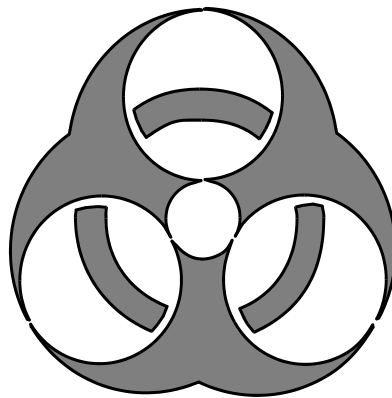




BIOLOGICAL SAFETY MANUAL



Revised April 2004

TABLE OF CONTENTS

Emergency Numbers	iv
Awareness Certification	v
Policy Statement	1
Scope and Application	1
Institutional Biosafety Committee	2
Biosafety Training	3
Medical Surveillance and Examinations	3
Hazard Identification	3
Protective Equipment	4
REM Services	4
Recombinant DNA (rDNA)	4
Biohazards	5
Routes of Exposure to Biohazards	5
Antigens	7
Principles of Biosafety	8
Laboratory Biosafety Level Criteria	9
Biosafety Level 1	9
Biosafety Level 2	10
Biosafety Level 3	12
Use of Animals	16
Vertebrate Animal Biosafety Level Criteria	16
Animal Biosafety Level 1	16
Animal Biosafety Level 2	17
Animal Biosafety Level 3	18
Bloodborne Pathogens Program	22
Decontamination and Disposal	22
Sterilization Procedures	23
Disposal Procedures	24
Sharps Handling Procedures	24
Clean and Contaminated Sharps Handling (Table)	26
Infectious Waste and Look-Alike Waste (Table)	27
Spills of Biohazardous Materials	27

TABLE OF CONTENTS

(Continued)

APPENDICES

Appendix A: Personal Protective Equipment (PPE) Policy & Hazard Assessment	29
Appendix B: Bio-Material Pick-up Certificate Disposal	30
Appendix C: Biological Safety Cabinets	31
Appendix D: Working Safely in a Biological Safety Cabinet	33
Ultraviolet Lamps	33
Biosafety Cabinet HEPA Filters	33
Additional Information	34
Appendix E: Biological Agent Risk Classification	36
NIH Agent Classification	37
Select Agents	47
Appendix F: Dangers of Cell and Tissue Culture Systems	50
Classification of Cell and/or Tissue Cultures According to Containment Level	50
Appendix G: Risk Classification For Oncogenic Agents	52
Criteria for Low Risk Oncogenic Agents	52
Criteria for Moderate Risk Oncogenic Viruses	52
Criteria for High Risk Oncogenic Viruses	53
Appendix H: Biosafety Reference Material	54

EMERGENCY NUMBERS

West Lafayette Campus

All Emergencies 9-1-1
Radiological and Environmental Management (REM) 765-494-6371

Ft. Wayne Campus

Fire and Ambulance (9) 9-1-1
University Police 219-481-6900
REM Contact 219-481-5744

North Central Campus

All Emergencies 219-785-5220
REM Contact 219-785-5527

Calumet Campus

All Emergencies 219-989-2911
REM 219-989-2724

REM Biosafety Contact List

REM Director 765-494-2350
BioSafety/Environmental Health 765-494-1496
BioSafety Cabinet Information 765-494-1496
BioSafety Cabinet Certification & Maintenance 765-494-7968
Biohazardous Waste 765-494-0238

ONLINE ADDRESSES

REM Home Page

<http://ww.purdue.edu/rem>

Office of the Vice President for Research

<http://www.purdue.edu/research/vpr/rschadmin/rschoversight/rdna/index.shtml>

Institutional Biosafety Committee

ibcomm@purdue.edu

PURDUE BIOLOGICAL SAFETY AWARENESS CERTIFICATION

The Occupational Safety and Health Administration (OSHA) requires that employees be made aware of the biological hazards at their place of employment.

After reading the "Purdue Biological Safety Manual," please complete and return a copy of this form to your supervisor or Designated Trained Individual. By signing below you acknowledge that you are aware of the Purdue Biological Safety Program and the policies and procedures applicable to your work. Your supervisor will provide additional information and training as appropriate.

Name _____ Phone _____

University ID Number _____

Department _____

Job Classification (if employee) _____

Building _____ Room _____

Course No. (if student) _____

Supervisor, instructor, or P.I. for your area _____

Signature: _____ Date: _____

Supervisors and instructors:

Please retain the completed documentation forms in your departmental safety training files.

PURDUE UNIVERSITY POLICY STATEMENT

It is the policy of Purdue University to take every reasonable precaution to provide a work environment that is free from recognized hazards for its employees in accordance with the General Duty Clause of the Indiana Occupational Safety and Health Law (IC 22-8-1.1 Section 2).

The Purdue University 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 workplace.

SCOPE AND APPLICATION

The Biological Safety Program applies to all personnel at Purdue University's West Lafayette Campus and Regional Campuses, University research farms and agricultural centers, and related facilities and operations engaged in the use of biohazardous agents.

LABORATORY SUPERVISORS AND PRINCIPAL INVESTIGATORS

Laboratory supervisors and principal investigators are responsible for biological safety in the laboratory. They must ensure that workers know and follow biological safety rules, that protective equipment is available and in working order, and that appropriate training has been provided; provide regular, formal biosafety inspections of their facilities and equipment; know the current legal and University requirements concerning biological safety; determine the required levels of protective apparel and equipment; and ensure that facilities and training for use of any agent are adequate.

LABORATORY STAFF

Laboratory workers are responsible for planning and conducting each operation in accordance with recognized biological safety procedures and for developing and practicing good personal hygiene habits.

INSTITUTIONAL BIOSAFETY COMMITTEE

The Purdue University Institutional Biosafety Committee (IBC) is the campus-based committee that has the responsibility for reviewing and approving all proposals, activities, and experiments involving an organism or product of an organism that presents a risk to humans. This includes, but is not limited to, work with potential pathogens, work with human clinical samples and primary cell lines and work with DNA from pathogenic organisms. Principal investigators (PIs) must submit to the IBC an application to use rDNA and other biohazards in their research. While certain rDNA protocols are exempt from the Guidelines, a determination of this exemption may only be made by the Chair of the IBC. The IBC convenes as a group semi-annually or more frequently as needed to fulfill its responsibilities.

The IBC review is conducted in accordance with the guidance and requirements of NIH, the Centers for Disease Control (CDC), and Purdue University policies and the Biosafety Manual. All PIs have an obligation to be closely familiar with health and safety guidelines applicable to their work and to adhere to them. Purdue's Radiological and Environmental Management (REM), the Office of Research Administration (ORA) and the Biosafety Officer work to support the IBC in carrying out its responsibilities.

Office of the Vice President for Research web site:

<http://www.purdue.edu/research/vpr/rschadmin/rschoversight/rdna/index.shtml>

Direct IBC questions to:

ibcomm@purdue.edu

BIOSAFETY TRAINING

Biosafety Training Information:

1. The written Biological Safety Program is available on the REM web site at <http://www.purdue.edu/rem/home/booklets/bioman.pdf>.
2. A biological safety training PowerPoint study guide is on the REM Website
3. The specific health hazards related to the biohazardous agents used in the work area must be reviewed. Measures employees can take to protect themselves from these hazards, including specific procedures, emergency procedures, and personal protective equipment to be used must be reviewed. Note: A "Certification of Hazard Assessment" should be posted in the work area identifying these hazards. This certification of hazard assessment should be reviewed at least annually and updated anytime a new task which presents a hazard is introduced into the lab. (See Appendix A).

Documentation: Training required by the Biological Safety Program should be documented using the form at the beginning of this publication (page 3). Group training can be documented by attaching an attendance sheet to the tear-out form. Copies of either form should be kept in each work area or department. Student training should be documented in the same fashion.

MEDICAL SURVEILLANCE AND EXAMINATIONS

Note: Acute medical care will normally be provided by the Purdue University Student Health Center in accordance with University policies and procedures. Requests for special surveillance and examinations should be arranged through REM.

All medical surveillance and examinations must be performed by or under the supervision of a licensed physician and must be provided without cost to the employee, without loss of pay, and at a reasonable time and place.

HAZARD IDENTIFICATION

LABELS

Supervisors must ensure labels on incoming containers of biohazardous agents are not removed or defaced. They must also ensure laboratory containers of biohazardous agents are labeled where required. Laboratory containers, including bottles, flasks, sample vials, etc., must be marked, labeled or coded **in all cases**. This will aid in preventing any confusion concerning agent identification. The label should be legible, dated, and should identify the owner of the agent. If codes, acronyms, formulae, or abbreviations are used, post a legend/key near the inside of the entrance to the room. An alphabetical listing of over 1000 chemicals that is suitable to use as a key for deciphering label abbreviations, acronyms, and chemical formulae can be found on REM's Booklets and guidelines web page.

<http://www.purdue.edu/rem/home/files/guide.htm>.

PROTECTIVE EQUIPMENT

Users of biohazardous agents must ensure fume hoods, biological safety cabinets, and other protective equipment are adjusted and functioning properly prior to initiating an activity requiring their use. Physical Facilities will ensure fume hood and biological safety cabinet performance is periodically evaluated and repairs are made when necessary.

USE OF RESPIRATORS

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 Purdue University Respiratory Protection Program. Contact REM for additional information.

REM SERVICES

The Department of Radiological and Environmental Management (REM) offers a variety of biological safety services. These services are provided at no cost and include:

1. Annual certification of Laminar Flow Clean Benches (LFCB), Biological Safety Cabinets (BSCs), and fume hoods.
2. Autoclave testing (upon request).
3. Employee training.
4. Biosafety consultation.
5. Laboratory audits.
6. Treated infectious waste disposal.

RECOMBINANT DNA (rDNA)

Recombinant DNA (rDNA) activities at Purdue University are subject to the National Institutes of Health (NIH) "Guidelines for Research Involving Recombinant DNA Molecules" regardless of the source of funds that support the activities. While certain rDNA protocols are exempt from the guidelines, a determination of this exemption may only be made by the Chair of the IBC. The IBC convenes as a group semi-annually or more frequently as needed to fulfill its responsibilities.

The IBC review is conducted in accordance with the guidance and requirements of NIH, the Centers for Disease Control (CDC), and Purdue University policies and the Biosafety Manual. PIs are required to submit an application to use rDNA, biohazardous agents, or unfixed human fluids and tissues prior to the initiation of the research. If at any time the research expands beyond the current protocol description, an amendment describing the changes must be submitted to the IBC before work is initiated. Initial protocol application forms and protocol amendment forms are available from the following web site:

<http://www.purdue.edu/Research/vpr/compliance/rdna/index.html>.

BIOHAZARDS

"Biohazardous agent" means an agent that is biological in nature, capable of self-replication, and has the capacity to produce deleterious effects upon biological organisms. Biohazardous agents include, but are not limited to, bacteria; fungi; viruses; rickettsiae; chlamydia; prion, parasites; recombinant products; allergens; cultured human and animal cells and the potentially biohazardous agents these cells may contain; infected clinical specimens; tissue from experimental animals; plant viruses, bacteria and fungi; toxins; and other biohazardous agents as defined in laws, regulations or guidelines. "Biohazardous material" means any material that contains or has been contaminated by a biohazardous agent.

ROUTES OF BIOHAZARD EXPOSURE

Exposure and subsequent infection of an individual with a biohazardous agent can occur by several routes, i.e., aerosol inhalation, splash, animal bites, sharps, and similar situations where direct contact can occur.

AEROSOLS

Some of the laboratory operations which release a substantial number of droplets are almost trivial in nature, such as breaking bubbles on the surface of a culture as it is stirred, streaking a rough agar plate with a loop, a drop falling off the end of a pipette, 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 in some applications, or opening a lyophilized culture, among many others. Most of these only take a second or so 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.* The possibility of aerosol production should always be considered while working with infectious organisms.

CONTACT

The control of potential exposure by the contact route requires that 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, use of care in the performance of procedures, and cleaning and disinfecting work surfaces. Procedures where exposure via direct contact may occur include: decanting of liquids, pipetting, removal of screw caps, vortex mixing of unsealed containers, streaking agar surfaces, and inoculation of animals.

It should also be recognized that dispersal of contaminants to other surfaces can occur by their transfer on the gloves of the laboratory worker, by the placement of contaminated equipment or laboratory ware, and by the improper packaging of contaminated waste.

ORAL

Mouth pipetting is prohibited. Mechanical pipetting devices are required. Indirect oral exposures can be avoided through the use of the personal hygienic practice of regular hand washing, no eating or drinking in the work area, and by not placing any objects, including fingers, into the mouth. The wearing of a N-95 dust and vapor mask or face shield will protect against the splashing of biohazardous material into the mouth.

SPLASH

The wearing of a face shield, safety glasses, or goggles will protect workers against splashing biohazardous material into the eyes.

SHARPS

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, in addition, result in the dispersal of biohazardous material onto surfaces and into the air. Depending upon the route of inoculation of animals, the use of a needle and syringe may also result in the contamination of the body surfaces. Because of the imminent hazard of self-inoculation, the use of the needle and syringe should be limited to those procedures where there is no alternative, and then the procedure should be conducted with the greatest of care. Inoculation can also result from animal bites and scratches.

ANIMAL EXPOSURE

Both research and non-research animals have the potential to cause injury, transmit zoonotic disease, and/or cause allergic reaction to those who have contact. These animal hazards can occur by either direct contact from handling an animal or just by being in close proximity, i.e., working or passing through an animal housing room. Understanding routes of disease transmission, disease or allergy signs and symptoms, personal protective equipment (PPE), waste handling, and emergency contacts is very important.

ANIMAL EXPOSURE OCCUPATIONAL HEALTH PROGRAM

<http://www.purdue.edu/Research/ORR/animals/occhealth.shtml>

ANTIGENS

Antigens are substances that can induce a detectable immune response. Proteins are usually the most potent antigens. In order to be immunogenic, a substance must be recognized as "foreign". This is why reactions to human environmental proteins (e.g., skin scales) are rare. Airborne antigen exposure can result in allergic rhinitis, allergic asthma, or hypersensitive pneumonitis. Contact sensitizations include conjunctivitis, dermatitis, or hives. Occupational antigens may produce tolerable or easily treatable symptoms or may not be perceived as being related to work. Allergic reactions may be induced after less than one year to several years after initial exposure.

Hypersensitivity pneumonitis can occur after the inhalation of particles below the sizes of 2-3 microns. Sensitized individuals may subsequently respond to very low levels of environmental antigens. Bacteria, fungi, and protozoa can release antigens in a size range and concentration that can produce hypersensitivity pneumonitis. Intact bacteria and small fungus spores can penetrate the lower airways. In addition, soluble antigens from most organisms can become airborne when substrates on which they are growing are disturbed and cause sensitization. Examples of hypersensitivity pneumonitis include farmer's lung, furrier's lung, ventilation pneumonitis, and suberosis.

Allergic rhinitis and allergic asthma are often induced after several years of low exposure to some antigens. Once sensitized, people may respond only to relatively high levels of environmental antigen. Particle size is relatively unimportant as upper airway deposition allows antigens to diffuse slowly (often causing delayed symptoms) while small particle antigens may cause immediate reaction.

The most effective control measures to prevent allergies from developing in employees are to prevent or minimize exposures to potential allergens. The use of laboratory fume hoods and biological safety cabinets can serve as effective containment devices for allergens. Use of ventilation systems and filtration devices can act to keep exposures down. Good housekeeping (including wet methods), personal hygiene, and laboratory techniques can serve to keep dust from becoming airborne. Use of NIOSH-approved dust/mist/fume respirators, either single-use or with disposable filters, should be used where short, intermittent high exposures are otherwise unavoidable. There is a dose relationship that affects the rate of employees becoming sensitized, and once sensitized, the worker must essentially avoid exposure. Therefore, employing means to keep exposures low, even where no complaints have been made, can decrease the probability an employee will develop an allergic reaction in the future.

PRINCIPLES OF BIOSAFETY

The term "containment" is used in describing safe methods for managing biohazardous agents in the laboratory environment where they are being handled or maintained. Primary containment, *i.e.*, the protection of personnel and the immediate laboratory environment from exposure to biohazardous agents, is provided by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

Secondary containment, *i.e.*, the protection of the environment external to the laboratory from exposure to biohazardous agents, is provided by a combination of facility design and operational practices. The purpose of containment is to reduce exposure of laboratory workers and other persons to, and prevent escape into the outside environment of, potentially biohazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

Safety Equipment (Primary Barriers)

Safety equipment includes biological safety cabinets and a variety of enclosed containers. The biological safety cabinet is the principal device used to provide containment of aerosols generated by many microbiological procedures. Two types of biological safety cabinets (Class II, III) used in microbiological laboratories are described in Appendix C. Open-fronted 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 to personnel and the environment.

Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, and safety glasses. These personal protective devices are often used in combination with biological safety cabinets and other devices which contain the agents, animals, or materials being used. In some situations in which it is impractical to work in biological safety cabinets, personal protective devices may form the primary barrier between personnel and 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.

LABORATORY BIOSAFETY LEVEL CRITERIA

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

I. BIOSAFETY LEVEL 1

Biosafety Level 1 is suitable for experiments involving agents of no known or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns of the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science. The following standard and special practices apply to agents assigned to Biosafety Level 1:

A. Standard Microbiological Practices

1. Laboratory doors are kept closed when experiments are in progress.
2. Work surfaces are decontaminated daily and after any spill of biohazardous material.
3. All contaminated liquid or solid wastes are decontaminated before being disposed of or otherwise handled.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, storing of food, and applying cosmetics are not permitted in the work area.
6. Persons wash their hands after they handle biohazardous materials and animals and when they leave the laboratory.
7. All procedures must be carefully performed to minimize the creation of aerosols.
8. The wearing of laboratory coats, gowns, or uniforms is recommended.

B. Special Practices

1. Contaminated materials are to be decontaminated away from the laboratory and placed in a durable leak-proof container that is covered before being removed from the laboratory.
2. An insect and rodent control program is in effect.

C. Containment Equipment

Special containment equipment is generally not required for manipulations of agents assigned to Biosafety Level 1.

D. Laboratory Facilities

1. The laboratory should be designed so that it is easily cleaned.
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. If the laboratory has windows that open, they should be fitted with fly screens.
6. An autoclave for decontamination of infectious laboratory wastes should be available in the same building as the laboratory.

II. BIOSAFETY LEVEL 2

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:

Laboratory personnel have specific training in handling pathogenic agents and are directed by the principle investigator;

Access to the laboratory is limited when work is being conducted; and

Certain procedures in which biohazardous aerosols are created need to be conducted in biological safety cabinets or other physical containment equipment.

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

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted by the supervisor when work with biohazardous agents is in progress. Laboratory doors are kept closed when experiments are in progress.
2. Work surfaces are decontaminated at least once a day and after any spill of biohazardous material.
3. All contaminated liquid or solid waste is decontaminated before being disposed or otherwise handled.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators solely intended for this purpose. Food storage cabinets and refrigerators should be located outside the work area.
6. Persons wash their hands after handling biohazardous agents and animals, and when leaving the laboratory.
7. All procedures are performed carefully to minimize the creation of aerosols.
8. Laboratory coats, gowns, gloves, or uniforms must be worn in the laboratory. Laboratory clothing or gloves must not be worn in non-laboratory areas.
9. Serological procedures with inactivated antigens known or shown to be free of residual infectivity can be performed on the open bench.

B. Special Practices

1. Contaminated materials to be decontaminated away from the laboratory are placed in a durable, leak-proof and properly labeled container, which is closed before being removed from the laboratory.
2. Access to the laboratory is limited by the laboratory supervisor when experiments are being conducted. In general, 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 immunodeficient or immunosuppressed. The supervisor has the final responsibility for assessing

- each individual circumstance and determining who may enter or work in the area.
3. The laboratory supervisor will assure that only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., immunizations) enter the laboratory or animal rooms.
 4. When biohazardous materials or infected animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol is posted on all laboratory and animal room access doors and on such other items (i.e., equipment, containers, materials) as appropriate to indicate the presence of biohazardous agents. 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.).
 5. An insect and rodent control program is in effect.
 6. Animals not involved in the experiment being performed are not permitted in the laboratory.
 7. All wastes from laboratories and animal rooms must be appropriately decontaminated before being disposed.
 8. The use of hypodermic needles and syringes is restricted to gavage, parenteral injection, and aspiration of fluids from laboratory animals and diaphragm vaccine bottles. Hypodermic needles and syringes are not used as a substitute for automatic pipetting devices in the manipulation of biohazardous fluids. Serial dilutions of biohazardous agents should not be done in diaphragm bottles with needles and syringes because of the hazards of autoinoculation and of aerosol exposure. Cannulas should be used instead of sharp needles whenever possible.
 9. If activities of lesser biohazard potential are conducted in the laboratory concurrently with activities requiring Biosafety Level 2, all activities will be conducted at Biosafety Level 2.
 10. Gloves will be worn for all procedures requiring the handling of biohazardous materials or infected animals. If feasible, hold small laboratory mammals with restraint devices when they are receiving injections or otherwise being handled provides an additional level of protection for personnel.
 11. Serological procedures with inactivated antigens shown to be free of residual infectivity can be performed on the open bench.
 12. All spills, accidents, and overt or potential exposures to biohazardous materials must be immediately reported to the laboratory supervisor. A written record must be prepared and maintained. Appropriate medical evaluation, surveillance, and treatment must be provided.
 13. When appropriate, considering the agent(s) handled, baseline serum samples are collected from and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.
 14. A safety or operations manual identifying known and potential hazards and specifying practices and procedures to minimize or eliminate such risks should

be prepared or adopted. Personnel should be advised of special hazards and are required to follow standard practices and procedures.

C. Containment equipment

1. Biological safety cabinets (Class II) or other appropriate personal protective or physical containment devices are used whenever:
2. 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 animals intranasally, and harvesting infected tissues from animals or eggs.
3. High concentrations or large volumes of biohazardous agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

D. Laboratory facilities

1. The laboratory should be kept clean.
2. Bench tops should be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat. The use of plastic-backed absorbent toweling over work surfaces facilitates clean up and minimizes aerosols from spills.
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, preferably foot or elbow operated.
5. If the laboratory has windows that open, they should be fitted with fly screens.
6. An autoclave for decontamination of biohazardous laboratory wastes should be available in the same building with the laboratory.

III. BIOSAFETY LEVEL 3

Biosafety Level 3 is suitable for experiments involving agents of high potential risk to personnel and the environment. 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. Access to the laboratory is controlled by the supervisor. The laboratory has special engineering and design features and physical containment equipment and devices. All procedures involving the manipulation of biohazardous material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices.

The following standard and special practices apply to agents assigned to Biosafety Level 3:

A. Standard microbiological practices

1. Laboratory doors are kept closed when experiments are in progress.
2. Work surfaces are decontaminated at least once a day and after any spill of biohazardous material.
3. All contaminated liquid or solid wastes are decontaminated before being disposed of or otherwise handled.

4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.
6. Persons wash their hands when they leave the laboratory.
7. All procedures are conducted carefully to minimize the creation of aerosols.

B. Special practices

1. Access to the laboratory is controlled by the laboratory supervisor and is restricted to persons whose presence is required for program or support needs. 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 immunodeficient or immunosuppressed. The supervisor has the final responsibility for assessing each individual circumstance and determining who may enter or work in the area.
2. The laboratory supervisor will assure that only persons who have been advised of the potential biohazard, 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.
3. When biohazardous materials or infected animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol is posted on all laboratory and animal-room access doors and on such other items (*i.e.*, equipment, containers, materials) as appropriate to indicate the presence of biohazardous agents. The hazard warning sign should identify the agent, list the name of the laboratory supervisor or other responsible person(s), and indicate any special conditions of entry into the area (immunizations, respirators, *etc.*).
4. All activities 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.
5. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when an experiment is finished. The use of plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets facilitates clean-up following the completion of activities.
6. An insect and rodent control program is in effect.
7. Laboratory clothing that protects street clothing (*e.g.*, solid-front or wrap-around gowns, scrub suits, coveralls, *etc.*) is worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing is not worn outside the laboratory and is decontaminated before being laundered.
8. Gloves are worn when handling biohazardous materials or animals. Gloves should be removed aseptically and autoclaved with other laboratory wastes before being disposed of.
9. Molded surgical masks or respirators are worn in rooms containing infected animals.
10. Animals and plants not related to the experiment being conducted are not permitted in the laboratory.

11. All laboratory and animal room waste is decontaminated before being disposed of or reused.
12. Vacuum lines are protected with high-efficiency particulate air (HEPA) filters and liquid traps.
13. The use of hypodermic needles and syringes is restricted to gavage, parenteral injection, and aspiration of fluids from laboratory animals and diaphragm vaccine bottles. Hypodermic needles and syringes are not used as a substitute for automatic pipetting devices in the manipulation of biohazardous fluids. Serial dilutions of biohazardous agents should not be done in diaphragm bottles with needles and syringes because of the hazards of autoinoculation and of aerosol exposure. Cannulas should be used instead of sharp needles.
14. If activities of lesser biohazard potential are conducted in the laboratory concurrently with activities requiring Biosafety Level 3, all work will be conducted at Biosafety Level 3.
15. Serologic procedures with inactivated antigens shown to be free of residual infectivity can be performed on the open bench.
16. All spills, accidents, and overt or potential exposures to biohazardous materials must be immediately reported to the laboratory supervisor. A written report must be prepared and maintained. Appropriate medical evaluation, surveillance and treatment must be provided. Baseline serum samples should be collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.
17. A safety or operations manual which identifies known and potential hazards and which specifies practices and procedures to minimize or eliminate such risks should be prepared or adopted. Personnel should be advised of special hazards and must read and follow required practices and procedures.

C. Biosafety equipment

1. Biological safety cabinets (Class II or III) or other physical containment devices are used for all procedures and manipulations involving biohazardous material.
2. Activities requiring Biosafety Level 3 physical containment can be conducted in Biosafety Level 2 laboratories if:
 - a. All standard and special practices specified for the Biosafety Level 3 are followed, and
 - b. All operations and procedures are contained in Class III biological safety cabinets, and
 - c. Materials are removed from these cabinets only through an attached autoclave or in a non-breakable, sealed container that is passed through an attached disinfectant dunk tank or fumigation chamber.

D. Laboratory facilities

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Separation is provided by either a double-door change room and shower or an airlock or other access facility that requires passage through two sets of doors to enter the laboratory. Access to other laboratory area is designed to prevent entrance of free-living arthropods.

2. The surfaces of walls, floors and ceilings are water resistant and can be easily cleaned. Openings in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is of simple, sturdy construction.
5. A foot- or elbow-operated hand washing sink is provided near each laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. Access doors to the laboratory are self-closing and self- locking.
8. An autoclave for decontamination of laboratory wastes is available preferably within the laboratory. Biohazardous wastes which must be removed to another area in the same building for decontamination must be held and transported in a covered, leak proof container.
9. An exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The building exhaust system can be used for this purpose if the exhaust air is not recirculated to any other area of the building. Personnel must verify that proper directional airflow (into the laboratory) is achieved. However, air within the laboratory can be recirculated. The exhaust air from the laboratory is discharged directly to the outside or through the building exhaust system so that it is dispersed away from occupied buildings and air intakes. The exhaust air from the laboratory that does not come from the biological safety cabinet can be discharged to the outside without being treated.
10. In laboratories that have supply air systems, the supply air and exhaust air systems are interlocked to assure inward (or zero) airflow at all times.
11. The HEPA-filtered exhaust air from Class II 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 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.

USE OF ANIMALS

All animal protocols involving the use of rDNA; infectious or transmissible agents; human blood, body fluids or tissues; toxins; carcinogenic, mutagenic, teratogenic chemicals; or physically hazardous chemicals (reactive, explosives, etc.) must be submitted to the Purdue Animal Care and Use Committee (PACUC) for review and approval, call (765)-494-7206 or email from their website (<http://www.purdue.edu/Research/ORA/animals/contact.shtml>). PACUC has developed procedures that are designed to protect personnel and students that have contact with animals. These procedures should be consulted and followed when working with animals that may harbor biohazardous agents. Refer to the National Research Council Guide for the Care and Use of Laboratory Animals or view <http://www.aphis.usda.gov/> for further animal use information.

VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA

I. ANIMAL BIOSAFETY LEVEL 1

A. Standard practices

1. Doors to animal rooms are self-closing and are kept closed when experiments are in progress.
2. Work surfaces are decontaminated following use or spills of biohazardous materials.
3. Eating, drinking, smoking, and storing food are not permitted in animal rooms.
4. Personnel wash their hands after handling viable cultures and animals and before leaving the animal room.
5. All procedures are carefully conducted to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. Special practice

1. Bedding materials from cages used for animals infected with agents transmissible to humans are decontaminated (preferably by autoclaving) before being discarded.
2. Cages used for animals infected with agents transmissible to humans are washed and/or rinsed with water heated to at least 180°F for at least 20 minutes.
3. The wearing of laboratory coats, gloves, gowns or a uniform in the animal room is recommended. Coats and gloves worn in the animal room are not worn in the laboratory or in other animal housing areas.

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

4. It is recommended, but not required, that the animal facility be provided with inward directional airflow and that exhaust air be discharged to the outside without being recirculated to other rooms.

II. ANIMAL BIOSAFETY LEVEL 2

A. Standard practices

1. Doors to animal rooms are self-closing and are kept closed when infected animals are present.
2. Work surfaces are decontaminated after use or spills of biohazardous materials.
3. Eating, drinking, smoking, and storing of food for human use are not permitted in animal rooms.
4. Personnel wash their hands after handling cultures and animals and before leaving the animal room.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. Special practices

1. Cages are decontaminated, preferably by autoclaving, before being cleaned and washed.
2. Surgical-type masks are worn by all personnel entering animal rooms housing non-human primates.
3. Laboratory coats, gowns, or uniforms are worn while in the animal room. Protective clothing is not worn elsewhere.
4. Access to the animal room is restricted by the laboratory or animal facility supervisor to personnel who have been advised of the potential hazard and whose presence is required when experiments are 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. Persons at increased risk may include children, pregnant women, and individuals who are immunodeficient or immunosuppressed. The supervisor has the final responsibility for assessing individual circumstances and determining who may enter or work in the animal room.
5. The laboratory supervisor will assure that only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization, if available) enter the animal room.
6. Hazard warning signs incorporating the universal biohazard warning symbol are posted on access doors to animal rooms when materials containing or animals infected with agents assigned to Biosafety Level 2 or higher are present. The hazard warning sign should identify the agent(s) in use, list the name of the animal room supervisor or other responsible person(s) and indicate any special conditions of entry into the animal room (e.g., immunizations, respirators).
7. Special care is taken to avoid contaminating skin with biohazardous material; gloves are worn when handling infected animals and when skin contact with biohazardous materials is unavoidable.
8. All wastes from the animal room are appropriately decontaminated (preferably by autoclaving) before being disposed of. Infected animal carcasses are

incinerated after being transported from the animal room in leak-proof, sealed containers.

9. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. 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 biohazardous materials. Needles are not bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe are promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.
10. If floor drains are provided, the drain traps are always filled with water.
11. When appropriate, considering the agents handled, baseline serum samples from animal-care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or the function of the facility.

C. Containment equipment

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

D. Animal facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A hand washing sink is available in the room where infected animals are housed.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended, but not required, that the direction of airflow in the animal facility be inward and that exhaust air be discharged to the outside without being recirculated to other rooms.
5. An autoclave to decontaminate biohazardous laboratory waste is available in the same building that contains the animal facility.

III. ANIMAL BIOSAFETY LEVEL 3

A. Standard practices

1. Doors to animal rooms are self-closing and self locking and are kept closed when work with infected animals is in progress.
2. Work surfaces are decontaminated at least once a day or after spills of biohazardous materials.
3. Eating, drinking, smoking, and the storing of food for human use are not permitted in the animal room.
4. Personnel wash their hands after handling cultures or animals and before leaving the laboratory.
5. All procedures are carefully performed to minimize the creation of aerosols.

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

B. Special practices

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
2. NIOSH approved respiratory protection devices are worn by personnel entering rooms that house animals infected with agents assigned to Biosafety Level 3. Personnel using respirators must comply with the provisions of the Purdue University Respiratory Protection Program.
3. Wrap-around or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns must remain in the animal room and must be decontaminated before being laundered.
4. The supervisor or other responsible person limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program service purposes when infected animals are present. 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.
5. The laboratory supervisor or other responsible person will assure that only persons who have been advised of the potential hazard and meet any specific requirements (*e.g.*, immunization, if available) may enter the animal room.
6. Hazard warning signs incorporating the universal biohazard warning symbol are posted on access doors to animal rooms containing animals infected with or materials containing agents assigned to Biosafety Level 3. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (*e.g.*, the need for immunizations or respirators).
7. Personnel wear gloves when handling infected animals or biohazardous agents. Gloves are removed aseptically and autoclaved with other animal room waste before being disposed of or reused.
8. All wastes from the animal room are autoclaved before being disposed. All animal carcasses are incinerated. Carcasses are transported from the animal room to the incinerator in leak-proof, sealed containers.
9. Hypodermic needles and syringes are used only for gavage or parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (*i.e.*, the needle is integral to the syringe) are used. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused. When possible, cannulas should be used instead of sharp needles (*e.g.*, gavage).
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.

11. If vacuum lines are provided, they are protected with HEPA filters and liquid traps.
12. Boots, shoe covers, or other protective footwear, and disinfectant footbaths are available and used when indicated.

C. Containment equipment

1. Personal protective clothing and equipment and/or other physical containment devices are used for all procedures and manipulations of biohazardous materials or infected animals.
2. Animals are housed in partial-containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar-flow cabinets), solid wall and bottom cages covered by filter bonnets or other equivalent primary containment systems.

D. Animal facilities

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 from other activities may also be provided by a double-door change 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 provided near each animal-room exit door.
4. Windows in the animal room are closed and sealed.
5. Animal room doors are self-closing and self-locking and are kept closed when infected animals are present.
6. An autoclave for decontaminating wastes is available, preferably within the animal facility. Materials to be autoclaved outside the animal room are transported in a covered, leak-proof container.
7. An exhaust-air ventilation system is provided. The system creates inward directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow is proper (*i.e.*, into the animal room). The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.
8. The HEPA-filtered exhaust air from Class II 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

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.

BLOODBORNE PATHOGENS PROGRAM

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 that may result from the performance of an employee's duties.

As defined in the standard, blood means human blood, human blood components, and products made from human blood. Other potentially infectious materials means the following human body fluids: semen, cell lines, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; any unfixed tissue or organ.

The full text of blood pathogens standard and the University's compliance program are too lengthy to detail here. Departments with occupational exposure to blood or other potentially infectious materials can review the Purdue University Bloodborne Pathogen Exposure Control Plan at <http://www.purdue.edu/rem/home/booklets/bpecp.pdf>.

DECONTAMINATION AND DISPOSAL

1. All biohazardous materials and all contaminated equipment or apparatus should be sterilized before being washed and stored or discarded. Autoclaving is the preferred method. Each individual working with biohazardous material should be responsible for its sterilization before disposal.
2. Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.
3. To minimize hazard to emergency response personnel, all biohazardous materials should be placed in an appropriately marked refrigerator or incubator, sterilized, or otherwise confined at the close of each workday.
4. 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.
5. 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 = MAY PRODUCE AN EXPLOSION

6. All laboratory rooms containing biohazardous materials should designate two separate areas or containers labeled:

BIOHAZARDOUS - TO BE AUTOCLAVED

Or

NON-INFECTIOUS - TO BE CLEANED

7. 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.

8. Floor drains should be flooded with water or disinfectant at least once each week to prevent dry traps and the release of sewer gases.
9. Floors should be swept with push brooms only. The use of 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.
10. Stock solutions of suitable disinfectants should be maintained in each laboratory for disinfection purposes.

STERILIZATION PROCEDURES

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 conditions to achieve sterility will vary in relation to the volume of material treated, its contamination level, the moisture content, and other factors.

1. Steam Autoclave
 - a. Laundry: 121C (250F) for 30 minutes with 15 minutes prevacuum of 27 inches Hg.
 - b. Trash: 121C (250F) for 1 hour with 15 minutes prevacuum of 27 inches Hg.
 - c. Glassware: 121C (250F) for 1 hour with 15 minutes prevacuum of 27 inches Hg.
 - d. Liquids: 121C (250F) for 1 hour for each gallon.
 - e. Animals: 121C (250F) for 8 hours with 15 minutes prevacuum of 27 inches Hg.
 - f. Bedding: 121C (250F) for 8 hours with 15 minutes prevacuum of 27 inches Hg.
2. Gas Sterilants
 - a. Ethylene oxide gas- Sixteen-hour exposure to a concentration of 750 mg/liter at 30 to 60% relative humidity and at ambient temperatures (>70F).
 - b. Paraformaldehyde- Sixteen-hour exposure to a concentration of 1.0 mg/liter at 40 to 60% relative humidity and at ambient temperatures (>70 F).
3. Disinfectants
 - a. *Mercurials* - Not recommended for general use; they have poor activity against vegetative bacteria and are useless as sporicides. Although the mercurials exhibit good activity against viruses (1:500 to 1:1000 concentration), they are toxic and therefore are not recommended.
 - b. *Quaternary Ammonium Compounds* - These are acceptable as general use disinfectants to control vegetative bacteria and non-lipid-containing viruses. However, they are not active against bacterial spores at the usual concentrations (1:750).
 - c. *Phenolic Compounds* - These are recommended for the killing of 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.
 - d. *Chlorine Compounds* - These are recommended for certain disinfecting procedures provided the available chlorine needed is considered. Low concentrations of available chlorine (50-500 ppm) are active against vegetative bacteria and most

viruses. For bacterial spores, concentrations of approximately 2500 ppm are needed. The corrosive nature of these compounds, their decay rates and lack of residuals is such that they are recommended only in special situations.

- e. *Iodophors* - Although these show poor activity against bacterial spores, they are recommended for general use (75-150 ppm). They are effective against vegetative bacteria and viruses. Their advantages are:
 - i. Iodophors possess a wide spectrum of anti-microbial and antiviral activity.
 - ii. Iodophors have a built-in indicator. If the solution is brown or yellow, it is still active.
 - iii. Iodophors are relatively harmless to man.
 - iv. Iodophors can be readily inactivated and iodophor stains can be readily removed with solutions of $\text{Na}_2\text{S}_2\text{O}_3$ (sodium thiosulfate).
- f. *Alcohols* - In concentrations of 70 to 95%, alcoholic solutions are good general-use disinfectants but they exhibit no activity against bacterial spores.
- g. *Formaldehyde Solutions* - At concentrations of 8%, formalin exhibits good activity against vegetative bacteria, spores, and viruses.
- h. *Activated Gluteraldehyde* - Two percent solutions exhibit good activity against vegetative bacteria, spores, and viruses. Its use, however, must be limited and controlled because of toxic properties and damage to eyes.
- i. *Formaldehyde-Alcohol* - Solutions of 8% formalin in 70% alcohol are considered very good for disinfection purposes because of their effectiveness against vegetative bacteria, spores, and viruses. For many applications this is the disinfectant of choice.

DISPOSAL PROCEDURES

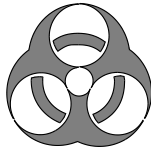
Biohazardous waste disposal must be handled in accordance with procedures established by REM. Contact REM (765-494-0238) for specific information on disposal procedures. These procedures include Universal Precautions, sterilization and disinfection, containment, storage, training, and record keeping. See appendix B for a copy of the REM biohazardous waste pick-up form. Call REM at 49-40121 to schedule waste pickup.

SHARPS HANDLING PROCEDURES

Sharps are items that are capable of puncturing, cutting, or abrading the skin, i.e., broken plastic or broken glassware, glass or plastic pipettes, scalpels, razor blades, needles, hypodermic needles, etc...

1. Do not place any sharps into the regular trash.
2. Needles and razor blades must be disposed of in puncture proof plastic containers.
3. Clean broken glass should be collected in a cardboard box or other strong, secure disposable container. When you want the box removed, tape it shut and label it "SHARP OBJECTS/GLASS - DISCARD". It is prudent to affix a "safe for disposal" sticker to the box as well.

4. Sharps and/or materials contaminated with human blood or blood products, or with any agent capable of being infectious to humans, must be treated and disposed of as “category 1” infectious waste. This includes:
 - Proper processing (disinfection and disposal) in accordance with the procedures issued by the REM. Note: Disinfection is accomplished by either chemical means (bleach) or by autoclave.
 - Storage in a secure area that restricts access to the general public and is protected from the environment and vermin.
 - Placement in leak proof, rigid, puncture-resistant containers that are tightly sealed to prevent expulsion.
 - Labeling with the biohazard symbol.

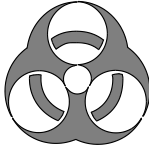


“Category 2” items have the general appearance of infectious or medical waste, but do not otherwise fit the category 1 description. These are also known as look-alike infectious waste, and will be removed by REM personnel along with the infectious waste.

5. Chemically contaminated sharps must be decontaminated with a suitable cleaning agent.

If You Are Injured From a Sharp

1. Wash the area with soap and water
2. Report to PUSH Urgent Care for medical care
3. Contact your supervisor
4. Supervisor-contact REM, 41496

CLEAN AND CONTAMINATED SHARPS			
Uncontaminated Clean Sharps	Biological Contamination	Chemical Contamination	Radioactive Contamination
STEP 1			
Deposit in rigid , puncture-proof container	Deposit in Sharps container with biological symbol 	Decontaminate with cleaning agent. Grossly contaminated sharps should be disposed of through REM's Hazardous Materials Management section	Deposit in rigid puncture-proof container.
STEP 2			
Label container "Sharps"	Complete "Bio- Material Pickup & Treatment Certification" form. Indicate waste category 1 or 2.	Deposit in rigid puncture proof container.	Label container "Sharps" and affix "Radioactive" label.
STEP 3			
Discard in household or solid waste trash container.	Dispose via REM's Hazardous Materials Management Program. Call 40121 to schedule a pickup.	Label container "Sharps" and discard in household or solid waste container	Dispose of material via REM's Radiological Waste Management Program

<u>INFECTIOUS & “LOOK-ALIKE” WASTES</u>	
Infectious Biological Waste	“Look-Alike” Biological Waste
STEP 1	
Deposit in RED bag or other receptacle displaying the biological symbol.	Deposit in bag or other appropriate container.
STEP 2	
Complete “Bio-Material Pickup & Treatment Certification” form. Indicate waste category 1.	Complete “Bio-Material Pickup & Treatment Certification” form. Indicate waste category 2.
STEP 3	
Dispose via REM’s Hazardous Materials Management Program. Call 40121 to schedule a pickup.	Dispose via REM’s Hazardous Materials Management Program. Call 40121 to schedule a pickup.

SPILLS OF BIOHAZARDOUS MATERIALS PROCEDURES

Plan in advance for an emergency. For example, what supplies and equipment should you maintain in your area to assist you in the event of an accidental spill, e.g., personal protective equipment, disinfecting solutions, spill control materials? What training do you need to handle an emergency in your area? What information can be made available to an emergency response team?

A minimally biohazardous material that is 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 of biohazardous material with the generation of aerosols will require cleanup personnel wearing protective clothing and respiratory protection. With *M. 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*. Therefore, if the agent is known, the recommended procedure and protective equipment should be used.

AGENTS REQUIRING BIOSAFETY LEVEL 2 OR HIGHER:

1. Evacuate the room immediately, close doors, remove all contaminated clothing, and decontaminate body surfaces.
2. Allow enough time (at least 30 minutes) for droplets to settle and aerosols to be reduced by the ventilation system before entering.
3. Don protective clothing and approved respiratory protective equipment.
4. Decontaminate the spill with an appropriate disinfectant (e.g., 1:10 solution of household bleach in water).
5. Decontaminate and dispose of contaminated items.
6. Following cleanup, responders should wash or shower with a germicidal soap.

Note: REM should be consulted before cleanup is started to ensure that proper techniques will be employed.

BIOHAZARDOUS MATERIAL SPILLS WITHIN A BIOLOGICAL SAFETY CABINET

1. Initiate cleanup at once, while the cabinet continues to operate, using an appropriate disinfectant. Avoid the use of organic solvents (alcohols).
2. Prevent the generation and escape of aerosols and contaminants from the cabinet during decontamination.
3. Formaldehyde gas decontamination can be used for final decontamination (arranged by REM)

APPENDIX A

PERSONAL PROTECTIVE EQUIPMENT (PPE) POLICY & HAZARD ASSESSMENT

Reference: Personal Protective Equipment (PPE) Policy
(<http://www.purdue.edu/rem/home/booklets/PPEPolicy.pdf>)

The Personal Protective Equipment (PPE) policy implements the requirements of OSHA regulations 29 CFR 1910.132 thru 29 CFR 1910.140. The policy applies to the use of PPE in **all** laboratories at the West Lafayette Campus, regional Campuses, University Research Farms and Agricultural Centers, related facilities and operations. Purdue University policy is that personal protective equipment be provided, used, and maintained in a sanitary and reliable condition. PPE such as protective clothing, respiratory devices (respirators), shields, and barriers shall be used to protect against chemical, radiological, biological, or mechanical hazards and irritants capable of causing injury or impairment through absorption, inhalation, or physical contact.

Hazard assessment is the process (required by law) of identifying the hazards associated with a defined task and prescribing personal protective equipment along with other relevant protection measures which must be employed to reduce the risk from the hazards. The supervisor shall assess each work assignment to determine if hazards are present or likely to be present and require the use of personal protective equipment. The PPE policy contains appendices with tables, required forms, and the Guidelines For Hazard Assessment and Personal Protective Equipment Selection to help with the hazard assessment process.

Training Requirements:

- The supervisor shall provide adequate training to each employee who is required to use PPE as per the PPE Policy.

Documentation Requirements:

- Certification of Hazard Assessment - (PPE Policy, *Appendix A1 or A2 or A3 [depending on situation]*, pages 7-9).
- Appendix A1 (Single Task)
<http://www.purdue.edu/rem/home/forms/CertST.pdf>
- Appendix A2 (Position/Title)
<http://www.purdue.edu/rem/home/forms/CertPT.pdf>
- Appendix A3 (Location) - **best suited to most laboratory environments**
<http://www.purdue.edu/rem/home/forms/CertL.pdf>
- Hazard Assessment Template
<http://www.purdue.edu/rem/home/forms/HazAsTmp.doc>
- Personal Protective Equipment Certification of Training (or PPE Policy, *Appendix B*, page 10) <http://www.purdue.edu/rem/home/forms/CertT.pdf>

Posting Requirements:

- Certification of Hazard Assessment - (PPE Policy, *Appendix A1 or A2 or A3 [depending on situation]*, pages 7-9).

APPENDIX B

PURDUE UNIVERSITY BIOMATERIALS PICKUP & TREATMENT CERTIFICATION

Contact REM at 40121 for Removal

Completed, signed certification page(s) must be attached to or near items for pickup.

Requesting Person's Name: _____
Building/Room: _____
Phone: _____
Other Contact Person: _____

REM Use Only	
Date:	_____
Time:	_____

TREATMENT CERTIFICATION

Building: _____ Room: _____ Phone: _____ Date: ____ / ____ / ____

Name of Principal Investigator: _____

Waste Categories:

1. Category 1, Biological Waste: (Known, assumed or suspected of being infectious to humans before treatment, includes **all** items containing or contaminated with human blood or blood products.)

Describe composition of waste material: _____

Number of Bags: _____ Number of Boxes: _____

Treatment Method: _____

2. Category 2, "Look-Alike" Wastes: (Animal tissue, fluids, cell cultures, petri dishes **NOT** fitting category 1 description.)

Describe composition of waste material: _____

Number of Bags: _____ Number of Boxes: _____

Treatment Method (None Required): _____

Signed: _____

Date picked up: _____ (REM use only)

APPENDIX C

BIOLOGICAL SAFETY CABINETS

Biological Safety Cabinets (BSCs) are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with biohazardous agents. There are three classifications of BSCs, each designed for specific applications.

A **Class I BSC** provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. Unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum of 75 linear feet per minute is maintained through the front opening. Not to be used with volatile chemicals.

A **Class II BSC**, when used in conjunction with good microbiological techniques, provides an effective partial containment system for safe manipulation of moderate and high-risk microorganisms (*i.e.*, Biosafety Level 2 and 3 agents). Class II BSCs have an inward face velocity (75 linear feet per minute) and provide containment in protecting the laboratory worker and the immediate laboratory environment from biohazardous aerosols generated within the cabinet. The Class II vertical laminar flow BSC is an open-fronted, ventilated cabinet with an average inward face velocity at the work opening of at least 75 feet per minute. This cabinet provides a HEPA-filtered, recirculated mass airflow within the workspace. The exhaust air from the cabinet is also filtered by HEPA filters. Design, construction, and performance standards for Class II cabinets have been developed by and are available from the National Sanitation Foundation (<http://www.nsf.org/>).

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the biological safety cabinets. Of particular note are those activities that may disrupt the inward directional airflow through the work opening of Class II cabinets. Repeated insertion and withdrawal of the worker's arms in and from the work chamber, opening and closing doors to the laboratory or isolation cubicle, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is used are demonstrated causes of the escape of aerosolized particles from within the cabinet. Strict adherence to recommended practices for the use of BSCs is as important in attaining the maximum containment capability of the equipment, as is the mechanical performance of the equipment itself.

The use of a Class II cabinet in the microbiology laboratory offers the additional capability and advantage of protecting material contained within it from extraneous airborne contaminants. This capability is provided by the HEPA-filtered, recirculated mass airflow within the workspace.

It is imperative that Class II BSCs are tested and certified *in situ* at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supercede the critical certification prior to use in the laboratory.

A **Class III BSC** is a totally enclosed ventilated cabinet of gas-tight construction. Operations within the Class III are conducted through attached rubber gloves. When in use, the Class III cabinet is maintained under negative air pressure of at least 0.5 inches water gauge. Supply air is drawn into the cabinet through HEPA filters. The cabinet exhaust air is filtered by two HEPA filters, installed in series, before discharge outside of the facility. The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility's ventilation system.

APPENDIX C

The Class III cabinet provides the highest level of personnel and product protection. This protection is provided by the physical isolation of the space in which the biohazardous agent is maintained. When these cabinets are required, all procedures involving biohazardous agents are contained within them. Several Class III cabinets are therefore typically set up as an interconnected system. All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet 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 a personal suit area and a Class II cabinet. This is one in which the laboratory worker is protected from a potentially contaminated environment by a one-piece positive pressure suit ventilated by a life-support system. This 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.

Note: Horizontal laminar flow "clean benches" are present in a number of clinical, pharmacy, and laboratory facilities. These "clean benches" 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 the handling of toxic, biohazardous, or sensitizing materials.

The National Sanitation Foundation (NSF) monitors and test designs, construction, and performance standards for vertical laminar flow biological safety cabinets. NSF publishes listings of these devices that meet their reference standards. Utilization of this standard and list should be the first step in selection and procurement of a biological safety cabinet (<http://www.nsf.org/>).

APPENDIX D

WORKING SAFELY IN A BIOLOGICAL SAFETY CABINET

The Biological Safety Cabinet (BSC) is a special and unique piece of laboratory equipment. Depending on the class, a BSC is used for working with low-, moderate-, or high-risk biological agents and materials. Cabinets are designed for personnel and environmental protection, and product protection.

The Class II cabinet is equipped with HEPA (high efficiency particulate air) filtration for laminar flow and/or exhaust air. It is the HEPA filtration system(s) that distinguishes the BSC from the common chemical fume hood. The Class II BSC provides protection to those using the cabinet, the biologicals within, and the laboratory itself. It is designed for use with low- or moderate-risk agents.

The Class III BSC, commonly referred to as a glove box, is a self-contained gas-tight enclosure that provides a complete and total physical barrier between the worker and the materials within. It is designed for use with high-risk biologicals. It gives ultra protection to personnel from highly biohazardous agents contained within, protects the agents from contamination, and protects the laboratory environment from exposure to these agents.

TRAINING

Training on the proper work practices and procedures for working with Biological Safety Cabinets is available by contacting REM at 4-7968 or 4-1496.

ULTRAVIOLET LAMPS

Ultraviolet (UV) lamps are not required in biosafety cabinets. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer.

BIO SAFETY CABINET HEPA FILTERS

HEPA filters, whether part of a building exhaust system or part of a cabinet, will require replacement when they become so loaded that sufficient air flow can no longer be maintained. BSC flow rates are monitored and certified annually by a REM approved outside vender. Filters with insufficient flow must be decontaminated before removal. Call REM at 4-7968 to arrange for services from a vender.

APPENDIX D

ADDITIONAL INFORMATION

1. Cabinet selection should be determined by assessing the risk to personnel, product, and the environment.
2. The filters do not provide protection against gases and vapors. The HEPA filters provide protection against particulate agents and materials.
3. BSC should be certified after installation, but before being used; whenever it is moved; and at least annually thereafter. Call REM at 4-7968 to schedule repairs and certifications of BSCs. Before a BSC is relocated, a risk assessment that considers the materials used in the BSC must be done to determine the need for decontamination. Contact Robert Golden, Biological Safety Officer, at 4-1496 for help in determining the type of decontamination required.
4. Do not work with biohazardous agents if cuts, sores, or abrasions are present on hands.
5. Wash hands with a good germicidal soap and water before and immediately after handling biologicals.
6. Wear protective gloves when handling biohazardous agents; preferably "double-gloved."
7. Carefully remove and change gloves if they become contaminated during work activities. (Hands should be washed immediately.)
8. Wear a lab coat or disposable gown while working with biohazardous agents. Lab coats should be worn buttoned over street clothing. Do not wear potentially contaminated clothing outside the laboratory.
9. Cabinet blowers should be operated at least 3 to 5 minutes before beginning work to allow the BSC to remove any particulates in the cabinet.
10. Before beginning work, adjust the stool height so that the worker's face is above the front opening of the BSC.
11. Place necessary materials in the BSC before beginning work to minimize the number of arm movement disruptions to the air barrier of the cabinet.
12. When working in a BSC, move arms and hands slowly in the cabinet, wait a few seconds for air currents to settle, and then begin to work.
13. Move arms in and out slowly, perpendicular to the face opening of the cabinet, to reduce the risk of compromising the partial barrier containment provided by the BSC.
14. When working in a BSC, the worker should work with arms raised slightly above the front grille instead of resting flatly on it. This alleviates the problem of room air flowing directly into the work area rather than being drawn into the BSC through the front grille.
15. Do not place any object directly on the grillwork at the opening of or at the back of the cabinet. This greatly interferes with the laminar airflow curtain.
16. All work should be performed at least 4 inches from the front grille on the work surface.
17. Decontaminate work surfaces with an appropriate disinfectant before you begin work, when work is completed, and after a spill.
18. Perform all procedures carefully and in a neat and orderly manner.
19. Avoid the generation of aerosols, droplets, splashes, and spills.

APPENDIX D

20. Keep an appropriate disinfectant agent within the cabinet for decontamination purposes.
21. Keep the number of items in a BSC to an absolute minimum. Only the materials and equipment required for the immediate work should be placed inside the BSC.
22. 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.
23. Dispose of used needles, or other sharps in a puncture-resistant biohazardous waste container.
24. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent in and out movement of the arms to place objects in the container is disruptive to the cabinet air barrier and can disrupt personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used inside the BSC.
25. Label all materials. All abbreviations or acronyms should be listed on the acronyms key posted near the inside of the entrance to the room.
26. Use mechanical pipetting devices. Do not pipette using mouth suction.
27. Do not eat, drink, smoke, or apply cosmetics in the laboratory.
28. Decontaminate or, where appropriate, autoclave all disposable materials and wastes before removal from the laboratory.
29. Do not tape the autoclave biohazard collection bag to the outside of the BSC. The frequent in and out movement of the arms to place objects in the bag is disruptive to the cabinet air barrier and can disrupt personnel and product protection.
30. All non-disposable items, including containers and equipment, that have been used should be cleaned and decontaminated before removing them from the BSC, and then autoclaved when appropriate.
31. Only when absolutely necessary, touch-plate micro burners equipped with a pilot light to provide a flame on demand may be used. An open flame in a BSC creates turbulence that disrupts the pattern of air supplied to the work surface. This type of burner minimizes the internal cabinet air disturbance and heat buildup.

APPENDIX E

BIOLOGICAL AGENT RISK CLASSIFICATION

Class 1 (NIH Risk Group One)

Agents of no or minimal hazard under ordinary conditions or handling.

Class 2 (NIH Risk Group Two)

Agents of ordinary potential hazard. This class includes agents which 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 (NIH Risk Group Three)

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

Class 4 (NIH Risk Group Four)

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

NOTE: The following list of biological agents is taken from the Guidelines for Research Involving Recombinant DNA Molecules, April 2002. The complete NIH guideline can be viewed at <http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>.

APPENDIX E

NIH APPENDIX B **CLASSIFICATION OF HUMAN ETIOLOGICAL AGENTS ON THE BASIS OF** **HAZARD**

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (see Sections V-C, V-D, V-E, and V-F, Footnotes and References of Sections I through IV. Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the NIH Guidelines.

Appendix B - Table 1 **Basis for the Classification of Biohazardous Agents by Risk Group (RG)**

Risk Group 1 (RG1)

Agents that are not associated with disease in healthy adult humans

Risk Group 2 (RG2)

Agents that are associated with human disease, which is rarely serious, and for which preventive or therapeutic interventions are often available

Risk Group 3 (RG3)

Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)

Risk Group 4 (RG4)

Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

APPENDIX E

Appendix B-I Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions), *Escherichia coli*-K12 (see Appendix C-II-A, *Escherichia coli*-K-12 Host-Vector Systems, Exceptions), adeno-associated virus (AAV) types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.

Those agents not listed in Risk Groups (RGs) 2, 3, and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Appendix B-II Risk Group 2 (RG2) Agents

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

Appendix B-II-A Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

- *Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
- *Actinobacillus*
- *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- *Aeromonas hydrophila*
- *Amycolata autotrophica*
- *Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- *Arizona hinshawii* - all serotypes
- *Bacillus anthracis*
- *Bartonella henselae*, *B. quintana*, *B. vinsonii*
- *Bordetella* including *B. pertussis*
- *Borrelia recurrentis*, *B. burgdorferi*
- *Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3)
- *Campylobacter coli*, *C. fetus*, *C. jejuni*
- *Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- *Clostridium botulinum*, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. histolyticum*, *Cl. novyi*, *Cl. septicum*, *Cl. tetani*
- *Corynebacterium diphtheriae*, *C. pseudotuberculosis*, *C. renale*
- *Dermatophilus congolensis*

APPENDIX E

- *Edwardsiella tarda*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- *Haemophilus ducreyi*, *H. influenzae*
- *Helicobacter pylori*
- *Klebsiella* - all species except *K. oxytoca* (RG1)
- *Legionella* including *L. pneumophila*
- *Leptospira interrogans* - all serotypes
- *Listeria*
- *Moraxella*
- *Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum*, *M. bovis* BCG vaccine strain, *M. chelonae*, *M. fortuitum*, *M. kansasii*, *M. leprae*, *M. malmoense*, *M. marinum*, *M. paratuberculosis*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, *M. xenopi*
- *Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- *Neisseria gonorrhoeae*, *N. meningitidis*
- *Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- *Rhodococcus equi*
- *Salmonella* including *S. arizonae*, *S. choleraesuis*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. meleagridis*, *S. paratyphi*, A, B, C, *S. typhi*, *S. typhimurium*
- *Shigella* including *S. boydii*, *S. dysenteriae*, type 1, *S. flexneri*, *S. sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus aureus*
- *Streptobacillus moniliformis*
- *Streptococcus* including *S. pneumoniae*, *S. pyogenes*
- *Treponema pallidum*, *T. carateum*
- *Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
- *Yersinia enterocolitica*

Appendix B-II-B Risk Group 2 (RG2) - Fungal Agents

- *Blastomyces dermatitidis*
- *Cladosporium bantianum*, *C. (Xylohypha) trichoides*
- *Cryptococcus neoformans*
- *Dactylaria galopava* (*Ochroconis gallopavum*)
- *Epidermophyton*
- *Exophiala (Wangiella) dermatitidis*
- *Fonsecaea pedrosoi*

APPENDIX E

- Microsporium
- Paracoccidioides braziliensis
- Penicillium marneffeii
- Sporothrix schenckii
- Trichophyton

Appendix B-II-C Risk Group 2 (RG2) - Parasitic Agents

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

APPENDIX E

- Trypanosoma including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. cruzi*
- *Wuchereria bancrofti* filaria worms

Appendix B-II-D Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses

- Eastern equine encephalomyelitis virus
- Venezuelan equine encephalomyelitis vaccine strain TC-83
- Western equine encephalomyelitis virus

Arenaviruses

- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Bunyaviruses

- Bunyamwera virus
- Rift Valley fever virus vaccine strain MP-12
- Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Calciviruses

Coronaviruses

Flaviviruses (Togaviruses) - Group B Arboviruses

- Dengue virus serotypes 1, 2, 3, and 4
- Yellow fever virus vaccine strain 17D
- Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except *Herpesvirus simiae* (Monkey B virus) (see Appendix B-IV-D, Risk Group 4 (RG4) -Viral Agents)

- Cytomegalovirus
- Epstein Barr virus
- Herpes simplex types 1 and 2
- Herpes zoster
- Human herpesvirus types 6 and 7

APPENDIX E

Orthomyxoviruses

- Influenza viruses types A, B, and C
- Other tick-borne orthomyxoviruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Papovaviruses

- All human papilloma viruses

Paramyxoviruses

- Newcastle disease virus
- Measles virus
- Mumps virus
- Parainfluenza viruses types 1, 2, 3, and 4
- Respiratory syncytial virus

Parvoviruses

- Human parvovirus (B19)

Picornaviruses

- Coxsackie viruses types A and B
- Echoviruses - all types
- Polioviruses - all types, wild and attenuated
- Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, Risk Group 3 (RG3) - Viruses and Prions) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, Footnotes and References of Sections I through IV)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses

- Rabies virus - all strains
- Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)

- Rubivirus (rubella)

Appendix B-III Risk Group 3 (RG3) Agents

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

APPENDIX E

Appendix B-III-A Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

- Bartonella
- Brucella including *B. abortus*, *B. canis*, *B. suis*
- Burkholderia (*Pseudomonas*) *mallei*, *B. pseudomallei*
- Coxiella burnetii
- Francisella tularensis
- Mycobacterium bovis (except BCG strain, see Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia), *M. tuberculosis*
- Pasteurella multocida type B -"buffalo" and other virulent strains
- Rickettsia akari, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R. siberica*, *R. tsutsugamushi*, *R. typhi* (*R. mooseri*)
- Yersinia pestis

Appendix B-III-B Risk Group 3 (RG3) - Fungal Agents

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

Appendix B-III-C Risk Group 3 (RG3) - Parasitic Agents

None

Appendix B-III-D Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses

- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))
- Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Arenaviruses

- Flexal
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

APPENDIX E

- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Flaviviruses (Togaviruses) - Group B Arboviruses

- Japanese encephalitis virus
- Yellow fever virus
- Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Poxviruses

- Monkeypox virus

Prions

- Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, Footnotes and References of Sections I through IV, for containment instruction)

Retroviruses

- Human immunodeficiency virus (HIV) types 1 and 2
- Human T cell lymphotropic virus (HTLV) types 1 and 2
- Simian immunodeficiency virus (SIV)

Rhabdoviruses

- Vesicular stomatitis virus

Appendix B-IV Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Appendix B-IV-A Risk Group 4 (RG4) - Bacterial Agents

None

Appendix B-IV-B Risk Group 4 (RG4) - Fungal Agents

None

APPENDIX E

Appendix B-IV-C Risk Group 4 (RG4) - Parasitic Agents

None

Appendix B-IV-D Risk Group 4 (RG4) - Viral Agents

Arenaviruses

- Guaranito virus
- Lassa virus
- Junin virus
- Machupo virus
- Sabia

Bunyaviruses (Nairovirus)

- Crimean-Congo hemorrhagic fever virus

Filoviruses

- Ebola virus
- Marburg virus

Flaviruses (Togaviruses) - Group B Arboviruses

- Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)

- Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses

- Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

Appendix B-V Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work. A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

APPENDIX E

Herpesviruses

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

Papovaviruses

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

Retroviruses

- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

Appendix B-V-1 Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

APPENDIX E

SELECT AGENTS

Requirements for Facilities Transferring or Receiving Select Agents

The Public Health Security and Bioterrorism Preparedness Act was passed on June 12, 2002. This Act is a joint venture between the Health and Human Services dept. and the United States Department of Agriculture. This Act increased the Select Agent list to over 80 bio agents. The Center for Disease Control and Animal and Plant Health Inspection Service are the enforcement agencies (<http://www.cdc.gov/od/sap/>). This law is concerned with the possession, security, transfer, or receiving of the following select infectious agents and toxins:

HHS NON-OVERLAP SELECT AGENTS AND TOXINS

- Crimean-Congo haemorrhagic fever virus
- *Coccidioides posadasii*
- Ebola viruses
- Cercopithecine herpesvirus 1 (Herpes B virus)
- Lassa fever virus
- Marburg virus
- Monkeypox virus
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*

South American haemorrhagic fever viruses

- Junin
- Machupo
- Sabia
- Flexal
- Guanarito

Tick-borne encephalitis complex (flavi) viruses

- Central European tick-borne encephalitis
- Far Eastern tick-borne encephalitis
- Russian spring and summer encephalitis
- Kyasanur forest disease
- Omsk hemorrhagic fever
- Variola major virus (Smallpox virus)

APPENDIX E

- Variola minor virus (Alastrim)
- *Yersinia pestis*
- Abrin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Shiga-like ribosome inactivating proteins
- Tetrodotoxin

HIGH CONSEQUENCE LIVESTOCK PATHOGENS AND TOXINS/ SELECT AGENTS (OVERLAP AGENTS)

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei* (formerly *Pseudomonas mallei*)
- *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)
- Botulinum neurotoxin producing species of *Clostridium*
- *Coccidioides immitis*
- *Coxiella burnetii*
- Eastern equine encephalitis virus
- Hendra virus
- *Francisella tularensis*
- Nipah Virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus
- Botulinum neurotoxin
- *Clostridium perfringens* epsilon toxin
- Shigatoxin
- Staphylococcal enterotoxin
- T-2 toxin

USDA HIGH CONSEQUENCE LIVESTOCK PATHOGENS AND TOXINS (NON-OVERLAP AGENTS AND TOXINS)

- Akabane virus
- African swine fever virus
- African horse sickness virus
- Avian influenza virus (highly pathogenic)

APPENDIX E

- Blue tongue virus (Exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- *Cowdria ruminantium* (Heartwater)
- Foot and mouth disease virus
- Goat pox virus
- Lumpy skin disease virus
- Japanese encephalitis virus
- Malignant catarrhal fever virus (Exotic)
- Menangle virus
- *Mycoplasma capricolum*/M.F38/*M. mycoides capri*
- *Mycoplasma mycoides mycoides*
- Newcastle disease virus (VVND)
- Peste Des Petits Ruminants virus
- Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus
- Vesicular stomatitis virus (Exotic)

LISTED PLANT PATHOGENS

- *Liberobacter africanus*
- *Liberobacter asiaticus*
- *Peronosclerospora philippinensis*
- *Phakopsora pachyrhizi*
- Plum Pox Potyvirus
- *Ralstonia solanacearum* race 3, biovar 2
- *Schlerophthora rayssiae* var *zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae*
- *Xylella fastidiosa* (citrus variegated chlorosi)

APPENDIX F

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.

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.

Primary and permanent cell lines from mouse, hamster, human, rat, *etc.* should therefore be handled as if they carry low risk infections. 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. Cells from the herpes and Epstein-Barr virus transformed cultures should be handled as moderate risk viruses. All established or permanent cultures of human lymphocytes should be handled on the assumption they harbor the Epstein-Barr virus, a moderate risk agent. Under no conditions should an individual handle lymphoid cells of a line derived from him or herself, or first-degree relative.

CLASSIFICATION OF CELL AND/OR TISSUE CULTURES ACCORDING TO CONTAINMENT LEVEL

I. MINIMAL CONTAINMENT

- A. Primary peripheral benign lymphocytes without passage.
- B. Primary explants of fibroblasts from benign tissues, up to and including first passage.

II. LOW CONTAINMENT

All other cell cultures except those containing:

- A. Viral agents of CDC Class 3 or Class 4,
- B. Specific exceptional viral agents of CDC Class 2:
 1. Herpes
 2. Adenovirus-Simian virus-10 hybrid viruses
 3. Human hepatitis-associated virus
 4. In case of cell cultures, if a given line is **known** by the investigator specifically not to release any of these specific viral agents, the facility may be category B unless otherwise contraindicated.
 5. Unknown isolates from malignant primate tissues,
 6. Oncogenic viruses considered by NCI as moderate or high risk.

III. MODERATE CONTAINMENT

- A. Cells containing specific exceptional viral agents of CDC Class 2:
 1. Herpes

APPENDIX F

2. Adenovirus-Simian virus-10 hybrid viruses
 3. Human hepatitis-associated virus
 4. In case of cell cultures, if a given line is **known** by the investigator specifically not to release any of these specific viral agents, the facility may be category B unless otherwise contraindicated.
- B. Unknown isolates from malignant primate tissues
- C. Oncogenic viruses considered by NCI as moderate risk.

IV. HIGH CONTAINMENT

Cells containing viral agents of CDC Class 4 or oncogenic viruses considered by the National Cancer Institute as high risk.

APPENDIX G

RISK CLASSIFICATION FOR ONCOGENIC VIRUSES

I. LOW RISK ONCOGENIC VIRUSES

- A. Adenovirus7-Simian virus 40 (Ad7-SV40)
- B. Avian leukosis virus
- C. Bovine leukemia virus
- D. Bovine papilloma virus
- E. Chick-embryo-lethal orphan (CELO) virus or fowl adenovirus-1
- F. Dog sarcoma virus
- G. Guinea pig herpes virus
- H. Lucke (Frog) virus
 - I. Hamster leukemia virus
- J. Marek's disease virus
- K. Mason-Pfizer monkey virus
- L. Mouse mammary tumor virus
- M. Murine leukemia virus
- N. Murine sarcoma virus
- O. Polyoma virus
- P. Rat leukemia virus
- Q. Rous sarcoma virus
- R. Shope fibroma virus
- S. Shope papilloma virus
- T. Simian virus 40 (SV-40)

II. MODERATE RISK ONCOGENIC VIRUSES

Criteria for Moderate Risk Oncogenic Viruses

- Suspected oncogenic virus isolated from man.
 - Virus that transforms human cells in vitro, as evidenced by a morphological and/or functional alteration that is transferred genetically.
 - Virus that produces cancer without the aid of experimental host modification in either a subhuman primate at any age or across another mammalian species barrier in juvenile or adult animals.
 - A genetic recombinant between an animal oncogenic virus and a microorganism infectious for man shall be considered moderate risk until its oncogenic potential for man is determined.
 - Any concentrated oncogenic virus or infectious transforming viral nucleic acid.
- A. Adenovirus
 - B. Adenovirus 2 - Simian virus 40 (Ad2-SV40)
 - C. Epstein-Barr virus (EBV)
 - D. Feline leukemia virus (FeLV)
 - E. Feline sarcoma virus (FeSV)

APPENDIX G

- F. Gibbon leukemia virus (GaLV)
- G. Herpesvirus (HV) ateles
- H. Herpesvirus (HV) saimir
 - I. Papovaviridae including human papilloma viruses
- J. Simian sarcoma virus (SSV)-1
- K. Yabapox virus

III. HIGH-RISK ONCOGENIC VIRUSES

At the present time, there are no known oncogenic viruses classified as high risk.

APPENDIX H

BIOSAFETY REFERENCE MATERIAL

Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. U.S. Government Printing Office Washington: 1995.

NIH Guidelines for Research Involving Recombinant DNA Molecules. U.S. Department of Health and Human Services, Public Health Service, 1995.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 42 CFR Part 72 Requirements for Facilities Transferring or Receiving Select Agents, 1996.

National Research Council Guide for the Care and Use of laboratory Animals, National Academy Press, Washington, D.C., 1996.

U.S. Department of Labor Occupational Safety and Health Administration CPL 2.106 Enforcement Procedures and Scheduling for Occupational Exposure to Tuberculosis, 1996.

U.S. Department of Labor Occupational Safety and Health Administration 29 CFR Part 1910.1030 Occupational Exposure to Bloodborne Pathogens, 1991.

BIOSAFETY WEB LINKS

Biosafety in Microbiological and Biomedical Laboratories (5th Edition, Feb 2007)
<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>

CDC Select Agent Program
<http://www.cdc.gov/od/sap/>

Purdue Human Research Subjects
<http://www.irb.purdue.edu/>

Animal and Plant Health Inspection Service
<http://www.aphis.usda.gov>

National Sanitation Foundation
<http://www.nsf.org>

National Institute of Health
<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

Purdue Radiological & Environmental Management
<http://www.purdue.edu/rem>

Office of the Vice President for Research
<http://www.purdue.edu/research/vpr/compliance/index.html>