Bunyaviridae/ Bunyavirus Group		2 arbovirus table	2 arbovirus table
211	Thetalymphocrypto virus	Herpesviridae/ Animal virus vector			
212	tick borne	Flaviviridae/ Flavivirus (Grp B Arbovirus)			
213	Tick-borne orthomyxoviridae,TBE	Orthomyxoviridae	Dhori & Thogoto (EC)	4	4 (Dhori and Thogoto)
214	Tocio	X-Arboviruses		3 arbovirus table	3 see arbovirus table
215	Tonate virus	Togaviridae/ Alphavirus (Grp A Arbovirus)		3 arbovirus table	3 arbovirus table
216	Toroviridae	Toroviridae			
217	Toscana virus	Bunyaviridae/ Phleboviruses		2 arbovirus table	2 arbovirus table

218	Turlock virus	X-Arboviruses			2 arbovirus table
219	unassigned herpesviruses HHV 7, HHV8	Herpesviridae			
220	Vaccinia virus	Poxviridae/ Orthopoxvirus		2 V	2
221	Variola (major and minor) virus	Poxviridae		R	Restricted
222	Venezuelan equine encephalomyelitis	Togaviridae/ Alphavirus (Grp A Arbovirus)	2, vaccine strain, TC-83	3	3, (2 for vaccine TC-83)
223	Vesicular stomatitis virus	Rhabdoviridae	Lab adapted:Indiana, San Juan, Glascow, Alagoas	3 (exotic, Piry)	3, 2, lab adapted strains
224	Wesselsbron virus	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
225	West Nile fever virus	Flaviviridae/ Flavivirus (Grp B Arbovirus)		3 arbovirus table	3 arbovirus table
226	Western equine encephalomyelitis	Togaviridae/ Alphavirus (Grp A Arbovirus)		2 ,V	2,V
227	Whitepox (Variola)	Poxviridae		R	Restricted
228	Yabapox virus (Tana and Yaba)	Poxviridae		2	2
229	Yellow fever virus (vaccine strain 17D)	Flaviviridae/ Flavivirus (Grp B Arbovirus)	3, see wild type strain	2	2
230	Yellow fever virus, wild type	Flaviviridae/ Flavivirus (Grp B Arbovirus)	2, see vaccine strain, 17D	3	3
231	Zinga (See Rift Valley Fever)	Bunyaviridae/ Phleboviruses	see Rift Valley Fever		

A. Class I - This group is limited to those bacteria, fungi, viruses and parasites which are unlikely to cause disease in healthy workers or animals.

B. Class 2. Agents of ordinary potential hazard

BACTERIAL AGENTS (Partial List)

Acinetobacter species

Actinobacillus - all species except A. mallei, which is in class 3
Aeromonas hydrophila
Arizona - all serotypes Arizona e
Bacillus anthracis
Bordetella - all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci chlamydiophila

Chlamydia trachomatis
Clostridium botulinum
Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum,
Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae
C. equi, C. haemolyticum, C. pseudotuberculosis,
C. pyogenes, C. renole
Diplococcus (Streptococcus) pneumoniae
Edwardsiella tarda
Erysipelothrix inopinata
Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing Kl antigen

Haemophilus ducreyi, H. influenzae
Hereflea vaginicola
Klebsiella - all species and all serotypes
LegioneHa pneumophila Leginilla
Leptospira interrogans - all serotypes
Listeria - all species
Mima polymorpha var. oxidans
Moraxella - all species
Mycobacteria - all species except those listed in Class 3
Mycoplasma - all species except Mycoplasma mycoides and Mycoplasma agalactiae, which are in Class 5.
Neisseria gonorrhoeae, N. meningitides
Pasteurella - afl species except those listed in Class 3
Salmonella - all species and all serotypes Shigella
Shigella - all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum, T. pallidurn, and T. pertenue
Vibrio parahemolyticus
Vibrio choleroe
Vibro fetus, V. comma, including biotype El Tor, and V. parahemolyticus
Yersinia enterocolitico

FUNGAL AGENTS

Actinomycetes (including Nocardia species and Actinomyces species and Arachnia propionica) Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

PARASITIC AGENTS

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi

Schistosoma mansoni
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

VIRAL, RICKETIFSIAL AND CHLAMYDIAL AGENTS

Adenoviruses - human - all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegalovffmes

Echoviruses - all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus

Hepatitis - associated antigen material
Herpes viruses - except Herpesvirus similae
(Monkey B virus) which is in Class 4
Human Immunodeficiency Virus (HIV*)
Corona viruses

Infectious bronchitis - like virus
Influenza viruses - all types except A/PR8/34 which is in
Class I

Langat Virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza viruses - all types except
Parainfluenza virus 3, SF4 strain, which is in
Class I

Polioviruses - all types, wild and attenuated
Corona viruses
Poxviruses - all types except Alastrim, Smallpox, and Whitepox which are in Class 5 and Monkey pox which depending on experiments is in Class 3 or Class 4

*HIV in the natural state is a Class 2 virus. If the virus is concentrated, a BL 2
Contaminant level may be used, but Level 3 practices must be used.
Rabies virus - all strains except Rabies street virus, which should be classified in Class 3 when inoculated into carnivores.
Reoviruses - all types
Respiratory syncytial virus
Rhinoviruses - all types
Rubella virus
Simian viruses - all types except Herpesvirus simiae
(Monkey B virus) and Marburg virus, which are in
Class 4

Sindbis virus
Tensaw virus
Turlock virus
Vacdnia virus
Varicefla virus
Vesicular stomatitis virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

C. Class 3 –
Agents involving special hazard or agents derived from outside the United States which require a USDA permit for importation unless they are specified for higher classification.

BACTERIAL AGENTS

Actinobacillus mallei**
Bartonella - all species
Brucella - all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains**)
Pseudomonas mallei
Pseudomonas pseudomallei**
Yersiriia pestis

**USDA permit also required for interstate transport

UNGAL AGENTS

Coccidioides imniitis
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii

PARASITIC AGENTS

Schistosoma mansoni

VIRAL, RICKETTSIAL AND CHLAMYDIAL AGENTS
Alastrim, Smallpox, Monkey Pox and Whitepox, when used in vitro
Arboviruses - all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. West Nile and Semliki Forest viruses may be classified up or down, depending on the conditions of use

Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Rickettsia - all species except Vole rickettsia when used transmission or animal inoculation experiments.
Psittacosis - Ornithosis - Trachoma group of agents
Rabies street virus, when used in inoculation of carnivores (see Class 2)
Vesicular stomatitis virus (USDA Permit required for transportation)
Yellow Fever virus- wild, when used in vitro

D. Class 4 - (Not allowed at Tuskegee University)

Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments and/or when other entomological experiments are conducted in the same laboratory area.

VIRAL, RICKETTSIAL AND CHLAMYDIAL AGENTS
Alastrim, Smallpox, Monkey Pox and Whitepox, when used in vitro
Ebola fever virus
Monkey pox, when used for transmission or animal inoculation experiments
Hemorrhagic fever agents, including Crimean hemorrhagic fever (Congo), Junin, and Machupo viruses and others as yet undefined

Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis complex, including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses
Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus - wild, when used for transmission or animal inoculation experiments

Classification of Oncogenic Viruses on the Basis of Potential Hazard

A. -Low-Risk Oncogenic Viruses
Rous sarcoma
SV-40
CELO
Ad7-SV40
Polyoma
Bovine papilloma
Rat mammary tumor

Avian leukosis
Murine leukemia
Murine sarcoma
Mouse mammary tumor
Rat leukemia
Hamster leukemia
Bovine leukemia
Dog sarcoma
Mason-Pfizer monkey virus
Marek's
Guinea pig herpes
Lucke (Frog)
Adenovirus
Shope fibroma
Shope papilloma

Moderate-Risk Oncogenic Viruses

Ad2-SV40
FELV
HV Saimiri
EBV
SSV-1
GALV
HV ateles
Yaba
FESV

Class 5 Agents

Animal Disease Organisms Which is Forbidden Entry in to the US by Law

Foot and mouth disease virus