BIOLOGICAL SAFETY MANUAL

VOLUME I
PRACTICES AND PROTOCOLS FOR HANDLING AND DISPOSAL OF INFECTIOUS MATERIALS

INSTITUTIONAL BIOSAFETY COMMITTEE
DEPARTMENT OF BIOLOGY

COLLEGE OF SCIENCE
AND
ENGINEERING TECHNOLOGY

UNIVERSITY OF ARKANSAS AT LITTLE ROCK
2801 SOUTH UNIVERSITY AVENUE
LITTLE ROCK, AR 72204-1099
ACCIDENT OR EMERGENCY PROCEDURES

IF YOU REQUIRE ASSISTANCE CALL:

For Emergencies .................. UALR Public Safety ................... 9-3400
............................................... Fire, Ambulance, Police ............... 8-911

For Medical Assistance ............. UALR Health Services ............... 9-3188
........................................ Ambulance ......................... 8-911

IF A PUBLIC HAZARD EXISTS, ALSO CALL:

For biological Hazard ............... IBC Office ....................... 9-3510 or 9-8983

For Chemical Hazard ................ Physical Plant ............... 9-3390 or 5-5102

FOR OTHER ASSISTANCE CALL:

Employee Assistance Program ........... 686-2588 or 1-800-542-6021

University Tower Building
1123 S. University Ave., Suite 700
Little Rock, AR 72204-1609
Biological Safety Manual
Volume I
Practices and Protocols for handling and
Disposal of Infectious Materials

Institutional Biosafety Committee

Compiling Editor – Maurice G. Kleve
Chairman, Institutional Biosafety Committee (IBC)
Director, Alzheimer Molecular Biology Laboratory (AMBL)

Biology Department
College of Science and Engineering Technology

University of Arkansas at Little Rock
2801 South University Avenue
Little Rock, AR 72204-1099

This Manual is Adapted and Complied from the
CDC-NIH Guidelines for Biosafety in Microbiological
and Biomedical Laboratories

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention
and
National Institutes of Health

and Biosafety Manuals for the

University of Iowa
University of Pennsylvania
University of Kentucky
University of Georgia
University of California, San Diego

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# Biological Safety Manual - Volume I

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Biological Safety Manual - Volume I

Manual Overview

Institutional Biosafety Committee
University of Arkansas at Little Rock

November 1998

The University of Arkansas at Little Rock's Biological Safety Manual provides information on work practices, procedures, and policies necessary to ensure the health and safety of individuals exposed to biohazardous agents in the work place. The contents of this manual are based on current federal and state work place safety guidelines and recommendations. Failure to comply with instructions contained in this manual may result in a violation of the regulations, codes and laws that establish these guidelines and recommendations.

In general, federal and state law holds that an employer must furnish employees a place of employment that is free from recognized hazards that are likely to cause death or serious physical harm. Paramount to a safe and healthful work place is the establishment of a program that defines and details the necessary practices that serve as its basis. This manual is designed to promote biosafety goals in departments, and in addition, details responsibilities of both the employer and the employee. By initiating protocols that are in keeping with current guidelines and recommendations, departments will also be able to assure compliance, especially in light of granting agency requirements.

Policies and procedures stated in this Manual require continuing evaluation, review, and approval by appropriate University officials. All statements reflect policies for procedures or procedures in existence at the time of writing, and the University reserves the right to change policies at any time and without prior notice.

The University of Arkansas at Little Rock is committed to the policy of providing equal opportunity for all persons and will not discriminate in admissions, programs, or any other educational function or service on the basis of sex, disability, age, race, national origin, color, or religion. In carrying out this commitment, the University follows the principle of affirmative action and operates within the federal laws and executive orders prohibiting discrimination. Inquiries concerning the application of any of the federal laws or regulations may be referred to the UALR Office of Human Relations.

The University of Arkansas at Little Rock makes every effort to meet special accommodations and access needs. For information on specific accommodations for individuals with disabilities, contact the department or organization sponsoring the class or event you wish to attend or call the Office of Disabilities Support Services at (501)569-3148.
ANNUAL STATEMENT OF IBC MEMBERSHIP
AND PROGRAM ORGANIZATION

January 2001

The membership of the Institutional Biosafety Committee for the calendar year 2001 is:

- Dr. Maurice G. Kleve, Chairman and Institutional Biosafety Officer
- Dr. James H. Peck, Committee member (On Sabbatical Leave)
- Dr. John M. Bush, Replacement committee member for Dr. Peck
- Dr. Thomas J. Lynch, Committee member
- Dr. Dennis A. Baeyens, Alternate committee member

The Institutional Biosafety Committee has established the Biosafety Level limit for the calendar year 2001 at BL2.

At the inclusion of this annual statement, four members of the Department of Biology faculty are authorized to conduct BL2 activities in rooms posted as BL2 areas.

BL2 authorized faculty are:

- Dr. William H. Baltosser
- Dr. John M. Bush
- Dr. Dale V. Ferguson
- Dr. Maurice G. Kleve

At the inclusion of this annual statement, one staff member of the Department of Biology is authorized to conduct BL2 activities in rooms posted as BL2 areas.

BL2 authorized staff are:

- Bobby Elder

At the inclusion of this annual statement, no students are authorized to conduct BL2 activities.

Maurice G. Kleve, IBO
BIOLOGICAL SAFETY PROGRAM

PROGRAM ADMINISTRATION

The National Institutes of Health (NIH), National Science Foundation (NSF), Center for Disease Control and Prevention (CDC), United States Department of Agriculture (USDA) and a other federal and state agencies require that universities follow all federal guidelines in the use, handling and disposal of biological agents that are infectious, toxic or of recombinant DNA (rDNA) in nature. This includes agents that are alive or the cellular and molecular constituents thereof. In compliance with this requirement, all institutions that deal with the these agents, must establish an Institutional Biosafety Committee (IBC) which oversees compliance with applicable regulations. At the University of Arkansas at Little Rock (UALR) biosafety is overseen by the IBC which was organized in 1990 by Dr. Charles Stevens, Dean of the College of Science and Engineering Technology as part of the UALR Chemical Safety Committee. The IBC consists of a chairman, who also serves as the Institutional Biosafety Officer (IBO), and at least two committee members that are knowledgeable faculty from an appropriate department.

The specific duties of the IBC are to set standard practices and protocols for the use, handling and disposal biological agents and rDNA at UALR and to review all research and teaching activities that utilize biological agents. The review determines if an activity meets regulatory requirements and is primarily accomplished through the examination of a detailed ‘Notice of Intent’ that describes the activity. The duties of the IBO are to: 1) oversee the submission and maintenance Notice of Intent forms; 2) Compile and distribute documentation that describes the standard practices and protocols established by the IBC; 3) organize appropriate training for personnel and students; 4) determine that protective equipment is available and in working order; 5) conduct biosafety inspections of facilities and equipment as needed; and certify compliance on all grant applications and federally and state funded projects.

INTRODUCTION

Laboratories handling biohazardous agents are special, often unique, environments that may pose infectious disease risk to persons in or near them. Fewer than 20% of all cases of laboratory-acquired infections are associated with a known incident. Exposure to infectious aerosols is a plausible, but usually unconfirmed, source of infections. The knowledge, techniques, and equipment needed to prevent most laboratory-acquired infections are readily available. This publication was prepared as an aid to researchers in the prevention of infection of laboratory workers and ancillary personnel. It serves as the written Biological Safety Program for facilities at The University of Arkansas at Little Rock. All personnel engaged in the use of infectious or hazardous biological (biohazardous) agents must participate in this program.

The goal of the University's biological safety program is to protect staff, students, and the environment from exposure to biohazardous agents, as well as the protection of experimental materials. Prior to removal from the clinical or research laboratory area, biohazardous/infectious wastes must be properly packaged and labeled for subsequent decontamination and disposal. The responsibility for identifying and disposing of biohazardous materials rests with principal investigators or laboratory supervisors. This responsibility cannot be shifted to inexperienced or untrained personnel.

Principal investigators or laboratory supervisors should call the Chairman of the IBC at 569-3510, if there is uncertainty about categorizing, handling, storing, treating, or discarding biologically derived material.
EMPLOYER RESPONSIBILITIES

The University is responsible for implementing and maintaining the Biological Safety Program in work areas. This responsibility typically rests with each department, either with a departmental safety committee or with one or more individuals selected to serve as coordinator(s). Employers are obligated to include the following elements in a biological safety program:

- A determination of the required levels of protective apparel, equipment and containment facilities and assurance that these are adequate
- Appropriate training to ensure workers know and follow proper biological safety procedures
- Protective equipment is available and in working order
- Regular biosafety inspections of facilities and equipment are performed
- Assurance of thorough knowledge and compliance with current legal and University requirements concerning biological safety

EMPLOYEE RIGHTS AND RESPONSIBILITIES

The University of Arkansas at Little Rock is responsible for providing a safe and healthful work environment. Biological Safety will be enforced by the University through the College of Science and Mathematics by the Institutional Biosafety Committee (IBC) that will establish rules and guidelines in compliance with existing standards and recommendations of the State of Arkansas as defined by the Arkansas Department of Health, Arkansas Pollution Control and Ecology, Arkansas Livestock and Poultry Commission, Arkansas State Plant Board, and the Arkansas Game and Fish Commission. The rules and guidelines established by the IBC shall also be in compliance with federal standards and recommendations established by the Occupational Safety and Health Administration (OSHA), the Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH), the United States Department of Agriculture (USDA) and any and all other regulatory agencies as applicable.

In a biosafety program, employees should be informed of their rights and responsibilities by including statements, such as:

**Employees have the right to be**

- informed of the health hazards of biological agents in their work areas
- properly trained to work safely with these agents

**Employees have the responsibility to**

- attend training programs concerning biological safety
- stay informed about biohazardous agents in their work areas
- use work practices and protective equipment required for safe performance of their job
- plan and conduct each operation in accordance with recognized biosafety procedures
- inform their supervisors of accidents, conditions, or work practices they believe to be a hazard to their health or to the health of others
INFORMATION AND TRAINING

Departments should provide employees with information and training to ensure they are apprized of biohazards in their work area. Training may take the form of individual instruction, group seminars, audiovisual presentations, handout material, or any combination of the above. Training should include the specific hazards associated with agents in the work area when generic training is insufficient to address specific hazards. A variety of training aids are available from the Office of the IBC. See Appendix C for a list of the training materials available from the IBC library.

Training should be provided at the time of an employee's initial assignment to a work area where biohazardous agents are present and prior to assignment involving new exposure situations. Employees should receive periodic refresher information and training. All training should be documented.

INFORMATION AND TRAINING PROVIDED BY DEPARTMENTS

Each department engaged in biosafety regulated activities should provide appropriate information and training in the use, handling and disposal of biohazardous materials. The Information and training provided by departments should include:

➤ the location and availability of the written Biological Safety Program

➤ the health hazards, signs and symptoms associated with exposures and infections from Biohazardous agents used in the work area

➤ the measures employees can take to protect themselves from these hazards, including specific procedures the University or department has implemented such as appropriate work practices, emergency procedures, and personal protective equipment

➤ the location and availability of reference material on the hazards, safe handling, storage, and disposal of biohazardous agents.

Faculty Training – At present faculty, staff and students involved in handling, storage, or disposal of biohazardous materials will be trained by:

Environmental Health and Safety
G154 University Hospital
University of Arkansas for Medical Sciences
4301 West Markham
Little Rock, AR 72205

Student Training – Students at the University of Arkansas at Little Rock should be aware of biohazards in teaching situations and be provided information and equipment to protect themselves from those hazards. Departments should provide student training at the beginning of each course in which biohazardous agents are used, with specific safety instructions provided at the beginning of each class period. Such instructions should include, on the first meeting of laboratory class each term, a review of the posted safety regulations found in each laboratory. A copy of this posting is shown on page 6. A copy of the biological spill clean-up procedure is presented on page 7 and a copy of the Arkansas Department of Labor Notice of Employees' Chemical Right to know is shown on page 8.
Universal Laboratory Precautions and Practices

Working in a biological laboratory with infectious agents or recombinant DNA molecules may present a biohazard for laboratory personnel. The following precautions and practices will greatly limit the chance of infection and should be exercised at all times. Limit routes of infection by:

1. **NOT** eating, drinking, smoking, adjusting contacts or applying cosmetics in the laboratory.
2. **NOT** mouth pipetting.
3. **NOT** transferring microorganisms to mouth by contaminated fingers or articles.
4. **NOT** exposing skin to contaminated liquids or articles.
5. **NOT** accidentally inoculating oneself with needles, sharp instruments or broken glass.
6. **NOT** transferring microorganisms to eyes by contaminated fingers.
7. **NOT** splashing or squirting infectious materials into the eyes.
8. **NOT** generating aerosols that cause inhalation of airborne microorganisms.

**Primary containment** of potentially infectious materials should be executed by safe and effective use of a Class II (or higher) biosafety cabinet.

**Aerosol generation** should be controlled by appropriate use of centrifuges, mixers, blenders, homogenizers, ultrasonic disruptors, grinders, lyophilizers, pipets, loop sterilizers, Bunsen burners, etc. that are designed to minimize aerosol formation.

**Personal protective equipment** should be used to protect personnel from hazardous materials and infectious agents. The following protective equipment is recommended for regular use.

**Barrier Protection** should be used at all times to prevent skin and mucous membrane contamination with blood or body fluids. Barrier protection should be used when working with ALL organismal tissues, cells or cultures. The type of barrier protection used should be appropriate for the type of procedures being performed and the type of exposure anticipated. Examples of barrier protection include lab coats, gloves and eye and face protection.

**Gloves** are to be worn when there is potential of hand or skin contact with blood, body fluids, tissues, cultures, or other potentially infectious materials, or items and surfaces contaminated with these materials.

**Face protection** should be worn during procedures that are likely to generate droplets of blood or body fluids to prevent exposure of mucous membranes of the mouth, nose and eyes.

**Protective body clothing** should be worn when there is potential for splashing blood or body fluids.

**Wash hands or other skin surfaces** thoroughly and immediately in contaminated with blood or body fluids to which universal precautions apply.

**Wash hands immediately after gloves are removed.**

**Avoid accidental injuries** that can be caused by needles, scalpel blades, laboratory instruments, etc. when performing procedures, cleaning instruments, handling sharp instruments, and disposing of used needles, pipettes, etc.

**Sharp items such as needles, syringes, scalpel blades, etc.** are to be placed in puncture resistant containers marked with a biohazard symbol for disposal.

**Spills of Infectious materials** should be dealt with immediately using a Basic Biological Spill Kit. Use the kit’s guideline procedures, modifying as necessary. As with any emergency, stay calm, call 911 if necessary, and proceed with common sense.
Contents and Use of the Basic Biological Spill Kit

1. Disinfectant, e.g., chlorine bleach (1:10 dilution), 500 ml, prepared fresh.
2. Absorbent material, e.g., paper towels, 1 roll.
3. Waste Container, e.g., autoclavable bags (3), sharps container (1).
4. Personal Protective Equipment, e.g., lab coat (1), gloves (3), eye and face protection.
5. Mechanical tools, e.g., forceps, dustpan and broom.

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. Spills Inside a BL-2 Laboratory:

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag. Don a disposable gown or lab coat, goggles and gloves. Have a complete biological spill kit ready to go before you start the clean-up. Initiate clean-up with disinfectant as follows:

- Cover spill with paper towels wet with disinfectant.
- Encircle the spill with disinfectant, being careful to minimize aerosolization.
- Decontaminate (disinfect) and remove all items within spill area.
- Remove broken glassware with forceps or broom and dustpan and dispose in sharps container.
- Do Not pick up any contaminated sharp object with your hands.
- Remove paper towels and any other absorbent material and dispose in biohazard bags.
- Apply disinfectant to the spill area and allow at least 10 min. contact time to ensure disinfection.
- Remove disinfectant with paper towels and dispose in biohazard bag.
- Wipe off any residual spilled material and reapply disinfectant before final clean-up.
- wipe equipment with compatible disinfectant (non-corrosive). rinse with water if necessary.
- Place contaminated disposable and reusables in separate biohazard bags for autoclaving.
- Reopen area to general use only after spill clean-up and decontamination is complete.
- Inform all personnel and laboratory supervisor about spill and clean-up as soon as possible.

Spill Inside a Biological Safety Cabinet

- Wear lab coat, safety goggles and gloves during clean-up.
- Allow cabinet to run during clean-up.
- Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 10 minutes contact time.
- Wipe up spillage and disinfectant with disposable taper towels.
- Wipe walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.
- Collect contaminated disposable material in biohazard bag and autoclave and discard in waste.
- Place contaminated reusable items in separate biohazard bag before autoclaving and final clean-up.
- Expose non-autoclavable materials to disinfectant for 10 minutes before removal from BSC.
- Remove protective clothing used during clean-up to a biohazard bag for further processing.
- Run cabinet at least 10 minutes after clean-up and before resuming work.
- Inform all users of the BSC as well as the laboratory supervisor about the spill and clean-up as soon as possible.

Spill Outside the Laboratory

Always transport biohazardous materials in an unbreakable well-sealed primary container placed inside a leak proof, closed and unbreakable secondary container, labeled with the biohazard symbol.

Should a spill of BL-2 material occur in the public, contact the IBC at 569-3510 or 569-3893. Do not attempt to clean-up the spill without the proper personal protective equipment and spill clean-up material.

Should the spill occur inside a car, leave the vehicle, close all doors and windows, and contact the IBC at 569-3510.
ARKANSAS DEPARTMENT OF LABOR
NOTICE
TO EMPLOYER AND EMPLOYEE

Act 556 of 1991 entitled the
PUBLIC EMPLOYEES' CHEMICAL RIGHT TO KNOW ACT

PURPOSE
The purpose of this law is to provide public employees access to training and information concerning hazardous chemicals in order to enable them to minimize their exposure to such chemicals and protect their health, safety and welfare.

PUBLIC EMPLOYERS' DUTIES
Public employers are responsible for the following as set out by the law:
1. Post adequate notice to inform employees of their rights
2. Ensure proper chemical labeling
   a. Existing labels on containers of hazardous chemicals are not to be removed
   b. If a chemical is transferred to another container, it must also be labeled with the name and appropriate warnings, as provided in this law
   c. A public employer is not required to label chemicals that have been transferred to a portable container by an employee when that employee is going to immediately use the chemical.
3. Maintain and make available safety data sheets available
   a. Chemical manufacturers and distributors must provide public employers with the appropriate MSDSs within the prescribed times
   b. Public employers must maintain current copies of each MSDS and have them available to employees and their designated representatives upon request within the prescribed time
   c. The employer must not require an employee to work with a chemical until an MSDS can be furnished except as indicated by this law
   d. An employee who declines to work with a chemical may not be penalized
   e. Public employers shall provide a copy of MSDSs to the Director of Labor upon request
4. Compile and maintain a workplace chemical list for hazardous chemicals used, generated, or stored in amounts of 55 gallons or 500 pounds or more
   a. The Workplace Chemical List must show the chemical or common name used on the MSDS and/or the container label, the Chemical Abstracts Service Number and the work area where it will normally be used, generated, or stored
   b. Chemical lists shall be filed with the Director of Labor no later than October 14, 1991, updated when necessary, and relied July 1 of each year
5. Provide employees with information and training
   a. The Director of Labor is responsible for maintaining a general information and training assistance program to aid public employers
   b. Additional training must be provided when a new hazard is introduced, when new information is received, or before new employees are assigned to a job
   c. Information and training programs must meet the requirements specified in the law and in the regulations of the Director of Labor.
   d. Information and training programs must be developed by January 15, 1992, and initial information and training must be provided prior to July 15, 1992. Employers must keep a record of the dates of training sessions given to their employees.
   e. The Director of Labor's rules and regulations concerning refresher training and training exemptions must be followed
   f. Handle trade secrets in accordance with provisions set out in the law
     a. The Director of Labor can request data substantiating a trade secret claim when asked to by an employee, designated representative, or public employer
     b. All information will be kept confidential

PUBLIC EMPLOYEES' RIGHTS
Public employees who may be exposed to hazardous chemicals must be informed and shall have access to the Workplace Chemical List, MSDSs for the chemicals on the list, and information and training as provided in this act.

A public employee cannot be disciplined, discharged or discriminated against for requesting information, filling a complaint, assisting an inspector of the Department of Labor, causing any complaint or proceeding to be instituted, testifying in any proceeding, or exercising any right afforded by this law.

Any waiver of the benefits or requirements of this law are a violation and are therefore null and void.

COMPLAINTS AND INVESTIGATIONS
The Director of the Department of Labor will investigate written or verbal complaints from public employees concerning violations of this law. The Director or his designated representative has the authority to enter the workplace and conduct a thorough investigation of the complaint as specified by this law.

ENFORCEMENT
If the Director of Labor finds a public employer in violation of this law, he shall issue an order to cease and desist the act or omission constituting the violation.

If the Director of Labor finds that a public employer has failed to provide the required information and training by the prescribed time, he may conduct the program and charge the employer for the costs incurred.

Violation of any act shall be cause for adverse personnel action against the responsible supervisor as set out in this act.

CAUSE OF ACTION - ATTORNEY FEES
Any citizen denied their rights under this law may commence civil action in circuit court and the court shall hear the petition within seven days.

The court shall have the jurisdiction to restrain violations of this act and to order all appropriate relief. Those who refuse to comply with these orders will be in contempt of court.

Attorney fees and court costs will be assessed to the defendant and plaintiff as set out by the law.

NO EFFECT ON OTHER LEGAL DUTIES
The provision of information to a public employee does not affect the liability of the employer with regard to the health and safety of the employee, or the employer's responsibility to prevent the occurrence of occupational disease.

The provision of information to an employee also does not affect any other duty or responsibility of a chemical manufacturer or distributor to warn users of a hazardous chemical.

ARKANSAS DEPARTMENT OF LABOR
10421 WEST MARKHAM
LITTLE ROCK, ARKANSAS 72205
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PRINCIPLES OF BIOSAFETY

BIOHAZARDOUS AGENTS/MATERIAL

"Biohazardous agent" means an agent that is biological in nature, capable of self replication, and possesses the capacity to produce deleterious effects upon biological organisms. Biohazardous agents include, but are not limited to those listed below.

A biohazardous material is any material that contains or has been contaminated by a biohazardous agent. This contamination may not actually include the living biohazardous agent but a product, exudate, secretion, etc. of a biohazardous agent.

Categories of Biohazardous Agents –

- viruses
- oncogenic viruses
- bacteria
- rickettsia
- chlamydia
- parasites
- fungi
- recombinant DNA (rDNA)
- cultured animal cells and potentially biohazardous agents they may contain
- human clinical specimens (tissues, fluids, etc.)
- tissues from experimental animals (including animal dander)

Containment and safe handling of potentially biohazardous materials require strict adherence to prudent microbiological practices. Procedures and practices are outlined within the section on Biosafety Level Criteria (see pages 13-22). Appendices A and B give the general class to which organisms have been assigned based on activities typically associated with the growth and manipulation of the quantities and concentrations of infectious agents required to accomplish identification or typing. In most cases, the class designation is the same as the biosafety level number designation. Additional precautions and increased levels of primary and secondary containment may be indicated in certain situations. Areas where increased safety measures may be needed are where activities involve large volumes and/or concentrated preparations ("production quantities"), manipulations are likely to produce aerosols, or those that are otherwise intrinsically hazardous.

When working with biohazardous materials, it's of primary importance that procedures are followed consistently. Additionally, specific safety knowledge regarding the material or agent, motivation and critical judgement are essential for ensuring the protection of all personnel, the public, and the environment.
ROUTES OF EXPOSURE

Unlike chemical and physical agents where "safe" doses are specified in technical literature, no such assignment has been made to biohazardous agents. Thus, controlling exposures to these agents becomes the greatest task in the prevention of subsequent infections. In order to accomplish this goal, it is necessary to understand the routes by which exposure to these agents occurs. The most common routes are:

- respiratory (from the generation of aerosols)
- contact
- oral (ingestion)
- ocular (mucous membrane)
- inoculation (parenteral)

GENERATION OF AEROSOLS

It is generally conceded that aerosols are the primary means by which infectious diseases are contracted or spread in the microbiological laboratory, although many are known to have occurred from animal bites, needle sticks, and similar situations where direct contact can occur.

There are many opportunities for aerosols to be generated through normal laboratory procedures. The number of organisms sufficient to cause infections in humans can be present in a single droplet. Studies have been conducted to determine the average number of droplets created by many typical operations, and it was found that some procedures are prolific aerosol generators.

Laboratory-associated infections represent an occupational hazard for all personnel working in institutions where infectious disease agents are handled. Of the nearly 4000 reported laboratory associated infections, just over 4% proved fatal. Less than 20% of the infections Laboratory acquired infections could be attributed to some known accident, such as accidental inoculation. Although not directly proven, aerosol production could account for a large part of those accidents listed as due to "unknown causes." The majority of laboratory-associated infections occurred at institutions engaged in research or clinical diagnostic work. It is interesting to note that the personnel involved were generally trained professionals, those individuals most knowledgeable about laboratory hazards.

Some of the laboratory operations which release a substantial number of droplets seem almost trivial in nature, such as breaking bubbles on the surface of a culture as it is stirred, streaking a rough agar place with a loop, using a vortex to mix a liquid and then removing the cap too soon, a drop falling off the end of a pipeffe, inserting a hot loop into a culture, pulling a stopper or a cotton plug from a bottle or flask, taking a culture sample from a vaccine bottle, opening and closing a petri dish, or opening a lyophilized culture, etc. Most of these only take a few seconds and are often repeated many times daily. Other more complicated procedures might be considered more likely to release organisms into the air, such as grinding tissue with a mortar and pestle, conducting an autopsy on a small animal, harvesting infected tissue from animals or eggs, intranasal inoculation of small animals, opening a blender too quickly, etc. Some incidents
have occurred by failing to take into account the possibility that accidents can happen, such as a tube breaking in a centrifuge. The possibility of aerosol production should always be considered while working with infectious organisms.

CONTACT

Control of potential exposure by contact requires procedures be conducted in a manner that avoids contamination of body or work surfaces. This is accomplished through the use of gloves and other personal protective clothing, protection of work surfaces with appropriate absorbent disposable covering, using care in the performance of procedures, and cleaning and disinfecting work surfaces. Dispersal of contaminants to other surfaces can occur by their transfer on the gloves of a laboratory worker, by placement of contaminated equipment or lab ware, and by improper packaging of contaminated waste.

ORAL OR INGESTION

A number of procedures carried out in the laboratory and animal facility offers the potential for either direct or indirect exposure by the oral route. The procedure that offers the greatest potential for exposure by ingestion is mouth pipetting. Clearly, such exposures are completely avoidable through the use of mechanical pipetting devices. Indirect oral exposures can be avoided by practicing good personal hygiene that includes regular hand washing and not placing any objects, including fingers, into the mouth. Wearing a surgical mask or face shield will serve as protection against splashes or splatters of biohazardous material into the mouth.

OCULAR OR MUCOUS MEMBRANE

The wearing of a face shield, safety glasses, or goggles will protect workers against splashing biohazardous material into the eyes. Avoid all hand and eye contact.

INOCULATION

The single procedure that presents the greatest risk of exposure through inoculation is the use of a needle and syringe. These are used principally for the transfer of materials from diaphragm-stoppered containers and for the inoculation of animals. Their use in the transfer of materials from diaphragm-stoppered containers can result in dispersal of biohazardous material onto surfaces and into the air. Depending on the route of inoculation of animals, using a needle and syringe may also result in contamination of body surfaces. Because of the imminent hazard of self-inoculation, the use of a needle and syringe should be limited to those procedures where there is no alternative. Perform such procedures with the greatest possible care. Keep in mind, the primary rule to remember when using a needle or syringe is to never recap! Place the uncapped needle or syringe into a sharps container. Do not overfill the container. Inoculation can also result from animal bites and scratches.

BIOHAZARDOUS/INFECTIOUS WASTE

In Arkansas, disposal of waste is regulated by the Department of Health (DOH). By definition, infectious waste includes the waste categories listed below. Infectious means containing pathogens with sufficient virulence and quantity so that exposure to an infectious agent by a susceptible host could result in an infectious disease when the infectious agent is improperly treated, stored, transplanted, or disposed of. At The University of Arkansas at little Rock,
infectious waste and biohazardous waste, such as cultures of bacteria used in recombinant DNA activities, are synonymous. Since a precise definition of infectious waste, based on the quantity and type of etiologic agents present, is virtually impossible to assess, the most practical approach to infectious waste management is to identify those categories of waste that have the greatest potential for transmitting disease. The following categories of waste are designated as infectious:

CULTURES AND STOCKS

➤ cultures from medical and pathology laboratories
➤ cultures and stocks of infectious agents from research and industrial laboratories
➤ wastes from the production of biologicals
➤ discarded live and attenuated vaccines
➤ culture dishes and devices used to transfer, inoculate, and mix cultures

PATHOLOGICAL WASTES AND HUMAN BODY FLUIDS

➤ tissues, organs, body parts and body fluids that are removed during surgery, autopsy, or other medical procedures
➤ specimens of body fluids and their containers
➤ cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid from humans

HUMAN BLOOD AND BLOOD PRODUCTS

➤ waste human blood
➤ products of blood
➤ items saturated and/or dripping with human blood
➤ items that were saturated with human blood that are now caked with dried human blood serum, plasma, and other blood components, and their container(s), which were used in either patient care, testing and laboratory analysis or the development of pharmaceuticals

SHARPS, NEEDLES, HYPODERMICS

➤ sharps that have been used in animal or human patient care or treatment or in medical, research, or industrial laboratories, including hypodermic needles, syringes, pasteur pipettes, scalpel blades, razor blades, and needles with attached tubing
➤ broken or unbroken glassware that were in contact with infectious agents
➤ used slides and cover slips
shards of contaminated broken glass

ANIMAL CARCASSES AND BEDDING

Animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research, production of biological material or testing of pharmaceuticals are infectious waste.

ISOLATION WASTES

These include all wastes that are biological or discarded materials contaminated with blood, excretion, exudates, or secretions from humans who are isolated to protect others from highly communicable diseases.

OTHER LABORATORY WASTES

These wastes include, but are not limited to:

- specimen containers
- disposable gloves, lab coats, masks and aprons
- disposable pipettes
- all cell culture materials
- all microorganisms constructed using rDNA

CONTAINMENT

The term containment is used in describing safe methods for managing biohazardous agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce exposure of laboratory workers and other persons, and to prevent escape of potentially biohazardous agents into the outside environment. Three elements of containment are laboratory practice and technique, safety equipment, and facility design.

LABORATORY PRACTICES AND TECHNIQUES

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with biohazardous agents or materials must be aware of potential hazards. They must be trained and proficient in the techniques required for safely handling of the material. When standard laboratory practices are not sufficient to control the hazard associated with a particular agent, additional measures may be needed.

Each laboratory should develop or adopt a biological safety plan identifying hazards that may be encountered, and specifying practices and procedures designed to minimize or eliminate risks. Personnel should be advised of hazards and should be required to follow the specified practices.

Safe practices and techniques must be supplemented by appropriate facility design, engineering features, safety equipment, and management practices.
SAFETY EQUIPMENT

Safety equipment includes biological safety cabinets and a variety of enclosed containers, e.g., the safety centrifuge cup, which is designed to prevent aerosol release during centrifugation. The biological safety cabinet (BSC) is the principal device used to provide containment of aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are described on pages 42-48. Open fronted Class I and Class II biological safety cabinets are partial containment cabinets that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection from an equipment standpoint. Structural diagrams of each class if BSC are provided on pages 46-47.

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

FACILITY DESIGN

Design of the facility is important in providing a barrier to protect persons working outside the laboratory from biohazardous agents that may be accidentally released inside the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory’s function. Described below are three facility designs in ascending order by level of containment. This is a brief description of the actual facility requirements for each level of containment. The requirements for each level are detailed in their entirety in the following section, Biosafety Level Criteria.

The Basic Laboratory—Provides general space appropriate for work with defined biohazardous agents that are not associated with disease processes in healthy adults or that do not colonize in humans. All activities are regularly conducted on the open bench using standard laboratory practices. The basic laboratory will be adequate for Biosafety Level 1 activities including recombinant DNA work and the use of Class I biohazardous organisms (see page 15 for a description of Biosafety Level 1 and page 61 for a listing of Class I organisms).

The containment Laboratory provides general space appropriate for work with biohazardous agents or potentially biohazardous materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. Work is commonly conducted on open benches with certain operations confined to biological safety cabinets. Conventional laboratory designs are adequate. Areas known to be sources of general contamination such as animal rooms and waste staging areas should not be adjacent to media processing areas, tissue culture laboratories, or patient care activities. Public areas and general offices to which non-laboratory staff requires frequent access should be separated from space that primarily supports laboratory functions. The containment laboratory is adequate for Biosafety Level 2 using Class 2 biohazardous infectious organisms (see pages 17 and 69 for a description of biosafety level 2 and class 2 organisms).
High Containment Laboratory—has special engineering features that make it possible for laboratory workers to handle hazardous materials. Unique features that distinguish this laboratory from the basic and containment laboratories are provisions for access control and a specialized ventilation system. The high containment laboratory may be an entire building or a single module or complex of modules within a building. In all cases, the laboratory is separated by a controlled access zone from areas open to the public and laboratory personnel from other areas. Facilities defined as high containment are adequate for Biosafety Level 3 and Class 3 organisms.

Maximum Containment Laboratory - containment lab; has special engineering and containment features that will allow safe conduct of activities involving biohazardous agents that are extremely hazardous to laboratory workers or that may cause serious epidemic disease. Although the maximum security lab is usually a separate building, it can be constructed as an isolated area within a building. The distinguishing characteristic is the provision for secondary barriers to prevent hazardous materials from escaping into the environment. Such barriers include: sealing all lab openings, installing air locks or liquid disinfectant barriers, adding a contiguous clothing change room, double door autoclave, biowaste treatment system, separate ventilation system, and an exhaust air decontamination system. Such systems are adequate for Biosafety Level 4 using Class 4 organisms. At the present time the University of Arkansas at Little Rock does not have facilities adequate for high containment activities and the Institutional Biosafety Committee does not allow activities and practices in research or teaching above Biosafety Level 2 or the use of organisms above Class 2.

BIOSAFETY LEVEL CRITERIA

Three biosafety levels are specified that consist of combinations of lab practices and techniques, safety equipment, and lab facilities. These are commensurate with the operations performed and with the hazard potential posed by the biohazardous agents with which the laboratory works.

The reference for this section is Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, May 1993, U.S. Department of Health and Human Services. A fourth biosafety level (the maximum containment lab) described in that reference is not included in this manual, as facilities, personnel and equipment do not presently exist at The University of Arkansas that would accommodate the safety regulations involved with Biosafety Level 4 work.

BIOSAFETY LEVEL 1 (BL1); THE BASIC LABORATORY

Level 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and that present a minimal potential hazard to lab personnel or the environment. The laboratory is not necessarily separated from general building traffic patterns and work is generally conducted on open bench tops. Lab personnel have specific training in the procedures used and are supervised by a scientist with general training in microbiology or a related science. Special containment equipment is generally not required for manipulations of agents assigned to BL1. Recombinant DNA work that only uses the K12 strains of E. coli as a recombinant host (or other Class I bacteria or organisms) as hosts, as designated in the NIH Guidelines for Recombinant DNA Research described in Volume 2 of the University of Arkansas at Little Rock, Biosafety Manual.

A. Standard Microbiological Procedures

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

2. Work surfaces are decontaminated daily and after any spill of biohazardous material.

3. All contaminated wastes are decontaminated before disposal. Materials to be decontaminated are placed in a durable, leak proof container and closed for removal from the laboratory. At present, all recombinant DNA work with class 1 organisms and all activities with class 2 organisms that require decontamination before disposal will be confined to Friibough Hall and the Science Laboratories Building, both of which have autoclave facilities. Before any recombinant DNA materials or Class 2 wastes can be transported, they must be decontaminated by sterilization in an approved autoclave (contact IBC for information). Materials to be decontaminated and transported off-site (such transport is not allowed at present time) from the laboratory must be packaged in accordance with University policies. Call IBC for a copy of the latest waste disposal procedures.

4. Mouth pipetting is prohibited.

5. Eating, drinking, smoking, handling contact lenses, or applying cosmetics in the lab is not permitted in work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food must be stored in cabinets or refrigerators solely intended for this purpose located outside the work area.

6. Persons will wash hands after handling biohazardous materials and animals, after removing gloves, and before they leave the laboratory.

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

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

9. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms.

B. Special Practices: None

C. Safety Equipment (Primary Barriers)

1. A special containment device or equipment such as a biological safety cabinet is generally not required for manipulations of agents assigned to BL1.

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 exists.

4. Protective eye wear should be worn for anticipated splashes of microorganisms or other hazardous materials.
D. Laboratory Facilities

1. The laboratory should be designed so that it is easily cleaned. Rugs should not be used because decontamination following a spill would be extremely difficult to achieve.

2. Bench tops should be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

3. Laboratory furniture should be sturdy and spaces between benches, cabinets, and equipment should be accessible for cleaning.

4. Each laboratory should contain a hand washing sink.

5. Windows that open should be fitted with fly screens.

BIOSAFETY LEVEL 2 (BL2); THE CONTAINMENT LABORATORY

Biosafety level 2 is similar to level 1 and is suitable for work involving agents that represent a moderate hazard for personnel and the environment. It differs in that:

A. Standard Microbiological Practices (in addition to those for BL1)

1. Access is restricted by the supervisor when work with biohazardous agents is in progress.

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

3. Serological procedures with inactivated antigens known or shown to be free of residual infectivity can be performed on the open bench.

B. Special Practices (in addition to those listed for BL1)

1. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. Persons at increased risk may include children, pregnant women, and individuals who are immunosuppressed. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the area.

2. The laboratory director establishes policies and procedures to assure that only persons who have been advised of potential hazards and who meet any specific entry requirements (e.g., immunizations) enter the laboratory or animal rooms.

3. When biohazardous materials or infected animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol (see following page) is posted on all laboratory and animal room access doors and on such other items (i.e., equipment, containers, materials) as appropriate. The hazard warning sign should identify the agent, list the name of the laboratory supervisor or other responsible person(s), and indicate any special requirements for entering the area (immunization, respirators, etc.).
ARKANSAS DEPARTMENT OF LABOR
NOTICE
TO EMPLOYER AND EMPLOYEE

Act 556 of 1991 entitled the
PUBLIC EMPLOYEES’ CHEMICAL RIGHT TO KNOW ACT

PURPOSE
The purpose of this law is to provide public employees access to training and information concerning hazardous chemicals in order to enable them to minimize their exposure to such chemicals and protect their health, safety and welfare.

PUBLIC EMPLOYERS’ DUTIES
Public employers are responsible for the following as set out by the law:
1. Post adequate notice to inform employees of their rights
2. Ensure proper chemical labeling
   a. Existing labels on containers of hazardous chemicals are not to be removed
   b. If a chemical is transferred to another container, it must also be labeled with the name and appropriate warnings, as provided in this law
   c. A public employer is not required to label chemicals that have been transferred to a portable container by an employee when that employee is going to immediately use the chemical.
3. Maintain and make material safety data sheets available
   a. Chemical manufacturers and distributors must provide public employers with the appropriate MSDSs within the prescribed times
   b. Public employers must maintain current copies of each MSDS and have them available to employees and their designated representatives upon request within the prescribed time
   c. The employer must not require an employee to work with a chemical until a MSDS can be furnished except as indicated by this law
   d. An employee who declines to work with a chemical may not be penalized
   e. Public employers shall provide a copy of MSDSs to the Director of Labor upon request
4. Compile and maintain a workplace chemical list for hazardous chemicals used, generated, or stored in amounts of 55 gallons or 500 pounds or more
   a. The Workplace Chemical List must show the chemical or common name used on the MSDS and/or the container label, the Chemical Abstracts Service Number and the work area where it will normally be used, generated, or stored
   b. Chemical lists shall be filed with the Director of Labor no later than October 14, 1991, updated when necessary, and rilled July 1 of each year
5. Provide employees with information and training
   a. The Director of Labor is responsible for maintaining a general information and training assistance program to aid public employers
   b. Additional training must be provided when a new hazard is introduced, when new information is received, or before new employees are assigned to a job
   c. Information and training programs must meet the requirements specified in the law and in the regulations of the Director of Labor
   d. Information and training programs must be developed by January 15, 1993, and initial information and training must be provided prior to July 15, 1992. Employers must keep a record of the dates of training sessions given to their employees
   e. The Director of Labor’s rules and regulations concerning refresher training and training exemptions must be followed
6. Handle trade secrets in accordance with provisions set out in the law
   a. The Director of Labor can request data substantiating a trade secret claim when asked to by an employee, designated representative, or public employer
   b. All information will be kept confidential

PUBLIC EMPLOYEES’ RIGHTS
Public employees who may be exposed to hazardous chemicals must be informed and shall have access to the Workplace Chemical List, MSDSs for the chemicals on the list, and information and training as provided in this act.
A public employee cannot be disciplined, discharged or discriminated against for requesting information, filing a complaint, assisting an inspector of the Department of Labor, causing any complaint or proceeding to be instituted, testifying in any proceeding, or exercising any right afforded by this law.
Any waiver of the benefits or requirement of this law are a violation and are therefore null and void.

COMPLAINTS AND INVESTIGATIONS
The Director of the Department of Labor will investigate written and oral complaints from public employees concerning violations of this law. The Director or his designated representative has the authority to enter the workplace and conduct a thorough investigation of the complaint as specified by this law.

ENFORCEMENT
If the Director of Labor finds a public employer in violation of this law, he shall issue an order to cease and desist the act or omission constituting the violation.
If the Director of Labor finds that a public employer has failed to provide the required information and training by the prescribed time, he may conduct the program and charge the employer for the costs incurred.
Violation of this act shall be cause for adverse personnel action against the responsible supervisor as set out in this act.

CAUSE OF ACTION - ATTORNEY FEES
Any citizen denied their rights under this law may commence civil action in circuit court and the court shall hear the petition within seven days.
The court shall have the jurisdiction to restrain violations of this act and to order all appropriate relief. Those who refuse to comply with these orders will be in contempt of court.
Attorney fees and court costs will be assessed to the defendant and plaintiff as set out by the law.

NO EFFECT ON OTHER LEGAL DUTIES
The provision of information to a public employer does not affect the liability of the employer with regard to the health and safety of the employees, or the employer’s responsibility to prevent the occurrence of occupational disease.
The provision of information to an employee also does not affect any other duty or responsibility of a chemical manufacturer or distributor to warn users of a hazardous chemical.

ARKANSAS DEPARTMENT OF LABOR
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LITTLE ROCK, ARKANSAS 72205
PH. (501) 682-4500
UNIVERSAL BIOSAFETY/BIOHAZARD WARNING SYMBOL

Signs incorporating this symbol and stating "BIOHAZARD" must appear in all biosafety designated areas. Additional information such as the person in charge, the nature of the hazard, general or specific instructions and emergency contact information may be added to such signage as required (see pages 39-41 for examples of signs presently authorized by the IBC).
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. Animals not involved in experiments being performed are not permitted in the laboratory.

6. A high degree of precaution must always be taken with contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be used 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.

   a. 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. 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.

   b. Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.

   c. 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. All needles, sharp equipment, and contaminated broken glass are placed in sharps buckets or sharps-containers for disposal by incineration or other approved means.

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

8. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated before it is sent for repair, maintenance or packaged for transport before removal from the facility.

9. Spills and accidents which result in overt or potential 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 (see page ) for instructions and procedures to follow in case of a biohazardous spill.

10. Baseline serum samples should be collected from and stored for all at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility. University Health Services will collect and store baseline serums on any individual with potential exposures (at present time the IBC does not allow activities with agents that require serum surveillance).

11. A safety or operations manual that identifies potential hazards and that specifies practices
and procedures to minimize or eliminate such risks should be prepared or adopted. Personnel are advised of special hazards and are required to follow standard practices and procedures.

C. Safety Equipment

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

b. High concentrations or large volumes of biohazardous 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.

1. Gloves are worn for all procedures requiring the handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves maybe appropriate. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are not worn outside the laboratory. Disposable gloves are not washed or reused.

2. Face protection (goggles, mask, face shield or other splatter guards) 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.

D. Laboratory Facilities (in addition to the requirements for BL1)

1. A method for decontamination of infectious or regulated laboratory wastes is available (e.g., autoclave, chemical disinfection).

2. An eyewash facility is readily available.

BIOSAFETY LEVEL 3 (BL3); THE HIGH CONTAINMENT LABORATORY

At the present time the University of Arkansas at Little Rock and the Institutional Biosafety Committee have limited activities on the UALR campus the Biosafety Level 2. This manual describes level 3 below but such activities are prohibited at this time.

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that can 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. The laboratory has special engineering and design features and physical containment equipment and devices.
All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices.

It is recognized that existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in BL2 facilities. However, the recommendations under "Standard Microbiological Practices," "Special Practices," and "Safety Equipment" for BL3 must be rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the laboratory director.

A. Special Practices (in addition to those listed for BL1 & BL2)

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

2. Access to is controlled by the laboratory director and is restricted 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 be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immuno-suppressed or immunocompromised 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 area.

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, if available), and comply with all entry and exit procedures may enter the laboratory or animal rooms.

4. Laboratory 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.

5. The laboratory director is responsible for insuring 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 director or other competent scientist proficient in safe microbiological practices and techniques.

6. All manipulations involving biohazardous materials are conducted in biological safety cabinets or other physical containment devices. No work in open vessels is conducted on the open bench.

7. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Contaminated equipment must be decontaminated before it is sent for repair or mainten-
ance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Plastic backed paper toweling is used on non-perforated work surfaces within biological safety cabinets to facilitate clean-up.

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

9. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from labs or animal rooms are decontaminated before being disposed of or reused.

10. 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.

11. Spills and accidents which result in overt or potential exposures to infectious materials are immediately reported to the laboratory director and the IBC office.

12. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained. See section on Exposure Evaluation (page 47).

B. Safety Equipment

1. Properly maintained biological safety cabinets are used (Class II or III) for all manipulations of infectious materials.

2. Outside of a BSC, appropriate combinations of personal protective equipment are used (e.g., special protective clothing, masks, gloves, face protection, or respirators), in combination with physical containment devices (e.g., centrifuge safety cups, sealed centrifuge rotors, or containment caging for animals).

3. This equipment must be used for manipulations of cultures and clinical or environmental materials that may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.

4. Face protection (goggles and mask, or face shield) is worn for manipulations of infectious materials outside of a biological safety cabinet.

5. Respiratory protection (molded surgical mask or respirator) is worn when aerosols cannot be safely contained (i.e., outside of a biological safety cabinet), and in rooms containing infected animals.

6. Protective lab clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls must be worn in, and not worn outside, the laboratory. Reusable laboratory clothing is to be decontaminated before being laundered.

7. Special care is taken to avoid skin contamination with contaminated materials; gloves must be worn when handling infected animals and when hands may contact infectious materials and contaminated surfaces or equipment. Disposable gloves should be discarded when contaminated. Never wash and reuse disposables.
C. Laboratory Facilities (in addition to those listed for BL1 & BL2)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of self-closing doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. A clothes change room (shower optional) may be included in the passageway.

2. The surfaces of walls, floors and ceilings are water resistant and can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

3. A foot or elbow operated hand washing sink is provided near each laboratory exit door.

4. Windows in the laboratory are closed and sealed.

5. Access doors to the laboratory are self-closing and self locking.

6. A method for decontaminating all laboratory wastes is available, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method).

7. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged directly to the outside with filtration and other treatment optional. Outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that directional airflow (into the laboratory) is proper.

8. In laboratories which have supplied air systems, the supply air and exhaust air systems are interlocked to assure inward (or zero) airflow at all times.

9. The HEPA-filtered exhaust air from Class II or Class III biological safety cabinets is discharged directly to the outside or through the building's exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class II or Class III biological safety cabinets is to be discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

10. 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.

11. Vacuum lines are protected with liquid disinfectant traps and their equivalent, which are routinely maintained and replaced as needed.
<table>
<thead>
<tr>
<th>Biosafety Level</th>
<th>Agents</th>
<th>Practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to cause disease in healthy adults.</td>
<td>Standard microbiological practices</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Open bench top sink required.</td>
</tr>
<tr>
<td>2</td>
<td>Associated with human disease.</td>
<td>BL-1 practices plus:</td>
</tr>
<tr>
<td></td>
<td>Hazard: auto-inoculation, ingestion, mucous membrane exposure</td>
<td>• limited access</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• biohazard warning signs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• sharps precautions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• biosafety manual defining waste decontamination or medical surveillance policies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary barriers: Class I or II biosafety cabinets or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats, gloves, face protection as needed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BL-1 plus:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• autoclave available</td>
</tr>
<tr>
<td>3</td>
<td>Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences</td>
<td>BL-2 practices plus:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• controlled access</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• decontamination of all waste</td>
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<tr>
<td></td>
<td></td>
<td>• decontamination of lab clothing before laundering</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• baseline serum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary barriers: Class I or II biosafety cabinets or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing, gloves, respiratory protection as needed</td>
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<tr>
<td></td>
<td></td>
<td>BL-2 plus:</td>
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<tr>
<td></td>
<td></td>
<td>• physical separation from access corridors</td>
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<tr>
<td></td>
<td></td>
<td>• self-closing, double door access</td>
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<tr>
<td></td>
<td></td>
<td>• exhausted air not recirculated</td>
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<tr>
<td></td>
<td></td>
<td>• negative airflow into laboratory</td>
</tr>
<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission</td>
<td>BL-3 practices plus:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• clothing change before entering</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• shower on exit</td>
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<tr>
<td></td>
<td></td>
<td>• all material decontaminated on exit from facility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary barriers: All procedures conducted in Class III biosafety cabinets or Class I or II biosafety cabinets in combination with full-body, air-supplied, positive pressure personnel suit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BL-3 plus:</td>
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<tr>
<td></td>
<td></td>
<td>• separate building or isolated zone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• dedicated supply/exhaust, vacuum, and decon systems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• other requirements outlined in the complete description of BL-4</td>
</tr>
</tbody>
</table>

TABLE 1: SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS
VERTEBRATE ANIMAL BIOSAFETY CRITERIA

These guidelines describe three combinations of practices, safety equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. They provide increasing levels of protection to personnel and the environment and are recommended as minimal standards for activities involving infected laboratory mammals. These three combinations are designated in each of the Animal Biosafety Levels 1-3 and describe animal facilities and practices applicable to work on animals infected with agents assigned to corresponding Biosafety Levels 1-3. No activities or organisms that exceed Animal Biosafety Level 3 are allowed by the IBC at this time.

See Appendix Q of the NIH Guidelines for additional requirements specific to large animals involved in research with rDNA. For a copy of the most recent NIH Guidelines, call the Office of Research and Sponsored Programs (569-6657; FAX 569-3039) or the Chairman of the Institutional Biosafety Committee (IBC), (569-3510; FAX 569-3271).

ANIMAL BIOSAFETY LEVEL 1

A. Standard Practices

1. Access to the animal facility is limited or restricted at the discretion of the laboratory or animal facility director.

2. Doors to animal rooms open inward, are self-closing and are kept closed when experiments are in progress.

3. Work surfaces are decontaminated following use or after any spill of viable materials.

4. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in animal rooms. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield.

5. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal room.

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

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

8. All wastes from the animal room are appropriately decontaminated, preferably by autoclaving, before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leak proof, covered containers.

B. Special Practices

1. The laboratory or animal facility director limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room.
2. The laboratory or animal facility director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization) may enter the animal room.

3. Bedding materials from animal cages are removed in such a manner as to minimize the creation of aerosols, and are disposed of in compliance with applicable institutional or local requirements.

4. Cages are washed manually or in a cage washer. Temperature of final rinse water in a mechanical washer should be 180°F.

5. The wearing of laboratory coats, gowns or uniforms in the animal facility is recommended. It is further recommended that lab coats worn in the animal facility not be worn in other areas.

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

C. Safety Equipment

Special containment equipment is generally not required for animals infected with agents assigned to Biosafety Level 1.

D. Animal Facilities

1. The animal facility should be designed and constructed to facilitate cleaning and housekeeping.

2. A hand washing sink is available in the animal facility.

3. Open windows should be fitted with fly screens.

4. Exhaust air is discharged to the outside without being recirculated to other rooms. It is recommended, but not required, that the direction of airflow in the animal facility is inward.

ANIMAL BIOSAFETY LEVEL 2

A. Special Practices (in addition to those listed for biosafety level 1)

1. Cages are appropriately decontaminated, preferably by autoclaving, before they are cleaned and washed. Equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated before it is sent for repair, maintenance, or packaged for transport and before removal from the facility.

2. The laboratory or animal facility director limits access to the animal room to personnel who have been advised of the potential hazard and who meet specific requirements (e.g., immunization). In general, persons who may be at increased risk of acquiring
infection or for whom infection might be unusually hazardous are not allowed in the animal room. Persons at increased risk may include children, pregnant women, and individuals who are immuno-deficient. The laboratory director has the final responsibility for assessing circumstances and determining who may enter or work in the animal room.

3. When the infectious agents in use in the animal room require special entry provisions (e.g., the need for immunizations and respirators) a hazard warning sign, incorporating the universal biohazard warning symbol, is posted on the access door to the animal room. The hazard warning sign identifies the infectious agents in use, lists the name and phone number of the animal facility supervisor or other responsible persons and indicates the special requirements for entering.

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. Considering the agents handled, baseline serum samples from animal care and other at-risk personnel are collected and stored, when appropriate. Additional serum samples may be collected periodically, depending on the agents handled or the function of the facility. The decision to establish a serologic surveillance program must take into account the availability of methods for the assessment of antibody to the agents of concern.

6. Laboratory 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.

7. A high degree of precaution must always be taken with contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments are restricted for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.

   a. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used for the 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. 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.

   b. Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.

   c. 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. Contaminated needles, sharp equipment, and broken glass are laced in sharps buckets or
sharps-containers for disposal by incineration or other approved means prior to disposal.

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

e. Spills and accidents resulting in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and necessary treatment is provided and written records are maintained. (See page ??, for a description of spill kit materials and equipment and practices and protocols required to contain and decontaminate biohazardous spills).

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

B. Safety Equipment (Primary Barriers)

1. Biological safety cabinets, other physical containment devices, and/or personal protection devices (e.g., respirators, face shields) are used whenever procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of tissues or fluid from infected animals or eggs, intranasal inoculation of animals, and manipulations of large volumes of infectious materials.

2. Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms housing non-human primates.

3. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.

4. Special care is taken to avoid skin contamination with infectious materials; gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.

C. Animal Facilities (in addition to those listed for BL1)

1. An autoclave to decontaminate biohazardous waste is available in the same building that contains the animal facility.

2. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.

3. The animal facility is designed and constructed to facilitate cleaning and housekeeping.

4. A hand washing sink is available in the room where infected animals are housed.

5. If the animal facility has windows that open, they are fitted with fly screens.

ANIMAL BIOSAFETY LEVEL 3

Activities and practices at animal biosafety level 3, which correspond to the human biosafety level 3 and Class 3 organisms are not currently allowed by the IBC.
A. Special Practices (in addition to those listed for level 1 and 2)

1. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. Equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or packaged for transport and before removal from the facility.

2. All wastes from the animal room are autoclaved before disposal. All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leak proof covered containers.

B. Safety Equipment

1. Personal protective clothing equipment is used for all activities manipulations of infectious materials or infected animals.
   
   a. Wrap-around or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns should be appropriately contained until decontamination or disposal.

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

   c. Appropriate face/eye and respiratory protection are worn by all personnel entering animal rooms housing nonhuman primates. Personnel using respirators must comply with provisions of the University of Iowa's Respiratory Protection Program.

   d. Boots, shoe covers, or other protective footwear, and disinfectant foot baths are available and used when indicated.

2. Physical containment devices and equipment appropriate for the animal species are used for all procedures and manipulations of infectious materials or infected animals.

3. Housing infected animals in partial containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar flow cabinets), solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems can be used to reduce the risk of exposure to infectious aerosols.

C. Animal Facilities (in addition to those listed for BL1 & BL2)

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping, and is separated from areas that are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or other activities may also be provided by a double-doored clothes changing room (showers may be included), airlock, or other access facility that requires passage through two sets of doors before entering the animal room.
2. The interior surfaces of walls, floors, and ceilings are water resistant and easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.

3. A foot, elbow, or automatically operated sink for hand washing is near each animal-room exit door.

4. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and a HEPA filter.

5. Windows in the animal room are closed and sealed.

6. If floor drains are provided, they are protected with liquid traps that are always filled with water or disinfectant.

7. A non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to provide for directional flow of air into the animal room. The exhaust air is discharged directly to the outside and clear of occupied areas and air intakes. Exhaust air from the room can be discharged without filtration or other treatment. Personnel must periodically validate that proper directional airflow is maintained.

8. HEPA-filtered exhaust air from Class II or Class III biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building’s exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA filtered exhaust air from Class II or Class III biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

9. Animal room doors are self-closing and are kept closed when infected animals are present.
<table>
<thead>
<tr>
<th>Biosafety Level</th>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equipment (Primary Barriers)</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
</table>
| 1              | Not known to cause disease in healthy adults. | Standard animal care and management practices, including appropriate medical surveillance programs | As required for normal care of each species. | Standard animal facility  
• non recirculation of exhaust air  
• directional air flow recommended |
| 2              | Associated with human disease. Hazard: auto-inoculation, ingestion, mucous membrane exposure | ABL-1 practices plus:  
• limited access  
• biohazard warning signs  
• sharps precautions  
• biosafety manual  
• decontamination of all infectious wastes and of animal cages prior to washing | ABL-1 equipment plus primary barriers: containment equipment appropriate for animal species; PPE: laboratory coats, gloves, face and respiratory protection as needed. | ABL-1 facility plus:  
• autoclave available  
• handwashing sink available in the animal room |
| 3              | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences | ABL-2 practices plus:  
• controlled access  
• decontamination of clothing before laundering  
• cages decontaminated before bedding removed  
• disinfectant foot bath as needed | ABL-2 equipment plus:  
• containment equipment for housing animals and cage dumping activities  
• Class I or II biosafety cabinets available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPE: appropriate respiratory protection | ABL-2 facility plus:  
• physical separation from access corridors  
• self-closing, double door access  
• sealed penetrations  
• sealed windows  
• autoclave available in facility |
| 4              | Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission | ABL-3 practices plus:  
• entrances through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting;  
• all wastes are decontaminated before removal from the facility | ABL-3 equipment plus:  
• maximum containment equipment (i.e., Class III biosafety cabinet or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities | ABL-3 facility plus:  
• separate building or isolated zone  
• dedicated supply/exhaust, vacuum and decontamination systems  
• other requirements outlined in the complete description of ABL 4 |
RESEARCH INVOLVING RECOMBINANT DNA (RDNA)

Approval of research work involving recombinant DNA (rDNA) must be approved by the DNA Committee which is the Institutional Biosafety Committee (IBC), as defined in the NIH Guidelines for Research Involving Recombinant DNA Molecules. The Principal Investigator (PI) must complete a rDNA Registration Document and send it to the Chair of the Institutional Biosafety Committee. Copies of the NIH Guidelines and The University of Arkansas' Review Procedures (a rDNA Registration Document is included in the procedure's booklet) are available by calling either Research and Sponsored Programs or the IBC.

The PI must abide by the latest edition of the Guidelines for Research Involving Recombinant DNA Molecules (NIH). The following is a condensed statement of those guidelines.

Recombinant DNAs are defined as either molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that replicate in a living cell, or molecules that result from the replication of those so constructed. There is no apriori way of knowing the nature of possible biohazards generated by replication of such DNAs. For this reason, the NIH has established and published Guidelines for Research Involving Recombinant DNA Molecules that, when followed, allow rDNA methodology to be realized while advocating caution in view of potential hazards. Specific topics of importance in the NIH Guidelines include the Assignment of a Class, Level of Containment and the Responsibilities of the PI and the University.

ASSIGNMENT OF CLASS

Experiments involving recombinant DNA are divided into categories denoted as classes. Presently there are 5 classes (III-A, III-B, III-C, III-D, and III-E). It is the responsibility of the PI to read the Guidelines in order to determine which class is applicable to the work being conducted.

LEVEL OF CONTAINMENT

The Guidelines call for the use of good microbiological technique and for physical and biological barriers to prevent the dissemination of potentially hazardous biological agents. In all studies, the physical and biological level of containment must match or exceed the estimated potential hazard for each of the different classes of rDNA.

RESPONSIBILITIES

Because risks are present (albeit small), research on rDNA imposes special obligations on both the scientist and the University. The NIH Guidelines are designed to help a PI determine the safeguards to be used in a given situation. However, when a PI's knowledge and evaluation dictate an increase in containment, then it is the PI's responsibility to increase that containment. Specific obligations of a PI and the University are discussed below.

THE UNIVERSITY

Nearly all NIH and NSF grants are given to institutions rather than to individuals. The responsibilities of a PI are also the responsibilities of the institution under a grant. The Institutional Biosafety Committee, appointed by and advisory to the Dean of the College of Science and Engineering Technology and the Vice Chancellor and Provost, acts for the University
in this regard to ensure compliance with the guidelines. The Biological Safety Officer, as chair of the IBC, and a member of the rDNA Committee subcommittee, assists the University in meeting the institution's safety obligations in this area.

PRINCIPAL INVESTIGATOR (PI)

The PI has the primary responsibility to:

* be adequately trained and knowledgeable about good microbiological techniques;

* determine the appropriate level of biological and physical containment;

* adhere to procedures for dealing with accidental spills and overt personnel contamination. See Spills (pages ??43-46) and Exposure Evaluations and Follow-up (page ??47).

* determine the applicability of various precautionary medical practices, serological monitoring, and immunization when available;

* secure the IBC's approval for proposed research. (Approval of the IBC must be obtained prior to initiation of regulated activities.)

* report to the IBC and NIH new information bearing on the Guidelines;

* report within 30 days to the NIH and IBC all significant problems with and violations of the Guidelines, and all significant research-related accidents and illnesses; and

* comply with shipping requirements for recombinant DNA molecules. (See Appendix H of the NIH Guidelines.)

Any recombinant DNA research, teaching or other activities that involve recombinant DNA, whether sponsored by intramural or extramural funding, come under NIH Guidelines for Recombinant DNA Technology. Any member of the university, faculty, staff, or student, who intends to engage in activities covered by the NIH guidelines must complete the Recombinant DNA Registration Document shown on the following page.

Any member of the university, faculty, staff, or students who intends to engage in any and all research, teaching and other activities, that involve infectious agents, biological toxins, or recombinant DNA molecules must also complete a form:

NOTICE OF INTENT TO WORK WITH INFECTIOUS AGENTS, BIOLOGICAL TOXINS, OR RECOMBINANT DNA MOLECULES

which is available from the IBC. A copy the Recombinant DNA Registration Document and the Notice of Intent are shown on pages 30 to 35.
RECOMBINANT DNA REGISTRATION DOCUMENT

ApplicationGrant Number: __________________________ Submitted to: __________________________

Title: __________________________________________________________________________

Name of Principal Investigator and/or others responsible: __________________________

________________________________________________________________________________

Department: ______________________________________________________________________

For this proposed DNA research, briefly describe the following:

1. Description of project.
   
   a. The source(s) of DNA.
   b. Nature of the inserted DNA sequences.
   c. The host and vectors to be used.
   d. Will this rDNA be introduced into animals? If so, which species and by which route of introduction?
   e. Will a deliberate attempt be made to obtain expression of a foreign gene in the cloning vehicle? If so, what protein?

2. Assessment of classification and containment.

   Class: _____ Containment: Biosafety Level _____ Animal BL ______
   HV______ (See Appendix E of the Guidelines for certified host-vector systems)

3. Information on health surveillance (if recommended).

I certify that I have read and understand the NIH Guidelines and instructions of July 5, 1994, and agree to comply with them and understand that this project will not be initiated until facilities meet containment requirements as expressed by the IBC. I further agree to notify the IBC of any significant change in this project.

Principal Investigator __________________________ Date ______________

The rDNA Committee has reviewed this proposal and found it to be in compliance with NIH Guidelines.

IBC Chairperson __________________________ Date ______________

Comments:
NOTICE OF INTENT TO WORK WITH
INFECTIONOUS AGENTS, BIOLOGICAL TOXINS, OR
RECOMBINANT DNA MOLECULES

To: Institutional Biosafety Committee
Biology Department
Fristorgh Hall Rm 406 or Science Laboratories Building Rm 361
College of Science and Engineering Technology
University of Arkansas at Little Rock
2801 South University Avenue, Little Rock, AR 72204

PLEASE TYPE ANSWERS

FOR BIOSAFETY COMMITTEE USE

IBC File No. ___________________________ Date received: ________________

Category of Biohazard:

_____ Infectious Agent

_____ Biological Toxin

_____ Recombinant DNA

IBC Recommendation:

_____ Approved

_____ Approved with Modifications

_____ Not Approved

Approval Period: From __________________ To __________________

Date of IBC Notification to PI.: ____________________________

1. Principal Investigator: ____________________________

2. Department: ________________________________ Telephone: ____________

3. Location where project will be conducted: ________________ Bldg. ________ Rm.

4. Laboratory contact other than P.I.: ____________________ Telephone __________

5. Title of Project:
6. Brief non-technical abstract of planned work (use reverse if more space is needed):

7. Indicate applicable category of this project:


   _____ b. Renewal of previously approved project (no changes).

   _____ c. Renewal of previously approved project (with changes - specify on reverse side).

   _____ d. Teaching/Training (if category b is checked, go directly to line 30 and sign and date.

8. Research materials to be used: (indicate the binomial name and strain of the organism, name and strain of virus, type or biological toxin or describe recombinant DNA materials):

   INFECTIONOUS AGENTS OR BIOLOGICAL TOXINS (Complete as applicable)

9. Is the organism or virus that will be used attenuated or fixed by serial passage or other means:

   _____ Yes _____ No _____ N/A.

10. Is an antibiogram or Biological Agent MSDS sheet available and on file for the bacterial agent(s)

    that will be used: _____ Yes _____ No _____ N/A.
11. Indicate biosafety level required for this organism or virus per the UALR - Biological Safety Manual Vol. 1, Practices and Protocols for Handling and disposal of Infectious Materials*. BL __________

12. Are maximum working quantities of cultures ______ less than 10L, ______ greater than 10L.

RECOMBINANT DNA MOLECULES (Complete as applicable)

13. Indicate: 
   Host cells: ____________________________________________
   Vector: _____________________________________________
   Recombinant: ____________________________________________

Note: for large projects with many recombinants, make as complete a list as possible and attach.


15. If project is exempt from NIH Guidelines, give basis by indicating applicable sub-section of IIID of Guidelines (i.e., D1, D2, etc. __________); see UALR - Biological Safety Manual Vol. 2.

CONTAINMENT AND SAFETY EQUIPMENT

16. Will a biological safety cabinet (BSC) be used: ______ Yes ______ No.

17. If "yes" what is the name of the manufacture: __________________________________________

18. Class of BSC: ________ I, ________ IIA, ________ IIB1, ________ IIB2, ________ IIB3.

19. Date of last BSC certification: _______________ Name of Certifier _______________
20. Other safety equipment to be used: _______ safety pipettes, _______ centrifuge safety cups, _______ chemical fume hood, _______ other, specify:

21. Will a laminar flow "clean bench" be used for the planned activity: _______ Yes _______ No.

Does the user understand the safety limitations of this "hood" type: _______ Yes _______ No.

LABORATORY WASTES

22. Method of decontamination of biological or infectious wastes:

_______ autoclave    Location: ____________________________ Bldg. _______ Rm.

_______ incinerator

_______ chemical disinfectant (specify): ________________________________

23. Method of disposal of chemical wastes:

PERSONNEL PRACTICES

24. Has a reference serum sample been collected and stored for all personnel working on this project:

_______ Yes _______ No _______ N/A.

25. Is vaccination recommended for personnel working with the organism, virus, toxin, or other material that will be used in the planned project: _______ Yes _______ No _______ N/A.
26. If "yes", are personnel vaccinated: ________ Yes ________ No.

27. Has an emergency plan been developed in the event of a spill or other accident involving infectious or toxic biological materials: ________ Yes ________ No ________ N/A.

28. What is your previous work experience with the agent/materials specified in item #8:

29. Please indicate any additional information or comments pertinent to the Biosafety Committee's review of this protocol:

30. I have read and am familiar with the standard and special microbiological practices, containment equipment, and laboratory facilities recommended for the required biosafety level and applicable to this project. I agree that all faculty, staff, and students working on this project will follow these recommendations as a condition of the Institutional Biosafety Committee approval of this project.

Date ________________ Principal Investigator ________________________________

* Publications available from the Office of the: Institutional Biosafety Committee Science Laboratories Building, Rm 381 University of Arkansas at Little Rock 2801 South University Avenue Little Rock, AR 72204 1-501-569-3510 or 1-501-569-3270
GENERAL BIOSAFETY PRACTICES AND PROCEDURES
ADMINISTRATIVE CONTROLS

Medical Surveillance and Examinations

Departments must provide all employees who work with biohazardous agents an opportunity to receive medical attention, including any follow-up examinations that the examining physician determines to be needed, when examinations are necessary to:

* provide specific immunizations necessary to protect employee health
* evaluate the effects of possible exposure to a hazardous agent (e.g., symptoms of clinical infection, allergic symptoms or complaints related to pharmacological effects of end products, by-products, medium components or inactivated biological agents)
* detect changes in employee health that may indicate the need for a change in job procedures or job assignment
* detect patterns of disease in the work force or evaluate the effectiveness of control measures

Signage/Labeling

All areas and laboratories that contain biohazardous agents must be posted with an appropriate biohazard sign (see Figures 5-12).

BIOHAZARD FIGURE 5

BIOHAZARD RECOMBINANT DNA
BIOLOGICAL SAFETY LEVEL 1

LABORATORY DIRECTOR

SCLB CONTACT 569-____ OR 569-3510

BIOHAZARD FIGURE 6

BIOHAZARD INFECTION AGENTS
BIOLOGICAL SAFETY LEVEL 2

LABORATORY DIRECTOR

INFECTION AGENT

SCLB CONTACT 569-____ OR 569-3510

AUTHORIZED PERSONNEL ONLY
NOTICE
AUTHORIZED PERSONNEL ONLY

NOTICE
NO EATING, DRINKING, SMOKING, HANDLING CONTACT LENSES OR APPLYING COSMETICS IN THE LABORATORY

NOTICE
ALWAYS WEAR GLOVES WHEN HANDLING LIVE MATERIAL

NOTICE
NO MOUTH PIPETING

ALWAYS WASH HANDS AFTER REMOVING GLOVES AND BEFORE LEAVING THE LABORATORY
Laboratory supervisors and principal investigators must ensure that labels on incoming containers of biohazardous agents are not removed or defaced. Laboratory containers, including bottles, flasks, sample vials, etc., must be marked, labeled or coded in all cases. The label should be dated and should identify the owner of the agent.

**PHYSICAL/ENGINEERING CONTROLS**

Users of biohazardous agents must ensure biological safety cabinets and other protective equipment are adjusted and functioning properly prior to initiating an activity requiring their use. The IBC will arrange an annual certification of Laminar Flow Clean Air Benches and Biological Safety Cabinets, and should be contacted regarding any problems or for repair needs related to them.

**Biological Safety Cabinets**

Biological safety cabinets (BSCs) are among the most effective and commonly used primary containment devices in laboratories working with biohazardous agents. There are three types, each having different performance characteristics.

**Types of BSCs by Class Design**

Class I—Is an open-fronted, ventilated cabinet that must have a minimum inward face velocity of at least 75 feet per minute. The exhaust air from the cabinet is filtered by a high efficiency particulate air (HEPA) filter. This system provides protection to personnel and to the laboratory area. Product protection is not provided because the inlet air is not filtered. Note: Class 1 BSCs are no longer
being manufactured on a regular basis; many have been replaced by Class II BSCs.

Class II is a vertical laminar flow BSC that adds HEPA filtered recirculating airflow within the work space to the basic design of the Class I BSC. This enables it to protect the biologicals within as well as the lab and the worker. It is designed for use with low or moderate risk agents.

Class III—commonly referred to as a glove box, is a self-contained gas-tight enclosure that provides a complete barrier between the worker and the materials in the cabinet. It is designed for use with high risk biologicals. When in use it is maintained under negative air pressure. Supply and exhaust air are HEPA filtered. Exhaust air is discharged outside the facility by fans separate from the facility's ventilation system.

Diagrams and specifications for various biosafety cabinets, laminar flow hoods and hood accessories are found on pages 42-43.

Proper Use and Operation of BSC According to Cabinet Class
Class I and II biological safety cabinets

When used in conjunction with good microbiological techniques, these cabinets provide an effective partial containment system for safe manipulation of moderate and high-risk microorganisms (i.e., Biosafety Level 2 and 3 agents). The use of these cabinets alone is not appropriate for containment of highest risk biohazardous agents because aerosols may accidentally escape through the open front. As with any other piece of laboratory equipment, personnel must be trained in the proper use of the cabinet. Strict adherence to recommended practices is as important as the mechanical performance of the equipment itself.

The Class I BSC is useful for containment of mixers, blenders, and other equipment. This cabinet is not appropriate for handling research materials that are vulnerable to airborne contamination since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet.

Class II BSCs are classified into two types (A and B) based on construction, air flow velocities and patterns, and exhaust systems. Basically, Type A cabinets are suitable for work with microbiological research in the absence of volatile or toxic chemicals and radio-nuclides, since air is recirculated within the work area. Type A cabinets may be exhausted through HEPA filters into the laboratory, or to the outside through a "thimble" connection to the exhaust duct work.

Type B cabinets are hard-ducted to the exhaust system, and contain negative pressure plena. These features, plus an increased face velocity of 100 fpm, allow work to be done with toxic chemicals or radio-nuclides. Type B cabinets are further sub-typed into types B1, B2, and B3. Type B1 requires 70% of the air to be exhausted to the outside, and 30% recirculated. Type B2 has 100% of the exhaust vented to the outside. Type B3 is required to have 30% of the air exhausted through the outside vent. Call the IBC for information on a comparison of the design features and applications.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of BSCs. Of particular note are those activities that may disrupt inward directional airflow through the work opening and cause escape of aerosolized particles from within the cabinet (e.g., repeated insertion and withdrawal of worker's arms into and from the work chamber, opening and closing laboratory doors, improper placement of materials, improper operation of equipment within the work
chamber, or brisk walking past the cabinet while it is in use). These cabinets should be located away from traffic patterns and doors. Fans, heating and air conditioning registers, and other air handling devices can also disrupt airflow patterns if located adjacent to a BSC. Strict adherence to recommended practices for use of BSCs and proper placement in the laboratory are as important in attaining the maximum containment capability of equipment as is the mechanical performance.

IMPORTANT: Cabinets must be tested and certified at the time of installation, at least annually thereafter, and any time they are moved. Certification is site specific and is required before the cabinet is put into operation in the laboratory.

Class III cabinets

The Class III cabinet is a totally enclosed, ventilated cabinet of gas-tight construction and offers the highest degree of personnel, environmental, and product protection. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4.

All operations in the cabinet are performed through attached rubber gloves. The Class III cabinet is operated under negative pressure. Supply air is HEPA-filtered, and the cabinet exhaust air is filtered by two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside the facility.

All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the system. Several Class III cabinets are typically set up as an interconnected system. Double-doored autoclaves and chemical dunk tanks are also attached to the cabinet system to allow supplies and equipment to be safely introduced and removed.

Personnel protection equivalent to that provided by Class III cabinets can also be obtained with the use of a one-piece ventilated suit in a "suit area" and using Class I or Class II cabinets. The personnel suit is maintained under positive pressure with a life support system to prevent leakage into the suit. In this containment system, the worker is isolated from the work materials.

The personnel suit area must be essentially equivalent to a large Class III cabinet. The area is entered through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surfaces of the suit as the worker leaves the area. The exhaust air from the suit area is filtered by two HEPA filter units installed in series. The entire area must be under negative pressure.

Horizontal laminar flow hoods

These "clean benches" are present in a number of clinical, pharmaceutical, and laboratory facilities strictly for product protection. They provide a high quality environment within the work chamber for manipulation of non-hazardous materials. Caution: since the operator sits in the immediate downstream exhaust from the "clean bench," this equipment must never be used for handling toxic, biohazardous, radioactive, or sensitizing materials.

Working Safely in a Biological Safety Cabinet

Personal protection:

* Wear protective gloves when handling biohazardous agents; preferably "double-gloved." Change gloves if they become contaminated.
* Do not work with biohazardous agents if you have cuts, sores, or abrasions on your hands.
* Wash hands with a good germicidal soap and water before and after handling biologicals.
* Wear a lab coat or disposable gown while working with biohazardous agents.
* Do not wear contaminated clothing outside the lab.

Work practices

* Move arms and hands slowly in the cabinet, wait several seconds for air currents to settle, then begin work.
* Don’t place anything on the grillwork inside the cabinet. This greatly interferes with the airflow.
* Decontaminate work surfaces with an appropriate disinfectant before and after work, and after a spill.
* Perform all procedures carefully to avoid the generation of aerosols, splashes, and spills.
* Keep an appropriate disinfectant agent within the cabinet.
* Keep the number of items in a BSC to an absolute minimum.

General Practices

* Take special care and precautions when using syringes, needles and other sharps. Needles should never be re-sheathed, bent, broken, removed from a syringe, or otherwise manipulated by hand.
* Label all materials.
* Use mechanical pipetting devices. Do not pipette using mouth suction.
* Do not eat, drink, smoke, or apply cosmetics in the laboratory.
* Dispose of all lab materials properly.
* Non-disposable items should be cleaned and decontaminated before removing them from the BSC, then autoclaved.

Cabinets restrictions

* Cabinet selection should be determined by assessing the risk to personnel, product, and the environment.
* HEPA filters do not provide protection against gases and vapors. They do provide protection against particulate agents and materials.
* BSC should be certified after installation, but before being used, whenever moved, and at least annually thereafter.
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### Class

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### Type

| Type    | - | - | A | B1 | B2 | B3 | - |

### Inflow (ft/min)

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### Use:

- **Biohazards:** - + + + + + + +
- **Toxic, Volatiles:** - + - +/- + + + +
- **Sterile work:** + - + + + + + +

### Protection:

- **User:** - + + + + + + +
- **Product:** + - + + + + + +

---

**Figure:** Airflow characteristics of class I, II, III BSC and laminar flow clean benches. Adapted from Biosafety in the Laboratory: Prudent Practices for the Handling and disposal of Infectious Materials, National Research Council; National Sanitation Foundation Standard 49, Centers for Disease Control; HHS Publication (CDC) 93-8395, Biosafety In Microbiological and Biomedical Laboratories, Centers for Disease Control and Prevention and National Institutes of Health; and Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, CDC and NIH.
A "Thimble Unit" for ducting a Class II, type A BSC. Note: There is a 1" gap between the thimble unit (D) and the exhaust filter housing (C), through which room air is exhausted.

A. ballancing damper B. flexible connect to exhaust system
C. cabinet exhaust HEPA filter D. thimble unit
E. BSC

A typical layout for working "clean to dirty" within a class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipetts can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). Arrangement is reversed for left-handed operation.

One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. A glass splurger in flask B minimizes spatter.

A bag-in-out filter enclosure allows for the removal of the contaminated filter without worker exposure.

A. filters B. bags C. safety straps D. cinching straps E. shock cord located in the mouth of the PVC bag restricts the bag around the second rib of the housing lip.
THE TEN COMMANDMENTS OF BSC OPERATION

1. Ready Work Area:
   - turn off UV lamp, turn on fluorescent
   - check air grilles for obstructions, switch on blower
   - allow air to purge work space for five minutes

2. Pre-disinfect:
   - spray or swab all interior surfaces with appropriate disinfectant
   - allow to air dry

3. Assemble Work Material:
   - introduce only materials required to perform procedure
   - place materials so that clean and contaminated do not meet
   - place contaminated material container at right rear
   - ensure view screen is properly located and secure

4. Pre-purge Cabinet:
   - with blower on, allow air purge period with no activity in BSC

5. Prepare Self:
   - don protective clothing, gloves, mask, etc, as appropriate

6. Do the Procedures:
   - introduce hands into work, move slowly and deliberately
   - work from clean area to contaminated area
   - complete work by securing critical material
   - DO NOT remove hands from BSC until work is complete

7. Post-purge Cabinet:
   - with blower on, allow air purge period with no activity in BSC

8. Finish Personally:
   - remove protective clothing and dispose as appropriate
   - wash hands with disinfectant soap

9. Post-disinfect:
   - don gloves, move materials to incubator or autoclave as appropriate
   - spray or swab all interior surfaces with appropriate disinfectant

10. Shutdown Cabinet:
    - turn off blower and fluorescent lamp
    - turn on UV lamp
PERSONAL PROTECTIVE EQUIPMENT

The purpose of personal protective equipment (PPE) is to prevent or minimize the entry of material into the worker's body. This includes entry via apparent or inapparent skin lesions, or through the membranes of the eye, nose, or mouth. Examples of PPE are gloves, gowns, fluid-proof aprons, laboratory coats, head and foot coverings, face shields, eye protection, and masks.

Selection Precautions

* Equipment should be selected based on the specific work, exposure conditions that will be encountered, and the anticipated level of risk. Evaluate the task, the exposure associated with its performance, and select appropriate PPE that will be an effective barrier under anticipated conditions of exposure.

* PPE must be made available in the proper size.

* Laundering and repair or replacement must be available for those items requiring it.

* Proper disposal containers for contaminated equipment must be in place.

* The limitations of equipment must be understood.

Protective Clothing

Lab coats, gowns, or fluid-proof aprons, will be worn in exposure settings. Long sleeved garments are preferred to minimize contamination of skin or street clothes and to reduce shedding of microorganisms from the arms. If clothing must be autoclaved, it must be capable of withstanding high temperatures. Additional criteria for selecting clothing are: comfort, appearance, closures, anti-static properties and durability. Consideration must be given to having disposable clothing available for visitors, maintenance and service workers.

Hand Protection

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Contact HPO for assistance in selecting the appropriate gloves for protection from contact with toxic or corrosive chemicals.

The lower sleeve and the cuff of the laboratory garment should be overlapped by the glove. A long sleeved glove or disposable arm shield may be worn for further protection of the garment or the work. When gloves are contaminated while working in a biosafety cabinet they must be discarded into a waste container within the cabinet.

Disposable gloves are not to be washed or disinfected for re-use. Disinfecting agents may cause deterioration of the glove material or compromise the protective qualities. They must be changed as soon as possible when visibly soiled. Replace gloves if a tear, puncture, or similar defect is noticed. Place used gloves in a plastic bag marked with a biohazard symbol and bearing the word BIOHAZARD. This bag will be incinerated, along with other biohazardous materials.
Some people develop an allergy to the latex and/or powder in gloves they are using. A variety of options are available that can alleviate allergic reactions. Both powdered and powder-free hypo-allergenic latex gloves are available from suppliers. If you need help, please call IBC.

Utility gloves (rubber gloves), often used for housekeeping chores, are of more substantial construction than surgical or examination gloves and are permitted to be decontaminated and reused. They must be discarded if they are cracked, peeling, discolored, torn, punctured or exhibit other signs of deterioration.

Eye and Face Protection

Masks and eye protection or chin-length face shields must be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials (OPIM, as defined in the Bloodborne Pathogens Standard) may be generated with a potential for mucous membrane contamination. If eye wear is chosen over the use of a face shield, it must be worn in conjunction with a face mask, since the goal is to provide protection of the eyes, nose, and mouth. Where the use of respirators is necessary to limit exposure to biohazardous agents, the department must provide, at no cost to the employee, the proper respiratory protective equipment. Respirators must be selected and used in accordance with the requirements of the University of Iowa Respiratory Protection Program. Contact HPO for additional information.

WORK PRACTICE CONTROLS

Work practice controls are meant to reduce the likelihood of exposure by altering the manner in which a task is performed. In other words, these controls are dependent upon employee behavior. It is necessary to employ good work practices in tandem with effective engineering controls and personal protective equipment in order to achieve the goals of this program.

Standard Operating Procedure (SOP)

This manual serves as a generic standard operating procedure (SOP) relevant to safety and health concerns with work involving biohazardous agents. Area-specific operations should be included by the department, PI, or supervisor. The SOP is intended to provide employees with the necessary guidelines for conducting work in a safe and consistent manner.

The types and quantity (as in large scale quantities) of organisms and the operations performed all define at which biosafety level the lab must function. Each level has certain recommended criteria in the areas of standard microbiological practices, special practices, safety equipment, and laboratory facilities. See page 13-22 for biosafety level criteria and pages 22-27 for the vertebrate animal biosafety level criteria. Appendix A lists organisms in alphabetical order and identifies each one as to its biosafety level and whether it is bacterial, fungal, parasitic, viral, etc. Appendix B lists organisms by biosafety level required for different general groups, i.e., bacteria, fungi, parasites, etc.

Standard operating procedures should include the following provisions:

* use of containment devices such as, biological safety cabinets or glove boxes
* procedures for safe removal of contaminated waste
* decontamination procedures
Universal Blood and Body Fluid Precautions to Prevent Occupational HIV and HBV Transmission:

All occupational exposure to blood or other potentially infectious materials is regulated under the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR 1910.1030. Occupational exposure means reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials (OPIM) that may result from the performance of an employee’s duties. The standard mandates the use of universal precautions as an approach to exposure control. According to this concept, all human blood and certain human body fluids are treated as infectious. The universal precautions concept is only one part of the overall plan to reduce exposures. Other methods of control include engineering controls that isolate or remove the bloodborne pathogens hazard from the work place, work practice controls that alter the manner in which a task is performed, use of personal protective equipment, receipt of the hepatitis B vaccine and training in exposure control.

Potentially infectious materials (OPIM) as defined in the standard are:

* human blood, blood components, and blood products
* semen, vaginal secretions
* cerebrospinal fluid, synovial fluid, pleural fluid
* pericardial fluid, peritoneal fluid, amniotic fluid
* saliva in dental procedures
* blood-contaminated body fluids, unknown body fluids
* unfixed human tissues or organs (other than intact skin)
* HIV- or HBV-containing cell, organ, tissue cultures, culture mediums, or other solutions
* blood, organs, or other tissue from animals infected with HIV or HBV.

The full text of the OSHA Standard can be obtained from the IBC.

Decontamination and Disposal

The goal of decontamination is to protect personnel and the environment from exposure to biological agents, and to prevent contamination of experimental materials. The current specific rules and protocols for UALR Biohazardous Waste Disposal are presents on page

General Procedures

1. Biohazardous materials and contaminated equipment originating from the lab should be sterilized before being washed and stored or discarded. Autoclaving is the preferred method. Each individual working with biohazardous material is responsible for its sterilization.

2. Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day. To minimize hazard to firemen or disaster crews, all biohazardous
materials should be placed in an appropriately marked refrigerator or incubator, sterilized, or otherwise confined at the close of each work day.

3. Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or the simultaneous opening of both doors on a double door autoclave.

4. Dry hypochlorites or any other strong oxidizing material must not be autoclaved with organic materials such as paper, cloth, or oil oxidizer + organic material + heat = explosion potential.

5. Laboratory rooms containing biohazardous materials should designate, where appropriate, separate areas or containers labeled:

   BIOHAZARDOUS -- TO BE AUTOCLAVED and NON-INFECTIONOUS -- TO BE CLEANED

6. All floors, laboratory benches, and other surfaces in buildings where biohazardous materials are handled should be disinfected as often as deemed necessary by the supervisor. After completion of operations involving plating, pipetting, centrifuging, and similar procedures with biohazardous materials, the surroundings should be disinfected.

7. Floor drains should be flooded with water or disinfectant at least once each week in order to fill traps and prevent backflow of sewer gases.

8. Floors should be swept with push brooms only. The use of floor sweeping compound is recommended because of its effectiveness in limiting the generation of airborne organisms. Vacuum cleaners equipped with HEPA filtration may be used. In all infectious units, water used to mop floors should contain a disinfectant.

9. Stock solutions of suitable disinfectants should be maintained in each laboratory for disinfection purposes.

   Disinfection

Disinfection is used to eliminate the infectious or pathogenic agent or agents present. A disinfectant is selected according to the specific infectious agent known or suspected to be present, as each chemical compound has a selective germicidal activity.

Liquid disinfectants are available under a wide variety of trade names. The more active a compound is, the more likely it is to have undesirable characteristics such as corrosivity. No liquid disinfectant is equally useful or effective under all conditions and for all viable agents. The most practical use of liquid disinfectants is for surface decontamination. At sufficient concentrations they can be used as decontaminants for liquid wastes prior to disposal in the sanitary sewer.

Mercurials -- toxic, therefore not recommended.

Quaternary Ammonium Compounds -- acceptable to control vegetative bacteria and non-lipid-containing viruses. They are not active against bacterial spores at the usual concentrations (1:750) and may be neutralized by anionic detergents (soaps).

Phenolic Compounds -- recommended for killing vegetative bacteria including Mycobacterium
tuberculosis, fungi and lipid-containing viruses (0.5-2.0%). They are less effective against spores and non-lipid-containing viruses. They have an unpleasant odor (e.g., Amphyll, Vesphene II).

Chlorine Compounds -- recommended provided the available chlorine needed is considered. Low concentrations (50-500 ppm) are active against vegetative bacteria and most viruses. For bacterial spores, concentrations of 2500 ppm are needed. They are corrosive to metal surfaces and must be made up fresh. 10 ml laundry bleach per liter of water yields approximately 525 ppm. See Appendix ( ) for current practice established under this revision.

Iodophors -- recommended for general use (75-150 ppm) for killing vegetative bacteria and viruses. They show poor activity against bacterial spores. The advantages are that they:

* possess a wide spectrum of anti-microbial and antiviral activity.

* have a built-in indicator. If the solution is brown or yellow, it is active.

* are relatively harmless to man. (Wescodyne diluted 1:10 is a popular disinfectant hand-wash).

* can be readily inactivated and iodophor stains can be removed with solutions of Na₂S₂O₃ (sodium thiosulfate).

Alcohols -- good general use disinfectant in concentrations of 70 to 80% (ethyl or isopropyl). They exhibit no activity against bacterial spores.

Formaldehyde Solutions -- exhibit good activity against vegetative bacteria, spores, and viruses at concentrations of 5-8% formalin. These have an irritating odor and are carcinogenic.

Activated Glutaraldehyde -- use must be limited because of toxic properties and potential damage to eyes (e.g., Cidex, Sporicidin 3M Glutarex). Two percent solutions exhibit good activity against vegetative bacteria, spores, and viruses.

Formaldehyde-Alcohol -- Solutions of 8% formalin in 70% alcohol are good for disinfection purposes because of the effectiveness against vegetative bacteria, spores, and viruses.

A summary of disinfectant properties is presented in Table 3 on page 53.
# Properties of Common Disinfectants

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Practical requirements for use</th>
<th>Agent inactivated</th>
<th>Major characteristics</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of active ingredient</td>
<td>For lipophilic virus only</td>
<td>Broad spectrum</td>
<td>Temperature, °C</td>
</tr>
<tr>
<td>Liquid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quaternary ammonium compounds</td>
<td>2%</td>
<td>10</td>
<td>N.E.</td>
<td></td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>2%</td>
<td>10</td>
<td>N.E.</td>
<td></td>
</tr>
<tr>
<td>Chlorine compounds</td>
<td>3%</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Iodophor</td>
<td>2%</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>85%</td>
<td>10</td>
<td>N.E.</td>
<td></td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>85%</td>
<td>10</td>
<td>N.E.</td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>8%</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>2%</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Gas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.E., not effective; N.A., not applicable.

1 Variable results dependent on virus.
2 Neither flammable nor explosive in 90% CO₂ or fluorinated hydrocarbon mixture.
3 In vapor form: at concentrations of 7 to 73% by volume in air. In solid form: exposure to open flame.
4 Protected from light and air.
5 Microscope, camera, or other optical equipment.
6 By skin or mouth or both—refer to manufacturer's literature and/or Merck Index.

Sterilization

General criteria for sterilization of typical materials are presented below. It is advisable to review the type of materials being handled and to establish standard conditions for sterilization. Treatment necessary to achieve sterility will vary in relation to the volume of material treated, its contamination level, moisture content, and other factors. These are general criteria and should be used for biohazardous/infectious agents coming from BL2 areas. These materials are all processed in the medical waste incinerator, but must be handled several times during transport. Proper containment and treatment at the source reduce the potential for an accidental exposure.

Gas Sterilants

* Ethylene oxide gas -- Sixteen hours exposure to a concentration of 750 mg/liter (= OR -5%) at 30 to 60% relative humidity and ambient temperature (>70°F).

* Paraformaldehyde -- sixteen hours exposure to a concentration of 1.0 mg/liter at 40 to 60% relative humidity and at ambient temperatures (>70°F).

Steam Autoclave

* Laundry -- 121°C (250°F) for 30 minutes with 15 minutes prevacuum of 27 in. Hg.

* Glassware and trash -- 1 hour at 121°C (250°F) with 15 minutes prevacuum of 27 in. Hg.

* Liquids -- 1 hour at 121°C (250°F) for each gallon.

* Animals and bedding -- 8 hours at 121°C (250°F) with 15 minutes prevacuum of 27 in. Hg.

General Autoclaving Guidelines

1. Every autoclave and sterilizer should be inspected and serviced on a regular basis. This will help ensure the equipment is functioning properly.

2. Each unit should have a standard operating procedure written in sufficient detail to ensure that operators will use the equipment properly.

3. Each unit must be tested regularly with a commercial preparation of Bacillus stearothermophilus (a biological indicator).

4. Keep detailed records on biological tests, recording thermometers, and service work performed on the unit.

5. Do not autoclave flammable liquids, toxic chemicals, carcinogens, cytotoxic drugs, or radioactive materials. The careless autoclaving of hazardous materials may generate toxic vapors or explosive environments.

6. Do not autoclave bulk liquids without following the manufacturer's written instructions. See #6 in the next section on autoclave containers.

7. High density wastes or materials that insulate the agents from heat and steam penetration
are not suitable for steam sterilization. Items that are covered with dirt or film require additional retention times. The importance of properly cleaning items to be sterilized cannot be over emphasized.

8. Place all autoclaved infectious waste into red bags for disposal.

Autoclave Bags / Containers Guidelines

The proper packaging and containment of infectious materials are crucial to achieve effective sterilization. The most frequent reason for sterilization failure is the lack of contact between the steam and the microorganisms.

1. To facilitate steam penetration, bottle caps and stoppers should be loosened after placement into the chamber. If left sealed, they may not be properly sterilized.

2. Most bags that are marketed as autoclavable are not suitable if closed because steam will not penetrate them. Steam resistant bags must be left open or have holes punched into the top to allow the steam to penetrate. Do not transfer open bags to the autoclave.

3. Never close autoclave bags that have a printed warning stating they are to remain open during sterilization. If air remains trapped in the bag, the material may not be properly sterilized.

4. Autoclave bags that allow steam penetration tend to melt or crumble during the sterilization process. Autoclavable bags may be placed inside paper bags, or open steam resistant polypropylene bags.

5. If the autoclavable bags leak, they should be placed into a shallow stainless steel pan. Plastic pans are less effective because they do not transfer heat as fast or efficiently.

6. Sterilization of bulk liquids requires special care to prevent the containers from exploding.

* Each gallon of infectious liquid must be autoclaved for one hour at 250°F at 15 psi. Closures and lids must be loosened prior to sterilizing.

* Bulk solutions must be sterilized separately from all other items in a load dedicated to liquids only. Solutions are subjected to a cycle designed specifically for liquids.

* Sterilized liquids must be allowed to cool before unloading. Removing hot bottles may cause them to explode.

Loading the Autoclave

Follow manufacturer’s instructions before attempting to load the chamber.

1. Transfer infectious waste to the autoclave in a sealed secondary container. Again, do not transfer open bags or containers to the autoclave because this could cause the spread of infectious agents.

2. Wear appropriate personal protective equipment (i.e., laboratory coat, rubber apron,
gloves, eye protection, if necessary).

3. Avoid rough handling of waste containers to minimize formation of infectious aerosols.

Spills

Laboratories must develop procedures for dealing with spills and should have appropriate equipment and materials. A basic spill kit could include some concentrated disinfectant (chlorine bleach or Wescodyne), a package of paper towels, sponges, household “rubber” gloves, forceps for broken glass and an autoclavable container.

The potential health risk of the spilled agent must be considered. With Mycobacterium tuberculosis, for example, the risk of exposure from the spill of a small quantity might be many times that of a much larger spill of E. coli. A minimally biohazardous material (BL1 or Class 1 agent) spilled without generating significant aerosols may be cleaned up with a paper towel soaked in an effective decontaminating agent. A spill of a large volume with generation of aerosols will require personnel to wear protective clothing and respiratory protection.

Spills inside a Biological Safety Cabinet

Preparation Cleanup materials should be kept in the cabinet so they are available when a spill occurs.

Cleanup

* Do not remove anything from the cabinet, including your hands, to prevent dispersion outside the cabinet.

* Continue to operate the cabinet to clear the air of contaminants. Run it for at least 10 minutes following cleanup before using again.

* Use a clean cloth and appropriate disinfectant solution to: clean up the spill, wash interior surfaces of the cabinet. in moderate to high-risk spills, flood catch basins.

* Prevent the generation and escape of aerosols and contaminants from the cabinet during decontamination.

* Allow a 20-minute disinfectant contact period.

* Put all cleanup materials in a biohazard bag and autoclave before removing from the area.

BL1/Class 1 Agent Spills

* Wear disposable gloves.

* Soak paper towels in disinfectant and place over the spill area.

* Clean spill area with fresh towels soaked in disinfectant.
* Put all materials used in the cleanup into a biohazard bag and autoclave before disposing.

* Thoroughly wash hands with soap and water.

**BL2/Class 2 Agent Spills-Outside a BSC**

**Preparation**

* Evacuate the area immediately.

* If biological safety cabinet or fume hood is in the room, leave it in operation and immediately exit the room. Close and lock the door.

* Be certain "Biohazard" and "Do Not Enter" signs are on the door.

* Notify supervisor; if a large quantity is spilled, also notify HPO's Biological Safety Section.

**Clean-up**

* Thoroughly wash face and hands. Remove all contaminated clothing, and decontaminate (autoclave, if necessary).

* Allow at least 30 minutes for droplets to settle and aerosols to be reduced before reentering.

* Don protective equipment (long sleeved lab coat, disposable gloves, safety goggles and face shield, and disposable shoe covers, if needed).

* If the spill is large (>10 ml), apply sorbent booms or dike around the spill area to avoid spreading.

* Decontaminate with an appropriate disinfectant (see page 40). Pour the disinfectant slowly around the spill, not on the spill, to avoid aerosolizing the material.

* Cover spill area with paper towels or sorbent pads soaked in disinfectant.

* Allow a 20 minute disinfectant contact period.

* Wipe down all surfaces that may have been splashed.

* Clean up the liquid working from the outside of the spill area inward to avoid spreading the spill.

* Dispose of all cleanup items in the proper container and autoclave.

* Using an autoclavable dust pan and squeegee, transfer all contaminated glassware or sharp material into a sharp's bucket.

* For spills >10 ml, IBC's Biological Safety Section will help determine if area decontamination is necessary.
BL3/Class 3 Agent Spill Containment-Outside a BSC

Preparation If you drop or otherwise spill a container of biohazardous material, or are in the same room when this occurs:

* Hold your breath. Evacuate the room. Close the door behind you. Exit only into the anteroom or airlock space that exists between the BL3 room and the common corridor. Do not exit directly into any non restricted corridor or passage in general community use.

* Remove and immediately containerize contaminated protective garments at the doorway after exiting.

* Warn others of the spill, and isolate area.

* Notify the supervisor and HPO Biological Safety Section immediately.

* Wash hands and face, or shower. Use germicidal soap.

Cleanup Decontamination and cleanup will be directed by the supervisor:

* Wait at least 30 minutes before reentering the area to permit reduction of airborne particles by ventilation changes.

* Cleanup personnel must wear long-sleeved coats, caps, rubber boots, medium-to heavy-weight rubber gloves, and a NIOSH/MSHA approved full-face or half-face respirator equipped with a charcoal and HEPA filter. Note: A respirator program with training and fit-testing must be in place.

* Dike drains if needed. Slowly pour disinfectant around the spill's outer edges so it flows into the spill area. (To minimize aerosol formation, do not spray.)

* Cover spill area with paper towels or sorbent pads.

* Allow a 20-minute disinfectant contact period.

* Search for smaller areas that may have been splashed. Wipe these areas with paper towels or sorbent pads soaked in disinfectant. Wait several minutes and repeat.

* Once the 20-minute contact time has expired, clean up the liquid: work from outside the spill area inward to avoid spreading the spill work gently to minimize the production of aerosols

* Use an autoclavable (or expendable) dustpan and squeegee to transfer all cleanup materials to a deep autoclave pan. Put the dustpan and squeegee in the autoclave pan. Cover the pan tightly with foil or other means, and transfer it to an autoclave. Leave the rubber gloves worn to that point in the autoclave and put on a fresh pair.

* Wash the spill and adjacent area with disinfectant solution.

* Circumstances may warrant sealing the area and decontaminating it with formaldehyde gas. Equipment may also need gas treatment. Consult the supervisor or HPO’s Biological Safety Section for direction.
* Before leaving the area, the cleanup team should remove and decontaminate all clothing. Remove and bag the respirator last. Shower using germicidal soap.

Disposal

Biohazardous waste disposal must be handled in accordance with procedures established by the University. This waste must be segregated from general waste at the point of origin. Potentially infectious material or biohazard waste must be discarded directly into containers or plastic bags that are clearly identifiable and distinguishable from general waste. Containers must be marked with the universal biohazard symbol. Plastic bags must be distinctly colored red or orange, and marked with the universal biohazard symbol.

All infectious/biohazardous wastes (excluding liquids, blood, and blood products) are destined for incineration and must be placed in sealable, labeled, or color-coded, leak proof containers or bags. Be sure to label boxes with investigator's name and room or lab number. If the bag or container is contaminated on the outside or leaks, a second leak proof bag or container that is also labeled and sealable must be placed over the first and sealed to prevent leakage during handling, storage, and transporting.

Place all needles and sharps in properly labeled sharps disposal containers. These must be easily accessible to personnel, replaced before overfilling occurs, puncture resistant, leak proof, and sealable to assure containment. Use tape to secure the lids on the sharps' containers. Label with PI's name and room number.

Liquid wastes (e.g., blood, blood products) may be disinfected with a solution of 5.25% sodium hypochlorite (household bleach) diluted between 1:10 and 1:100, autoclaved. Once disinfected, these can be disposed of in the sanitary sewer system.

Custodial service will collect properly packaged waste and transport it to areas designated as waste collection points were the commercial waste handler used by UALR will transport the containers to and appropriate sanitary land fill. Waste disposal procedures are also outlined in the Waste Disposal Guidelines and Procedures Manual available from IBC.

The current IBC rules and protocols for biohazardous waste disposal at UALR are presented on page 60.
Institutional Biosafety Committee Rules
Protocols for Biohazardous Waste Disposal

1. Biosafety Level 1 - Recombinant DNA Wastes:

All laboratories designated Biosafety Level 1 - Recombinant DNA, will have waste containers appropriately labeled for the disposal of any materials that have been used in recombinant DNA work (gloves, paper towels, pipets, pipet tips, etc). Only autoclavable bag liners will be used in these containers. Material for BL1 disposal also includes culture plates of E. coli derived from the K12 strain or other species and strains authorized by the NIH recombinant DNA guidelines (see biosafety manual, vol. 2 for more information). All other bacterial cultures must be disposed of at Biosafety Level 2 - Infectious Agents.

2. Biosafety Level 2 - Infectious Agents:

All laboratories designated Biosafety Level 2 - Infectious Agents, will have at least one waste container, with cover, appropriately labeled for disposal of any materials that have the potential to cause infection (see biosafety manual, vol. 1 for specific information on USDA and NIH guidelines). Only autoclavable bag liners will be used in these containers.

3. Kill Jar Requirements:

All laboratories designated Biosafety level 1 will have a wide mouth jar, marked BL1, that is half filled with at least 15% Clorox. All liquid cultures or liquid wastes from liquid cultures will be treated in this kill jar for at least 1 hr. before disposal. BL2 laboratories will have a separate, wide mouthed, autoclavable kill jar, labeled BL2, for liquid cultures that have potential for infection.

4. Final Disposal of Biohazardous Wastes:

All bagged waste from steps 1 and 2 above, will be autoclaved for 30 minutes at 121°C and then discarded in the waste container provided by the autoclave in Rm 353. This waste will be picked up by housekeeping and disposed off in normal waste system. Kill jars for recombinant DNA work may be dumped down a sink drain after the 1 hr. treatment period (run water down the sink for several minutes to flush the drain). Kill jars for Infectious waste (BL2) must be autoclaved for 30 minutes at 121°C before dumping down a drain.
EXPOSURE EVALUATION AND FOLLOW-UP
MEDICAL EVALUATION

When a hazardous exposure incident occurs, a documentation of the medical evaluation, treatment and follow-up will be done by University Health Services (HS). They are located in the Donaghey Student Center in Rm 102A and can be reached by calling 569-3188. The Health Service are 8:00-6:00 Mon.-Thur./8:00-5:00 Friday. These steps should be taken following an exposure incident:

* Cleanse the body area thoroughly using mild soap and water.
* Report the incident immediately to your supervisor, who will contact the IBC.
* Call UALR HS for directions and to arrange evaluation and treatment.
* 

* On weekends, holidays, or after 4:30 on weekdays, go directly to University Hospitals and Clinics Emergency Treatment Center. They can also be reached at 356-2333.

Supervisors: A Worker’s Compensation form must be completed and sent to Staff Benefits within 24 hours. As soon as possible, document exposure route and circumstances of the incident. If this requires the intervention of a physician, call HP0’s Biosafety Professional (5-8501) for additional follow-up (see below).

CONTROL METHOD EVALUATION

HP0’s Biosafety Professional, in conjunction with the Principal Investigator and the employee(s) involved, will evaluate the circumstances of the exposure incident. The goal of this evaluation is to identify and correct problems in order to prevent recurrence of similar incidents.

The protocol for this include:

* Document route of exposure and circumstances under which the incident occurred.
* Evaluate the policies and “failures to control” at the time of the incident.
* Record engineering controls that were in place at the time.
* Record work practices and protective equipment or clothing that were used at the time of the incident.
* Determine what action(s) could prevent this or a similar incident in the future.
APPENDIX A

CLASSIFICATION OF BIOLOGICAL AGENTS.

A class designation is assigned to each biological agent based on the risk of acquiring the disease and the degree of severity from the disease. The original reference, "Classification of Etiological Agents on the Basis of Hazard," was revised by NIH in the rDNA Guidelines and by Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, May 1993. In most cases, the class number designation is the same as the Biosafety Level number designation. Class 5 agents are not permitted entry into the U.S. BL5 does not exist; BL4 contains the most stringent procedural and facility safety precautions.

Note: Due to continually changing nomenclature, an updated copy of the appendices will periodically be available. If you are aware of any of these changes, reclassifications, additions to these lists, etc., please send such information to HPO for inclusion in this appendix.

BASIS FOR AGENT CLASSIFICATION

Class 1 - Agents of no or minimal hazard under ordinary conditions.

Class 2 - Agents of ordinary potential hazard. This class includes agents that may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration, but which are contained by ordinary laboratory techniques.

Class 3 - Agents involving special hazard or agents derived from outside the United States which require a federal permit or importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.

Class 4 - 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 includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

Class 5 - Foreign animal pathogens are excluded from the United States by law or entry is restricted by USDA administrative policy.

NOTE: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for vaccine production or further passages of the vaccine strains.

CLASS 1 AGENTS

These are all bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes. If it is not listed below, it is a Class 1 agent.

Agent classification: B = Bacteria  M = Mycoplasma  P = Parasite
                          C = Chlamydia  R = Rickettsia  TSE = Trans. spong. encephal
                          F = Fungus    V = Virus     OV = Oncogenic Virus
<table>
<thead>
<tr>
<th>Organism/Biohazard Class/Agent Type</th>
<th>Abras/2/V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absettarou/4/V</td>
<td>Abu Hammad/2/V</td>
</tr>
<tr>
<td>Acado/2/V</td>
<td>Acara/2/V</td>
</tr>
<tr>
<td>Acinetobacter all species/2/B</td>
<td>Actinobacillus - all species/2/B</td>
</tr>
<tr>
<td>Actinomycetes (including Nocardia species,</td>
<td>Ad2-SV40/3/OV</td>
</tr>
<tr>
<td>Actinomycetes species and Arachnia propionica)/2/B</td>
<td>Ad7-SV40/2/OV</td>
</tr>
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<td>Adenovirus/2/OV</td>
<td>Adenoviruses-human-all types/2/V</td>
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<td>Aeromonas hydrophila/2/B</td>
<td>African horse sickness virus/5/V</td>
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<tr>
<td>African swine fever virus/5/V</td>
<td>Aguacate/2/V</td>
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<td>Akabane virus/5/V</td>
<td>Alastrim 3/5/V</td>
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<tr>
<td>Altuy/2/V</td>
<td>Almiwar/2/V</td>
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<td>Ananindeua/2/V</td>
<td>Ancyllostoma spp/ 2P</td>
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<td>Anhembi/2/V</td>
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<td>Apoi/2/V</td>
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<td>Arboviruses (unless listed in Class 2 or 4)/3/V</td>
<td>Archanobacterium haemolyticum/2/B</td>
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<td>Aride/2/V</td>
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<td>Arumowot/2/V</td>
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<td>Avian Leukosis/2/OV</td>
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<td>Bacillus anthracis *,**/2/B</td>
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<td>Barur/2/V</td>
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<td>Batama/2/V</td>
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<td>Bimiti/2/V</td>
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<td>Blastomyces dermatitidis*/2/F</td>
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<td>Borna disease virus/5/V</td>
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<td>Borrelia recurrentis, B vincenti, B burgdorferi/2/B</td>
<td>Botambi/2/V</td>
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<td>Bouboui/2/V</td>
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<td>Bovine spongiform encephalopathy/5/TSE</td>
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<td>Brucella-all species (B melitensis is in Class 5)/3/B</td>
<td>Brucella melitensis/5/B</td>
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<td>Brugia spp/2/P</td>
<td>Bujar/2/V</td>
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<td>Bushbush/2/V</td>
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<td>Bussuquara/2/V</td>
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<td>Disease/Pathogen</td>
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<td>Bwamba/2/V</td>
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<td>Cache Valley/2/V</td>
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<td>Camel pox virus/5/V</td>
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<td>Campylobacter jejuni/coli - all serotypes/2/B</td>
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<td>Cape Wrath/2/V</td>
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<td>Caraparu/2/V</td>
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<td>Chaco/2/V</td>
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<td>Chilibre/2/V</td>
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<td>Cladosporium (Xylohypha) trichoides/2/F</td>
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<td>Chlamydia pneumoniae*/2/C</td>
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<td>Chobbar gorge/2/V</td>
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<td>Clostridium botulinum**, Cl chauvoie,</td>
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<td>Cl septicum, Cl tetani**, Cl perfringens, etc/2/B</td>
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<td>Coccolithodes immitis/3/F</td>
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<td>Cochliomyia hominivora (screw worm)/5/P</td>
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<td>C pseudotuberculosis, C pyogenes, C renale /2/B</td>
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<td>Coxiella burnetii (Q Fever)/3/R</td>
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<td>Echoviruses-all types/2/V</td>
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<td>E coli (most lab strains, used in rDNA work and are</td>
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<td>Fibroma (Squirrel)/2/OV</td>
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<td>Candiru/2/V</td>
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<td>Cytomegaloviruses (CMV)/2/V</td>
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<td>Edwardsiella tarda/2/B</td>
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<td>Encephalomyocarditis virus (EMC)/2/V</td>
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<td>Epstein-Barr virus/2/V</td>
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<td>Erve/2/V</td>
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<td>Eyach/2/V</td>
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<td>Exophiala (Wangiella) dermatitides/2/F</td>
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<td>FeLV/3/OV</td>
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<td>Fibroma (Deer)/2/OV</td>
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<td>Flanders/2/V</td>
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<tr>
<td>Foot and mouth disease virus/5/V</td>
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Fort Morgan/2/V
Francisella tularensis**/3/B
Fusobacterium necrophorum/2/B
Gamboa/2/V
Glardia spp/2/P
Gomoka/2/V
Grand Arbaud/2/V
Guajara/2/V
Guanarito/4/V
Guaroa/2/V
Guinea Pig Leukemia/2/OV
Haemophilus ducreyi, /2/B
Hamster Leukemia/2/OV
Hanzalova/4/V
Hazarra/2/V
Hemorrhagic fever agents, including Crimean hemorrhagic fever, (Congo, Junin, and hemorrhagic fever agents Machupo viruses, and others as yet undefined/4/V
Hepatitis-associated antigen material*,**/2/V
Herpes viruses-except Herpesvirus simiae)/2/V
Herpesvirus simiae (Monkey B virus)/4/V
Histoplasma capsulatum var/ duboisi/3/F
Histoplasma (Zymonema farcinomosum)/5/F
Human Immunodeficiency Virus (HIV)*/2/V
Human T-cell lymphotropic viruses (HTLV types 1 & 2)/2/V
Icoaraci/2/V
Ileus/2/V
Influenza viruses-all types except (A/PR8/3/4) /2/V and (A/WS/3/3) which are in Class 1
Iritiu/2/V
Isospora spp/2/P
Itaquii/2/V
Japanaute/2/V
Johnston Atoll/2/V
Juan Diaz/2/V
Junin**/4/V
Jutlapa/2/V
Kaeng Khok/2/V
Kalsodi/2/V
Kammavan pettail/2/V
Kao Shuan/2/V
Karsh/2/V
Kemerovo/2/V
Ketapang/2/V
Keuralib/2/V
Kismayo/2/V
Klebsiella-all species except oxytoca/2/B
Kokoobra/2/V
Fowl plague virus/5/V
Frijoles/2/V
GalV/3/OV
Gan Gan/2/V
Goat pox virus/5/V
Gossas/2/V
Great Island/2/V
Guama/2/V
Guaratuba/2/V
Guinea Pig Herpes/2/OV
Gumbo Limbo/2/V
Haemophilus influenzae (H flu)/2/B
Hantavirus/3/V
Hart Park/2/V
Helicobacter pylori /2/B
HV Saimiri/3/OV
Highlands J/2/V
Histoplasma capsulatum/3/F
HIV/2/V
Hog cholera virus/5/V
Hookworms/2/P
Huacho/2/V
Hughes/2/V
HV ates/3/OV
Hymenolepis spp/2/P
Hypr/4/V
Ileri/2/V
Ileshe/2/V
Ingwavuma/2/V
Inkoo/2/V
Ippy/2/V
Iṣfahan/2/V
Itaporang/2/V
Jamestown Canyon/2/V
Jerry Slough/2/V
Joinjakaka/2/V
Jugra/2/V
Jurona/2/V
Kadam/2/V
Kalkalur/2/V
Kamese/2/V
Kannaman galam/2/V
Karimabad/2/V
Kasba/2/V
Kem Canyon/2/V
Keterah/2/V
Keystone/2/V
Klamath/2/V
Klebsiella oxytoca/1/B
Kolongo/2/V
Nairobi sheep disease virus (Ganjam virus)/5/V
Neisseria gonorrhoeae*, N meningitidis*/2/B
Newcastle disease virus (velogenic strains)/5/V
Nocardia asteroides, N brasiliensis, N otidiscaviarum, N transvalensis/2/B
Nugget/2/V
Nyando/2/V
Ohkotskiy/2/V
Olifantsvlei/2/V
Onchocerca spp/2/P
Orliboca/2/V
Pacora/2/V
Pahayokee/2/V
Papilloma (Bull head Trout)/2/OV
Paracoccidioides brasiliensis/2/F
Parainfluenza virus-all types except Parainfluenza virus 3, SF4 strain, which is in Class 1/2/V.
Pasteurella-all species except those listed in Class 3/3/B
Pasteurella multocida type B-3B ("buffalo" and other foreign virulent strains)/3/B
Peste des petits ruminants (small ruminant pest)/5/V
Pixuna/2/V
Point/2/V
Poliioviruses all types, wild and attenuated/2/V
Poxviruses-all types except Alastrim, Smallpox and Whitepox (Class 5), and Monkey pox, which, depending on experiment, is in Class 3 or Class 4/2/V
Pseudomonas mallei 2 (see Burkholderia)/3/B
Pseudomonas pseudomallei*/2/B (see Burkholderia)
Punta Toro/2/V
Q Fever (see Coxiella burnetii)(see Coxiella)/3/R
Rabies virus*,**-all strains except Rabies street virus, which is classified in Class 3/2/V
Rat Mammary tumor/2/OV
Reoviruses-all types/2/V
Restan/2/V
Rhinoviruses-all types/2/V
Rickettsia-all species except Vole rickettsia when used for transmission or animal inoculation experiments/3/R
Rio Grande/2/V
Rous Sarcoma/2/OV
Russian spring-summer encephalitis/4/V
Sabra arenavirus/3/V
Saboya/2/V
Sahalin/2/V
Salmonella-all species and serotypes/2/B
Sandfly f (Naples)/2/V
Ngaingan/2/V
Nique/2/V
Nkolbisson/2/V
Nola/2/V
Ntaya/2/V
Nyamanini/2/V
O'nyong -nyong/2/V
Okola/2/V
Omsk hemorrhagic fever/4/V
Orf virus/2/V
Ossa/2/V
Pacul/2/V
Palyam/2/V
Papovaviridae/2/V
Parana/2/V
Paravaccinia/2/V
Pata/2/V
Pathum Thani/2/V
Patois/2/V
Penicillium marneffii/2/F
Phnom-Penh Bat/2/V
Pichinde/2/V
Plasmodium spp/2/P
Polyoma/2/OV
Pongola/2/V
Ponteves/2/V
Precaurios/2/V
Pretoria/2/V
Prospect Hill/2/V
Puchong/2/V
Punta Salinas/2/V
Qalyub/2/V
Quaranfil/2/V
Rabbit Lymphoma/2/OV
Rat Leukemia/2/OV
RD-114/3/OV
Respiratory syncytial virus/2/V
Retroviruses/2/V
Rhodococcus equi/2/B
Rift Valley fever virus**/5/V
Rinderpest virus/5/V
Rio Bravo/2/V
Ross River/2/V
Royal Farm/2/V
Rubella virus/2/V
Sabo/2/V
Saint Floris/2/V
Salehabad/2/V
San angelo/2/V
Sandfly f (Sicilian)/2/V
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<th>Disease/Species</th>
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<tr>
<td>Sandjimba</td>
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<td>Sarcocystis spp.</td>
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<td>Sebokele</td>
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<td>Sembalam</td>
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<td>Shigella-all species and serotypes</td>
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<td>Shope Fibroma</td>
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<td>Shuni</td>
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<td>Simian Immunodeficiency Virus (SIV)</td>
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<td>Simian viruses-all types except...</td>
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<td>simiae (Monkey B virus) and Marburg...</td>
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<tr>
<td>which are in Class 4</td>
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<td>Sunday Canyon</td>
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* Biosafety Level 3 practices, containment, equipment and facilities are recommended for work involving production volumes or concentration of cultures, and for activities that have a high potential for aerosol or droplet production.

** A vaccine is available and recommended for all persons working with this agent.

References


2. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products Office, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782.

3. All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization - WHO Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).


APPENDIX B
CLASSIFICATION OF BIOLOGICAL AGENTS ACCORDING TO RISK

A class designation is assigned to each biological agent based on the risk of acquiring the disease and the degree of severity from the disease. The original reference, "Classification of Etiological Agents on the Basis of Hazard," was revised by NIH in the rDNA Guidelines and by Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, May 1993. In most cases, the class number designation is the same as the Biosafety Level number designation. Class 5 agents are not permitted entry into the U.S. BL5 does not exist; BL4 contains the most stringent procedural and facility safety precautions. (1)

Note: Due to continually changing nomenclature, an updated copy of the appendices will periodically be available. If you are aware of any of these changes, reclassifications, additions to these lists, etc., please send such information to HPO for inclusion in this appendix.

BASIS FOR AGENT CLASSIFICATION

Class 1 - Agents of no or minimal hazard under ordinary conditions.

Class 2 - Agents of ordinary potential hazard. This class includes agents that may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration, but which are contained by ordinary laboratory techniques.

Class 3 - Agents involving special hazard or agents derived from outside the United States which require a federal permit or importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.

Class 4 - 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 includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

Class 5 - Foreign animal pathogens are excluded from the United States by law or entry is restricted by USDA administrative policy.

NOTE: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for vaccine production or further passages of the vaccine strains.

CLASS 1 AGENTS

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

CLASS 2 AGENTS

Class 2 Bacterial

Acinetobacter-all species
Actinobacillus-all species
Actinomycetes (including Nocardia species)
Actinomyces species
Amycolata autotrophica
Bacillus anthracis*,**, Baccharis recentris,
B. burgdorferi
Burkholderia (Pseudomonas), except B. pseudomallei
and B. mallei, which are in Class 3
Chlamydia psittaci
Campylobacter jejuni/coli - all serotypes
Cl. chauvoei
Cl. histolyticum
Cl. septicum
Cl. perfringens, etc.
C. equi
C. pyogenes
Dermatophilus congolensis
Edwardsiella tarda
all enteropathogenic, enterotoxigenic,
enteroinvasive, and strains bearing K1 antigen coli
H. influenzae
Leptospira interrogans-all serotypes
Klebsiella-all species except oxytoca
Legionella-like agents*
Mycobacteria-all species except those listed Class 3

Neisseria gonorrhoeae*
Nocardia asteroides
N. otitidiscaviarum
Pasteurella-all species except those listed in Class 3

Rhodococcus equi
Shigella-all species and serotypes
Streptobacillus moniliformis
S. pyogenes
T. pallidum
Vibrio cholerae
Yersinia enterocolitica

Aeromonas hydrophila
Arachnia propionica
Archaeobacterium haemolyticum
Bordetella-all species
B. vincentii
Campylobacter fetus
Chlamydia pneumoniae*
Chlamydia trachomatis
Clostridium botulinum**
Cl. haemolyticum
Cl. novyi
Cl. tetani**
Corynebacterium diphtheriae**
C. pseudotuberculosis
C. renale
Epsipellothrix rhusiopathiae
Escherichia coli
Fusobacterium necrophorum
Haemophilus ducreyi
Helicobacter pylori
Listeria-all species
Legionella pneumophila*
Moraxella-all species
Mycoplasma-all species
except My. mycoids, and My.
agalactiae, which are in Class 5
N. meningitidis*
N. brasiliensis
N. transvalensis
Pseudomonas pseudomallei*
(see Burkholderia)
Salmonella-all species and serotypes
Staphylococcus aureus
Streptococcus pneumoniae
Treponema carateum
T. pertenue
V. parahemolyticus
Yersinia pestis*,**

Class 2 Fungal

Blastomyces dermatitidis
Cladosporium (Xylohypha) trichoides
Dactylaria gallopava (Ochoronis gallopavum)
Exophiala (Wangiella) dermatitides
Microsporum
Penicillium marneffii
Trichophyton spp.

Cladosporium bantianum
Cryptococcus neoformans
Epidermophytan
Fonsecaea pedrosi
Paracoccidioides brasiliensis
Sporothrix schenckii
Class 2 Parasitic Agents

Ancylostoma spp.
Babesia spp.
Coccidia spp.
Cysticercus cellulosae
Entamoeba spp.
Fasciola spp.
Hookworms
Isospora spp.
Loa loa filaria
Naegleria gruberi
Necator spp.
Plasmodium spp.
Strongyloides spp.
Taenia solium
Toxocara canis
Trypanosoma spp.
Ascaris spp.
Brugia spp.
Cryptosporidia spp.
Echinococcus granulosus
Enterobius
Giardia spp.
Hymenolepis spp.
Leishmania spp.
Microsporidia spp.
Naegleria fowleri
Onchocerca spp.
Sarcocystis spp.
Schistosoma spp.
Toxoplasma gondii
Trichinella spiralis
Wuchereria bancrofti (filaria)

Class 2 Viral, Rickettsial, and Chlamydial

Adenoviruses-human-all types
Arenaviruses
Coxsackie A and B viruses
Echoviruses-all types
Encephalomyelitis viruses*
Herpes viruses-except Herpesvirus simiae
(Monkey B virus) which is in Class 4
Human Immunodeficiency Virus (HIV)*
Influenza viruses-all types except A/PR8/34
and A/WS/33 which are in Class 1
Measles virus
Mumps virus
Papovaviridae
Parainfluenza virus-all types except Parainfluenza
virus 3, SF4 strain, which is in Class 1
Poliomyelitis all types, wild and attenuated
Poxviruses-all types except Alastrim, Smallpox, and
Whitepox (Class 5), and Monkey pox, which,
depending on experiment, is in Class 3 or 4
Rabies virus*,**-all strains except Rabies street virus,
which is classified in Class 3
Simian viruses-all types except Herpesvirus simiae
(Monkey B virus) and Marburg virus, which are
in Class 4
Vesicular stomatitis virus-Laboratory adapted strains
of low virulence only, otherwise, it is a
Class 3 organism.2
Varicella virus
Arboviruses (see list at end of BL2)
Coronaviruses
Cytomegaloviruses
Encephalomyocarditis virus (EMC)
Hepatitis-associated antigen material*,**
Epstein-Barr virus
Human T-cell lymphotropic viruses
Lymphohgranuloma venereum agent
Molluscum contagiosum virus
Orf virus
Paravaccinia virus
Reoviruses-all types
Respiratory syncytial virus
Retroviruses
Rhinoviruses-all types
Rubella virus
Simian Immunodeficiency Virus (SIV)
Tanapox
Vaccinia virus
Vole rickettsia
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<td>Seletar</td>
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<td>Serra do Navio</td>
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<td>Shamonda</td>
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<td>Silverwate</td>
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<td>Sixgun City</td>
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Soldado  
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West. equine enc.;**  
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Yaquinae Head  
Zaliv Terpeniya  
Zingila  
Sororoca  
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Tahyna  
Tanjong Rabok  
Tembe  
Tete  
Thottapalayam  
Timboteua  
Toure  
Trivittatus  
Turlock  
Umatilla  
Upolu  
Uukunlemi  
Vinces  
VS-New Jersey  
Wanowrie  
Whataroa  
Wongorr  
Yata  
Zegia  
Zirqa  
Stratford  
Tacaribe  
Tamiami  
Tatague  
Tembusu  
Tettnang  
Tibrogargan  
Tindholmur  
Tibec  
Trubanaman  
Tyuleny  
Umbre  
Urucuri  
Vellore  
Virgin River  
Wad Medani  
Warrego  
Witwatersrand  
Wyeomyia  
Yogue  
Zika

* Biosafety Level 3 practices, containment, equipment and facilities are recommended for work involving production volumes or concentration of cultures, and for activities that have a high potential for aerosol or droplet production.

** A vaccine is available and recommended for all persons working with this agent.

CLASS 3 AGENTS

Class 3 Bacterial Agents

Bartonella-all species  
Brucella-all species (B. melitensis is in Class 5)  
Burkholderia (Pseudomonas ) mallei 2  
Francisella tularensis**  
Mycobacterium bovis, M. tuberculosis  
Pasteurella multocida type B-(“buffalo” and other foreign virulent strains)2  
Yersinia pestis (antibiotic resistant strain)

Class 3 Fungal Agents

Coccidioides immitis  
Histoplasma capsulatum  
Histoplasma capsulatum var. duboisii
Class 3 Parasitic Agents

NONE

Class 3 Viral, Rickettsial, and Chlamydial

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 conditions of use and geographical location of laboratory.)
Coxiella burnetii (Q Fever)
Dengue virus, when used for transmission or animal inoculation experiments
Hantavirus
Lymphocytic choriomeningitis virus (LCM)
Monkeypox, when used in vitro
Rabies street virus
Rickettsia—all species except Vole rickettsia when used for transmission or animal inoculation experiments
Sabia arenavirus
Yellow fever virus—wild, when used in vitro

CLASS 4 AGENTS

Class 4 Bacterial, Fungal, Parasitic Agents

NONE

Class 4 Viral, Rickettsial and Chlamydial

Ebola fever virus
Monkeypox, 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
Guanarito Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex, including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever and Central European encephalitis viruses, Absettarou, Hanzalova, Hypr, and Kumlinge.i.Tick-borne encephalitis virus complex
Venezuelan equine encephalitis virus**, epidemic strains, when used for transmission or animal inoculation experiments

CLASS 5 AGENTS

Animal Disease Organisms and Vectors which are Forbidden Entry into the United States or restricted by law or USDA Policy.

African horse sickness virus
African swine fever virus
Akabane virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever agent
Bovine spongiform encephalopathy
Brucella melitensis
Camel pox virus
Cochliomyia hominivorax (screw worm)
Cowdia ruminantium (Pseudomonas ruminantium) (heart water)
Ephemeral fever virus
Foot and mouth disease virus
Fowl plague virus
Goat pox virus
Histoplasma (Zymonema farciminosum)
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Mycoplasma mycolides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious agalactia of sheep)
Nairobi sheep disease virus (Ganjam virus)
Newcastle disease virus (velogenic strains)
Paste des petits ruminants (pest of small ruminants)
Rift Valley fever virus**
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschchen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Theileria parva (East Coast Fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Viral hemorrhagic disease of rabbits
Wesselsbron disease virus

Organisms Which May Not Be Studied in the United States Except at Specified Facilities

Smallpox 3
Ailastrim 3
Whitepox 5

Classification of Oncogenic Viruses on the Basis of Potential Hazard 4

Low-Risk Oncogenic Virus (Class 2)

Adenoviruses
Rous Sarcoma
SV-40 (Simian)
CELO
Ad7-SV40  
Polyoma  
Bovine Papilloma  
Rat Mammary tumor  
Avian Leukosis  
Murine Sarcoma  
Murine Leukemia  
Mouse mammary tumor  
Rat Leukemia  
Hamster Leukemia  
Bovine Leukemia  
Dog Sarcoma  
Mason-Pfizer Monkey Virus  
Marek's  
Guinea Pig Herpes  
Guinea Pig Leukemia  
Lucke (Frog)  
Adenovirus  
Shope Fibroma  
Dog mast cell  
Rabbit Lymphoma  
Papilloma (Bull head Trout)  
Fibroma (Squirrel)  
Fibroma (Deer)  
Shope Papilloma  

Moderate-Risk Oncogenic Viruses (Class 3)

Ad2-SV40  
FeLV  
HV Saimiri  
EBV  
SSV-1  
GaLV  
HV ateles  
Yaba pox  
FeSV  
SLV  
RD-114  

References

1. The original reference for this classification was the publication Classification of Etiologic Agents on the Basis of Hazard 4th edition, July 1974, U.S. Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purpose of these Guidelines, this list has been revised by NIH in the rDNA Guidelines, Federal Register 59:34496, and subsequently by Biosafety in Microbiological and Biomedical Laboratories 3rd Edition, May 1993, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and the National Institutes of Health, Atlanta, Georgia 30333.
2. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products Office, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782.

3. All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization- WHO Collaborating Center for Smallpox Research, Centers for Disease Control, In Atlanta).


* Biosafety Level 3 practices, containment, equipment and facilities are recommended for work involving production volumes or concentration of cultures, and for activities that have a high potential for aerosol or droplet production.

** A vaccine is available and recommended for all persons working with this agent.

APPENDIX C

TRAINING RESOURCES

Primary training in all aspects of Biological Safety is provided by the Office of Environmental Safety and Health at the University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72207, 686-5000.

Annual training update is provided by the Institutional Biosafety Committee, Department of Biology, University of Arkansas at Little Rock, 2801 South University Avenue, Little Rock, AR 72204, 569-3510.

The following text materials are available by loan from the Institutional Biosafety Committee Office, Rm 381, Science Laboratory Building, for use by students and staff.


The following Video tapes are available by loan from the Institutional Biosafety Committee Office, Rm 381, Science Laboratory Building, for use by students and staff.

Biological Safety Cabinets
Safe Use of Biological Safety Cabinets (Eagleson Institute)

Biological Safety
Comparative Review of Class II Biological Safety Cabinets (Rhode Island ETS)

Laboratory Safety
Working Safety with HIV (NIH)
Universal Precautions in the Laboratory (Syntex)-15 min
Practicing Safe Science (HHMI)- 29 min
Controlling Your Risks: HIV in the Research Laboratory (HHMI)-28 min

APPENDIX D

DANGERS OF CELL AND TISSUE CULTURE SYSTEMS

Many biochemistry, physiology, microbiology, and cancer research laboratories use cell cultures as routine source materials. The actual hazards of this work are not clearly recognized and may be minimal, with certain exceptions. Some hazards may involve diseases that develop slowly over many years, (e.g., solid tumors or degenerative neurological diseases).

The mechanisms of environmental dissemination of mycoplasmas and cultured cells can also serve to disseminate potentially biohazardous agents into the environment. Any microbiological agent that is present in the cell culture can be released to the immediate environment, even during careful work. Handling procedures for cell cultures, therefore, must cause the least interference with the experimental work, but provide personnel protection consistent with the presumed hazards.

Most cell cultures are known to harbor viruses, either adventitiously or deliberately. In these cases the appropriate procedures for the known or presumed virus should be used with the cell culture. Biosafety Level 2 meets the minimal requirements for work involving many of the cell cultures presently being manipulated.

Primary and permanent cell lines from mouse, hamster, human, rat, etc., should be handled as if they carry low risk infections (as Class 2 agents). Human isolates from malignant tissues or those from tissues susceptible to, or likely to, harbor mammalian oncogenic viruses should be considered as moderate risk agents (as Class 3 agents). Cells from the herpes and Epstein-Barr virus
transformed cultures should be handled as moderate risk viruses (Class 3). All established or permanent cultures of human lymphocytes should be handled with the assumption they harbor the Epstein-Barr virus, a moderate risk agent. Under no conditions should individuals handle lymphoid cells of a line derived from themselves, or a first degree relative, viruses;

APPENDIX E

WORKING WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND OTHER RETROVIRUSES, INCLUDING SIMIAN IMMUNODEFICIENCY VIRUS (SIV)

Laboratory Hazards

HIV has been isolated from blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, cervical secretions, and tissue of infected persons and experimentally infected nonhuman primates. CDC has recommended that blood and body fluid precautions be used consistently when handling any blood contaminated specimens. This approach, referred to as "universal precautions," precludes the need to identify clinical specimens obtained from HIV+ patients or to speculate as to the HIV status of a specimen. Although the risk of occupationally acquired HIV is primarily through exposure to infected blood, it is prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat, vomitus and human breast milk. This reduces the potential for exposure to other microorganisms that may cause other types of infections.

In the laboratory, virus should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other than intact skin) from a human (living or dead), in HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

SIV has been isolated from blood, cerebrospinal fluid, and a variety of tissues of infected nonhuman primates. Limited data exist on the concentration of virus in semen, saliva, cervical secretions, urine, breast milk, and amniotic fluid. In the laboratory, virus should be presumed to be present in all SIV cultures, in animals experimentally infected or inoculated with SIV, in all materials derived from HIV or SIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

In the laboratory, the skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses. Whether infection can occur via the respiratory tract is unknown. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other virus-containing or potentially infected materials.

Recommended Precautions

In addition to these recommended precautions, persons working with HIV, SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogens Standard. Call HPO for a copy of the University’s Bloodborne Pathogens Guidelines.

1. BL-2 standard and special practices, containment equipment and facilities are recommended for activities involving all blood contaminated clinical specimens, body fluids and tissues from all humans or from HIV- or SIV-Infected or inoculated laboratories animals.
2. Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BL-2 facility, but using the additional practices and containment equipment recommended for BL-3.

3. Activities involving industrial-scale volumes or preparation of
   * concentrated HIV or SIV are conducted in a BL-3 facility, using BL-3 practices and containment equipment.

4. Nonhuman primates or other animals infected with HIV or SIV are housed in ABL-2 facilities using ABL-2 special practices and containment equipment.

Additional Comments

1. There is no evidence that laboratory clothing poses a risk for retrovirus transmission; however, clothing that becomes contaminated with HIV or SIV preparations should be decontaminated before being laundered or discarded. Laboratory personnel must remove laboratory clothing before going to non-laboratory areas.

2. Work surfaces are decontaminated with an appropriate chemical germicide after procedures are completed, when surfaces are overtly contaminated, and at the end of each work day. Many commercially available chemical disinfectants can be used for decontaminating laboratory work surfaces and some laboratory instruments, for spot cleaning of contaminated laboratory clothing, and for spills of infectious materials. Prompt decontamination of spills should be standard practice.

3. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BL-2.

4. It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV and SIV in conjunction with applicable federal, state and local laws. Such policies should consider confidentiality, consent for testing, administration of appropriate prophylactic drug therapy, counseling, and other related issues. If a laboratory worker has a parenteral or mucous-membrane exposure to blood, body fluid, or viral-culture material, the source material should be identified and, if possible, tested for the presence of virus. If the source material is positive for HIV antibody, virus, or antigen, or is not available for examination, the worker should be counseled regarding the risk of infection and should be evaluated clinically and serologically for evidence of HIV infection. The worker should be advised to report and seek medical evaluation of any acute febrile illness that occurs within 12 weeks after the exposure. Such an illness—particularly one characterized by fever, rash, or lymphadenopathy—may indicate recent HIV infection. If seronegative, the worker should be retested 6 weeks after the exposure and periodically thereafter (i.e., at 12 weeks and 6, 9 and 12 months after exposure). During this follow-up period exposed workers should be counseled to follow Public Health Service recommendations for preventing transmissions of HIV.

5. Other primary and opportunistic pathogenic agents may be present in the body fluids and tissues of persons infected with HIV. Laboratory workers should follow accepted biosafety
practices to ensure maximum protection against inadvertent laboratory exposure to agents that may also be present in clinical specimens or in specimens obtained from non-human primates.

Research involving other human (i.e., human T-lymphotrophic virus types I and II) and simian retroviruses occurs in many laboratories. Transmission of such viruses has not been reported in the laboratory setting. The precautions outlined above are sufficient while working with these agents.

Between 1989 and 1992, 69 persons with CD4+ T-lymphocyte depletion, but without evident HIV infection, were identified in the U.S. This condition has been provisionally termed "idiopathic CD4+ T-lymphocytopenia (ICL)." To date, investigations of persons with idiopathic CD4+ T-cell depletion indicate that ICL is rare, that these findings may represent various disorders, and in some cases, normal or transient variations in CD4+ T-lymphocyte counts. No epidemiologic or laboratory evidence of a transmissible agent of immunodeficiency has been found as of October 1992.


Effective June 24, 1994, Published in Federal Register, July 5, 1994 (59 FR 34496)
Amendment Effective July 28, 1994, Federal Register, August 5, 1994 (59 FR 40170)
Amendment Effective April 17, 1995, Federal Register, April 27, 1995 (60 FR 20726)
Amendment Effective December 14, 1995, Federal Register, January 19, 1996 (61 FR 1482)
Amendment Effective September 30, 1997, Federal Register, October 14, 1997 (62 FR 53335)
Amendment Effective October 20, 1997, Federal Register, October 29, 1997 (62 FR 56196)
Amendment Effective October 22, 1997, Federal Register, October 31, 1997 (62 FR 59032)
Amendment Effective February 4, 1998, Federal Register, February 17, 1998 (63 FR 8052)
Amendment Effective April 30, 1998, Federal Register, May 11, 1998 (63 FR 26018)