GUIDELINES FOR HANDLING PATHOGENIC MICROORGANISMS AND DISPOSING BIOHAZARDOUS WASTE

Biohazard
Recognition and Control

Institutional Biosafety Committee
University of Texas at Arlington

Institutional Biosafety Committee
Office of Research
The University of Texas at Arlington
301 S. Center St. Suite 412, Box 19145
Arlington, TX 76019
Version 1.2, Issued January 2003
Additional copies of this manual can be obtained from the Office of Research, extension 2105.

COVER EMBLEM—UNIVERSAL BIOHAZARD SYMBOL
Signifies actual or potential contamination of equipment, rooms, materials or animals by viable hazardous agents.

Table of Contents

Introduction

This booklet seeks to increase awareness of biological hazards frequently encountered in research, clinical, and teaching laboratories at the University of Texas at Arlington and to provide guidance on recommended practices. Biological hazards include infectious or toxic microorganisms, potentially infectious human substances, and research animals, or their tissues, from which transmission of infectious agents or toxins is reasonably anticipated. Principal Investigators contemplating research involving biological hazards are asked to register their research protocol with the Environmental Health & Safety Office. A description of the research registration process is found in Appendix A.

The object of safety awareness and practice is to assure laboratory personnel that—with proper precautions, equipment and facilities—most biohazardous materials can be handled without undue risk to themselves, their associates, their families, and the environment.

This document is intended not only for trained microbiologists, but also for individuals handling potentially biohazardous materials in other laboratory disciplines such as biochemistry, genetics, immunology, and molecular biology. Persons who have little microbiological training might not realize the potential hazard involved.

The safety principles described are based on sound safety practices, common sense, good housekeeping, thorough personal hygiene, and a plan for responding to accidents. It is likely that laboratories that are well organized and procedurally disciplined are not only effective scientifically, but safe as well.

Institutional Biosafety Committee (IBC) Charge

1. INTRODUCTION

The University of Texas at Arlington (UTA) Institutional Biosafety Committee (IBC) is a University-wide Standing Committee appointed by the President not reporting to the Academic Senate.

The University of Texas at Arlington follows the NIH Guidelines for Research Involving Recombinant DNA Molecules, effective June 24, 1994, published in Federal Register July 5, 1994, (59 FR 34496) and all subsequent amendments issued by the NIH Director with advice of the RAC and Biosafety in Microbiological and Biomedical Laboratories, 3rd Ed., May 1993. US Department of Health and Human Services (DHHS), Public Health Service (PHS). Center for Disease Control (CDC) and Prevention, and the National Institutes of Health (NIH). HHS Publication No. (CDC) 96-8395.

These policies and procedures comply with applicable federal law, state statutes, and University policy in maintaining the highest standards for the handling and use of recombinant DNA (with or without etiologic agents).

The responsibility for compliance with applicable federal law, state statutes, and University policy concerning hazardous materials/hazardous waste management, radiation safety, environmental regulatory affairs, and other general institutional safety requirements rests with the University Environmental Health and Safety Office in the Office of the Vice President for Finance and is not covered under this policy. A. Applicability

The responsibilities of the IBC are applicable to all activities which, in whole or in part involve research with recombinant DNA and /or other biohazardous materials if:

a. The research is sponsored by UTA, or

b. The research is conducted by or under the direction of any employee or agent of UTA in connection with his or her institutional responsibilities, or

c. The research is conducted by or under the direction of any employee or agent of UTA using any property or facility of UTA.

2. INSTITUTIONAL POLICY

a. It is the policy of UTA that, all research covered by this policy, including those considered exempt under the NIH Guidelines will be reviewed and approved by the University's Institutional Biosafety Committee.

b. The involvement of recombinant DNA in research covered by this policy will not be permitted until the IBC has reviewed and approved the research protocol in accord with and to the extent required by the NIH Guidelines. The IBC's review of research on a continuing basis will be conducted at appropriate intervals but not less than once per year.

c. UTA has established and will maintain one IBC in accordance with the NIH Guidelines. The IBC has the responsibility and authority to review, approve, disapprove or require changes in appropriate research activities so that the research involving recombinant DNA follows the NIH Guidelines.

d. UTA has provided and will continue to provide both meeting space for the IBC and sufficient staff to support the IBC's review and record keeping duties.

e. UTA encourages and promotes constructive communication among the research administrators, department heads, research investigators, IBC, other institutional officials as a means of maintaining a high level of awareness regarding the NIH Guidelines.

f. UTA will maintain documentation of IBC activities as prescribed by the NIH Guidelines.

g. UTA will exercise appropriate administrative overview carried out at least annually to insure that its practices and procedures designed for the research involving recombinant DNA are being effectively applied and are in compliance with the requirements of the NIH Guidelines and this policy.

h. UTA will comply with the requirements set forth in the NIH Guidelines regarding cooperative research projects. When research covered by this policy is conducted at or in cooperation with another entity, all provisions of this policy remain in effect for that research. UTA may accept, for the purpose of meeting the IBC review requirements, the review of an IBC established under another policy of compliance with NIH. Such acceptance must be in writing, approved and signed by UTA's Office of Research, approved and signed by correlative officials of each of the other cooperating institutions.

i. Copies of the general policy will be available to all faculties through the Office of Research, the offices of the Deans and the Program Heads, and the Chairperson of the IBC.

j. UTA will also provide each individual at the institution conducting or reviewing human subject research a summary of the rules and regulations including any future modifications and an outline of the procedures to be followed in any research involving recombinant DNA as covered by this policy.

k. Research covered by this policy will fall into one of the following categories.

i. rDNA (Recombinant deoxyribonucleic acid): (i.) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; (ii.) molecules that replicate as a result of the above (i.)

ii. RG2 (Risk Group 2): Agents that are associated with diseases that are rarely serious and for which preventive/therapeutic interventions are often available

iii. RG3 (Risk Group 3): Agents that are associated with serious/lethal human diseases for which preventive/therapeutic interventions may be available (high individual risk, but low community risk)

iv. (RG4) Risk Group 4: Agents that are likely to cause serious/lethal human diseases for which preventive/therapeutic interventions are not usually available (high individual risk and high community risk)

l. Research considered exempt will be reviewed and approved by the Chair of the IBC. The Chair will then forward the approved exemptions to the UTA Office of Research. All other categories/risk groups will be approved by the full IBC.

m. In addition to the NIH Guidelines for the Use of Recombinant DNA in Research, the Institutional Biosafety Committee will be charged with implementing policies and procedures in accordance with the USA Patriot Act of 2001 and the Public Health Security and Bioterrorism Preparedness Response Act of 2002.

3. IBC STRUCTURE

a. Institutional Establishment of the IBC

i. The IBC is established at The University of Texas at Arlington to review all research involving recombinant DNA and other biohazardous materials used in research protocols to include the CDC Select Agent list (Appendix A of 42 CFR Part 72). The IBC membership is appointed by the President of the University and shall be composed of no fewer than five members.

ii. The term of office of the Committee members shall be for two years, effective September 1 to August 31, and the President may reappoint members for additional terms. If for any reason a Committee member resigns, the President shall appoint another individual to serve the remainder of the un-expired term.

iii. To ensure continuity, initial appointments of Committee members will be for staggered terms so that one-half of the appointments expire August 31 of each academic year.

b. IBC Membership Requirements

i. The IBC is comprised of faculty, administrators, and community members from diverse backgrounds to promote complete and adequate review of research activities covered by this policy, and has the professional competence necessary to review the specific research activities, which will be assigned to it.

ii. The Chairperson shall be the Dean of Science and the President or designee shall appoint the Vice Chairperson of the Committee annually. The Assistant Vice President for Research shall be the responsible university official.

iii. The IBC is sufficiently qualified through the experience and expertise of its members, and the diversity of the members' backgrounds, including consideration of the racial and cultural backgrounds of members and sensitivity to such issues as community attitudes, to promote respect for its advice and counsel.

iv. The IBC will include at least two members who are not otherwise affiliated with the institution and who is not a part of the immediate family of a person affiliated with the institution; a UTA scientist with expertise in plant, plant pathogen, or plant pest containment principles; a UTA scientist with expertise in animal containment principles; a UTA laboratory technical staff representative; and, the UTA Safety Officer.

4. PROCEDURES

a. All principal investigators including faculty, staff, or students using rDNA or biohazardous materials in research , funded or non-funded, must submit a protocol with the IBC. All protocols should be filed with the Dean of the School of Science or through the current IBC designee. The Dean of the School of Science will then approve or refer a protocol for full IBC review and submit the protocols to the Office of Research for processing. The UTA Office of Research will maintain all files, records and minutes of all IBC activities.

General Principles

RISK ASSESSMENT

To apply biological safety principles rationally while handling a potential pathogen, one must perform a risk assessment, which considers:

Agent's biological and physical nature.
The sources likely to harbor the agent.
Host susceptibility.
The procedures that may disseminate the agent.
The best method to effectively inactivate the agent.

Globally, numerous government agencies have classified microorganisms pathogenic for humans into risk groups (RG) based on the transmissibility, invasiveness, virulence or disease-causing capability, and the lethality of the specific pathogen. Risk groupings of infectious agents (1 through 4, see Table 1) correspond to biosafety levels (BSL1-4) which describe recommended containment practices, safety equipment and facility design features necessary to safely handle these pathogenic microorganisms. The list of pathogenic microorganisms includes bacteria, viruses, fungi, parasites and other infectious entities. Beginning with Risk Group 1 agents, which are nonpathogenic for healthy human adults, the scheme ascends to Risk Group 4 in order of increasing hazard.

The risk group listing of the NIH *Guidelines* (see Appendix B) is an accepted standard, even when recombinant DNA technology is not used. It can be accessed electronically at: *http://www.nih.gov/od/orda/apndxb.htm*. The American Biological Safety Association also provides a comprehensive risk group listing which references agencies globally; this list is accessible at: *http://35.8.104.121/riskgroups/default.htm*. An excellent source of information about human pathogens is available from material safety data sheets posted by Health Canada at: *http://www.hc.sc.gc.ca/hpb/lcdc/biosafty/msds/index.html*.

TABLE 1

Relationship of Risk Groups to Biosafety Levels, Practices and Equipment

Risk Group	Biosafety Level	Examples of Laboratories	Laboratory Practices	Safety Equipment
1	Basic–Biosafety Level 1	Basic teaching and research	GMT[a]	None; open bench work
2	Basic–Biosafety Level 2	Primary health services, research, diagnostic, teaching and public health	GMT plus protective clothing, biohazard sign	Open bench plus BSC[b] for potential aerosols
3	Containment–Biosafety Level 3	Special diagnostic and research	As level 2 plus special clothing, controlled access, directional air flow	BSC and/or other primary containment for all activities
4	Maximum Containment–Biosafety Level 4	Dangerous pathogen units shower exit, special waste disposal	As level 3 plus airlock entry	Class III BSC or positive pressure suits, double-ended autoclave, filtered air

[a]GMT, good microbiological technique.
[b]BSC, biological safety cabinet.

Source: Modified from WHO 1993

Microorganisms in RG1 require standard laboratory facilities and standard microbiological practices, whereas those in RG4 require maximum containment. Some of the agents likely to be handled experimentally at UT-Arlington are RG2 and potentially some RG3 pathogens, designated as moderate and high hazard, respectively. These agents typically require more sophisticated engineering controls (e.g. facilities and equipment) than standard laboratories, as well as special handling and decontamination procedures.

- **RISK GROUP 1** agents are not associated with disease in healthy adult humans. Examples: *E. coli* K–12, *Saccharomyces cerevisciae*.

- **RISK GROUP 2** agents are associated with human disease, which is rarely serious, and for which preventive or therapeutic interventions are *often* available. Examples: *E. coli* O157:H7, *Salmonella*, *Cryptosporidium.*

- **RISK GROUP 3** agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available (high individual risk but low community risk). Examples: *Bacillus anthracis*, *Brucella abortus*, *Mycobacterium tuberculosis.*

- **RISK GROUP 4** agents 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). Examples: Ebola virus, *Cercopithecine herpesvirus* 1 (Herpes B or Monkey B virus).

Microorganisms classified as RG2 or higher have been reported to cause infection and disease in humans. The progression from invasion to infection to disease following contact with an infectious agent depends upon the dose, route of transmission, invasive characteristics of the agent and resistance of the person exposed, whether innate or acquired. Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. It is prudent to assume virulence and to handle such agents at the prescribed biosafety level.

ROUTES OF INFECTION

Pathogens are transmitted via several routes of infection, depending on the pathogen in question. The most common routes of infection are inhalation of infectious aerosols or dusts, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, or percutaneous self-inoculation (injection or incision). Appropriate precautions can be implemented to avoid such exposures.

EXPOSURE SOURCES

Clinical and Pathological Specimens

Any specimen from human patients or animals may contain infectious agents. Specimens most likely to harbor such microorganisms include blood, sputum, cerebrospinal fluid, urine, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, feces and tissues. Personnel in laboratories and clinical areas handling human blood or body fluids should practice *universal precautions,* an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and other bloodborne pathogens. Such personnel are required by law to be enrolled in the campus Bloodborne Pathogen Training Program (contact the Environmental Health & Safety Office, 817-272-2185, for information.)

Certain animals may harbor indigenous pathogens that are virulent for humans. For personnel handling these animals or their tissues/body fluids, we recommend an analogous approach to infection control, *general precautions,* which assumes these animals and their blood and body fluids to be potentially infectious. **At a minimum, any specimen from either human and non-human primates should be handled as if it were infectious.**

Cultures

Accidental spilling of liquid infectious cultures is an obvious hazard due to the generation of aerosols (airborne droplets containing microorganisms). However, even routine manipulations of cultures may release microorganisms via aerosol formation:

- Popping stoppers from culture vessels.
- Opening closed vessels after vigorous shaking.
- Spatter from flame-sterilizing utensils.
- Expelling the final drop from a pipette.

Manipulate cultures of infectious material carefully to avoid aerosols or spills. Centrifugation should involve the use of gasket-sealable tubes, carriers and rotors, when available. Seal microplate lids with tape or replace them with adhesive-backed Mylar film. Load, remove and open tubes plates and rotors within a biological safety cabinet or fume hood.

When preparing aliquots of infectious material for long term storage, consider that viable lyophilized cultures may release high concentrations of dispersed particles if ampules are not properly sealed. Breakage of ampules in liquid nitrogen freezers may also present hazards because of survival and dispersal of pathogens in the liquid phase.

Equipment used for manipulations of infectious materials, such as cell sorters and automated harvesting equipment, must be evaluated to determine the need for secondary containment and to consider decontamination issues.

Use of human or animal cell cultures in laboratories requires special consideration. Cell or tissue cultures in general present few biohazards, as evidenced by their extensive use and lack of infection transmitted to

laboratory personnel. Clearly, when a cell culture is inoculated with or known to contain an etiologic agent, it should be classified and handled at the same biosafety level as the agent.

Biosafety Level 2 containment conditions should be used for cell lines of human origin, even those that are well-established like HeLa and Hep–2, and for all human clinical material (e.g., tissues and fluids obtained from surgery or autopsy). Primate cell cultures derived from lymphoid or tumor tissue, cell lines exposed to or transformed by a primate oncogenic virus, and all nonhuman primate tissue should also be handled at BSL2. Use a biological safety cabinet, not a laminar flow clean bench, for manipulations with potential to create aerosols.

Animals

Exercise care and thoughtfulness when using animals to isolate and propagate microorganisms, study pathology or produce antibodies. Laboratory animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted microorganisms. In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols. During surgical procedures, necropsies and processing of tissues, aerosols can be produced unintentionally, or the operator can inflict self-injury with contaminated instruments. Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols and dust when changing bedding and cleaning cages.

LABORATORY EXPOSURE

Teaching Laboratories

Whenever possible, we recommend the use of avirulent strains of infectious microorganisms in teaching laboratories. However, even attenuated microbes should be handled with care. Students should be cautioned against and trained to prevent unnecessary exposure, as exposure to “avirulent” strains may become problematic in the immune-compromised individual. Establishment of safety consciousness is integral to the conduct of good science.

Research Laboratories

Experiments in research laboratories using high concentrations or large quantities of pathogens increase the risk of exposure. The use of animals in research on infectious diseases also presents greater opportunities for exposure.

Clinical Laboratories

Personnel in laboratories performing diagnostic work-up of clinical specimens from human or animal patients are often at risk of exposure to infectious agents. The absence of an infectious disease does not preclude the presence of pathogens. This is especially true of materials from patients who have received immunosuppressive therapy since such treatment may activate latent infectious agents.

APPLICABLE POLICIES

Infectious Agents and Toxins

All principal investigators involved with research involving Infectious Agents or Toxins (at any Biosafety Level) must complete and submit the form, "Control of Infectious Agents and Toxins" (Appendix G or www.uta/edu/research - Biosafety) to Dr. Ronald Smith, Department of Biology, Box 19498. This form shall be resubmitted and reviewed on an annual basis. Infectious Agents / Toxins requiring Biosafety Levels 2 and above must submit the "Control of Infectious Agents and Toxins" form **and** the research protocol to the Institutional Biosafety Committee for review.

Select Agent Registration

All principal investigators involved with research involving Select Agents (see Appendix C and Appendix D) must inform the Research Compliance Officer (Office of Research) in writing **before** ordering and / or receiving the agent **in addition to** the registration of the protocol with the Institutional Biosafety Committee. It is now required by law for all Select Agents to be registered with the CDC and the USDA. The Assistant Vice President for Research and Director of the Office of Research is the Institutional Official for Select Agent Registration and can be reached at extension 2105.

Protocol For Approval To Use Vaccinia Virus In Research

The UTA policy on the use of Vaccinia Virus in research follows national guidelines as issued by the Centers for Disease Control and Prevention (CDC). The University Institutional Biosafety Committee (IBC) recommends the policy that is administered by the Office of Research (OR).

1. Principal Investigators (PI's) must obtain written approval to use Vaccinia Virus from OR. Biosafety Level 2 practices and procedures must be followed for work with nonhighly attenuated strains of Vaccinia Virus. Lab personnel must wear appropriate personal protective equipment: lab coat, disposable gloves, and eye protection. When performing procedures with aerosol generating potential, personnel must work in a biological safety cabinet. Biosafety Level 1 practices and procedures may be followed for work with highly attenuated strains (MVA, NYVAC, ALVAC and TROVAC). For a complete description of Biosafety Level 1 and 2 guidelines consult the Biosafety in Microbiological and Biomedical Laboratories at http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf.

    A. The CDC recommends that all persons working with nonhighly attenuated strains of vaccinia virus be immunized, if medically eligible, before beginning work with virus. Accordingly, at UTA, PI's must offer all laboratory personnel who directly handle cultures or animals contaminated or infected with nonhighly attenuated vaccinia virus, recombinant vaccinia viruses derived from nonhighly attenuated vaccinia strains, or other Orthopoxviruses that infect humans, the opportunity to receive counseling and Small Pox immunization from The UTA Student Health Center. Recombinant, highly attenuated poxvirus strains (MVA, NTVAC, ALVAC, and TROVAC) are unable to replicate or replicate poorly in mammalian host cells; therefore, they do not create productive infections. Vaccination is not recommended for workers who do not handle nonhighly

attenuated virus cultures or materials or who do not work with animals contaminated or infected with these viruses. (The Occupational Safety and Health Board of NIH no longer requires vaccinia (smallpox) vaccination for personnel manipulating MVA or NYVAC strains in a laboratory where no other vaccinia viruses are being manipulated.)

B. The Vaccinia (Smallpox) vaccine that had prior FDA approval is no longer available. The current vaccine is a new formulation that is not FDA approved. Before it can receive FDA approval, it must be evaluated for safety and efficacy. Therefore, it is being made available by the Centers for Disease Control and Prevention as part of a clinical trial. Personnel interested in obtaining the vaccine must become a participant in the clinical trial.

   To obtain the vaccine, principal investigators must complete the "CDC Request For Vaccinia (Small Pox) Vaccine" form (www.uta/edu/research - Biosafety), starting at the section titled "Head of the laboratory doing research with the virus(es)." The completed form, a copy of the PI's curriculum vitae, and a brief abstract of the research project must be returned by mail to:

   The Office of Research
   PO 19145
   Arlington, Texas 76019

C. The UTA Student Health Center will counsel personnel in conjunction with review of the CDC document, "Important Information for Recipients of Small Pox Vaccine". To obtain a copy of this document, call the Office of Research at extension 2105. The UTA Student Health Center will complete the remainder of the Small Pox vaccine request form (www.uta.edu/research -Biosafety) and order the vaccine from CDC. The UTA Student Health Center will notify personnel when the vaccine is available.

D. If the UTA Student Health Center determines during counseling that the vaccine is medically contraindicated for a person, they will advise the individual to avoid contact with infectious vaccinia viruses in the workplace.

E. The UTA Student Health Center will verify vaccinia counseling or immunization by notifying the PI in writing as to whether each person who signed up: (1) was immunized or (2) declined or was medically ineligible to be immunized.

   If the person was immunized, he/she will be required to return to the UTA Student Health Center on the following days after immunization: 3 days, 5-7

days, 14 days and 17 days. It is the responsibility of the PI to require individuals to attend these follow-up visits.

Individuals who decline counseling and/or immunization must complete the "Declination for a Vaccinia Vaccination" form (www.uta.edu/research - Biosafety).

The PI must sign the vaccinia consent/declination form acknowledging the immunization status of the person and return the signed form to the Office of Research (www.uta.edu/research - Biosafety).

F. Experiments involving the generation of recombinant DNA in vaccinia virus must be registered with the IBC. To obtain a Registration Document for rDNA Experiments contact Dr. Ronald Smith in the UTA Biology Department at extension 2871. The completed document must be returned to Dr. Ronald Smith in the Biology Department, PO 19498.

G. If work with vaccinia virus also involves human source material, free Hepatitis B immunization must be offered to all University employees who are at risk of occupational exposure to human blood, body fluids and/or tissues.

Vaccinia (Smallpox Vaccine): Recommendations of the Immunization Practices Advisory (ACIP), MMWR, June 22, 2001 / Vol. 50 / No. RR-10

HEALTH STATUS

Some unusual circumstances warrant special considerations or measures to prevent infection of laboratory by certain microorganisms:

Please be informed that certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include: pregnancy, immunosuppression, and animal related allergies. If any of these conditions apply, you should discuss your situation with the Principal Investigator of the laboratory and/or the Department Chair and inform your personal physician/health care professional of your work.

Biohazard Containment

Although the most important aspect of biohazard control is the awareness and care used by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can prevent dissemination of pathogens should their accidental release occur.

PRACTICES AND PROCEDURES

The following practices, corresponding to **Biosafety Level 1**, are not only important for the prevention of laboratory infection and disease, but also reduce contamination of experimental material. These practices and procedures provide the foundation for the more restrictive Biosafety Levels 2 and above.

Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
- A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.
- An insect and rodent control program is in effect.

Special Practices

- None

Safety Equipment (Primary Barriers)

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

- It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
- Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.
- Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

Laboratory Facilities (Secondary Barriers)

- Laboratories should have doors for access control.
- Each laboratory contains a sink for handwashing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- If the laboratory has windows that open to the exterior, they are fitted with fly screens.

TABLE 2

Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Laboratory Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	PPEs: laboratory coats; gloves; eye protection as needed	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon systems Other requirements outlined in the text

Biological Safety Cabinets

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. Biological safety cabinets, when functioning correctly and when used in conjunction with good microbiological techniques, can control infectious aerosols. BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed (see *http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm*).

Laminar flow clean benches are not biological safety cabinets and should never be used for work with potentially hazardous materials, biological or chemical. These devices protect the material in the cabinet but not the worker or the environment.

The ultraviolet lamps within some biosafety cabinets provide only limited ability to inactivate microbes. Efficacy is limited to exposed surfaces and penetration of organic material is poor. Note that effectiveness decreases as the lamp ages. Furthermore, exposure to the ultraviolet light may cause eye damage.

LOADING MATERIALS/EQUIPMENT AND STARTUP

Turn on blower and fluorescent light and close drain valve.
Check pressure gauge to ensure proper operating conditions.
Check grills for obstructions.
Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
Disinfect the exterior of all containers prior to placing them in the cabinet.
Load only items needed for the procedure.
Arrange materials within the cabinet so that:
    movement within the cabinet is minimized;
    flow of procedure is from CLEAN to DIRTY
    (never place non-sterile items upstream of sterile items);
    rear or front exhaust grilles are unobstructed.
Once the cabinet is loaded, adjust view screen to proper position and wait three minutes before commencing procedures. NEVER use the view screen above the 8-inch mark.
RESTRICT traffic in the BSC vicinity.

RECOMMENDED WORK TECHNIQUES

Wash hands thoroughly with soap before and after procedure.
Wear sterile gloves and lab coat/gown; use aseptic technique.
Avoid blocking front grille. Work only on or over solid surface, adjust chair so armpits are at elevation of lower window edge.
Avoid rapid movement during procedures, particularly within the BSC, but also in the vicinity of the BSC.
Move hands and arms straight into and out of work area, never rotate hand/arm out of work area during procedure. Move laterally in work area.

FINAL PURGING AND WIPE DOWN

After completing work, run the BSC blower for three minutes before unloading materials from the cabinet.

Disinfect the exterior of all containers BEFORE removing from the work zone.
Decontaminate interior work surfaces of the BSC with an appropriate disinfectant effective against the agent in question.
Care should be taken to prevent towelettes from being sucked into exhaust plenums.

DECONTAMINATION AND SPILLS

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. The final surface decontamination of the cabinet should include a wipe-down of the work zone. Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.
Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.
Spills large enough to result in liquids flowing through the front or rear grills require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grill(s) into the drain pan. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. The drain pan should be emptied into a collection vessel containing disinfectant. Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel (817-272-2185) should be contacted for specific instructions.

MAINTENANCE

To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be verified as leak-proof. **The University of Texas at Arlington requires that all biological safety cabinets be certified annually.** This is imperative for BSCs intended for work at BSL2 or above. For more information about this service, contact the Environmental Health & Safety Office (817-272–2185).

DRIP PAN MAINTENANCE

Beneath the BSC work surface is a drip pan to collect large spills. This area should be routinely checked for cleanliness and, if a major spill has occurred, appropriately cleaned and disinfected (see **DECONTAMINATION AND SPILLS** above).

PURCHASING A BSC

Before ordering a biological safety cabinet, consult the Environmental Health & Safety Office (817-272-2185) for an evaluation of its suitability for the intended research and the available space. To ensure the adequacy of the installed mechanical ventilation and to facilitate coordination with the Physical Plant, exhausted biological safety cabinets must be approved by the Environmental Health & Safety Office and Physical Plant prior to purchase.

TABLE 3

Comparison of Biosafety Cabinet Characteristics

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front; exhausted through HEPA to the outside or into the room through HEPA (see Fig. 2)	YES	YES (1)
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a thimble unit	YES	NO
II, A2/B3	100	Same as II, A1, but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter	YES	YES (minute amounts (2))
II, B1	100	Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	YES	YES (minute amounts (2))
II, B2	100	No recirculation; total exhaust to the outside through hard-duct and a HEPA filter	YES	YES (small amounts)
III	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series	YES	YES (small amounts)

(1) Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I cabinet in to a room should not occur if volatile chemicals are used.

(2) In no circumstances should the chemical concentration approach the lower explosion limits of the compound.

Disposal of Wastes
Contaminated with Infectious Agents

These biohazardous waste disposal guidelines are designed to protect not only the public and the environment, but also laboratory and custodial personnel, waste haulers, and landfill/incinerator operators at each stage of the waste-handling process. Generators of biohazardous waste in the laboratory must

DECONTAMINATION means a process of reducing the number of disease-producing microorganisms and rendering an object safe for handling.

DISINFECTION means a process that kills or destroys most disease-producing microorganisms, except spores.

STERILIZATION means a process by which all forms of microbial life, including spores

assure that the labeling, packaging, and intermediate disposal of waste conforms to these guidelines.

WHAT IS INFECTIOUS WASTE?

The following items usually are considered to be infectious waste.

- Microbiological laboratory wastes such as cultures derived from clinical specimens and pathogenic microorganisms, and laboratory equipment that has come into contact with the cultures.
- Tissues, bulk blood, and body fluids from humans.
- Tissues, bulk blood or body fluids from an animal that is carrying an infectious agent that can be transmitted to humans.
- Contaminated sharps.

Other categories of waste that require decontamination before disposal are regulated recombinant DNA organisms and exotic or virulent plant and animal pathogens.

The following are usually not included in the definition of infectious waste, but should be placed in containers such as plastic bags prior to disposal to contain the waste. If these items were mixed with infectious wastes, they would have to be managed as though they were infectious.

- Items soiled or spotted, but not saturated, with human blood or body fluids. Examples: blood-spotted gloves, gowns, dressings, and surgical drapes.
- Containers, packages, waste glass, laboratory equipment and other materials which have had no contact with blood, body fluids, clinical cultures or infectious agents.
- Non-infectious animal waste, such as manure and bedding, and tissue, blood and body fluids or cultures from an animal which is not known or suspected to be carrying an infectious agent that can be transmitted to humans.

PACKAGING OF WASTE

Laboratory materials used in experiments with potentially infectious microorganisms, such as discarded cultures, tissues, media, plastics, sharps, glassware, instruments, and laboratory coats, must be decontaminated before disposal or washing for re-use. Collect contaminated materials in leak-proof containers labeled with the *Universal Biohazard Symbol*; autoclavable biohazard bags are recommended.

After autoclaving, biohazard symbols on containers are defaced to assure custodial/waste disposal personnel that containers are safe to handle. Uncontaminated sharps and other noninfectious items that may cause injury require special disposal even if they need not be decontaminated. Sharps need to be collected in rigid puncture-proof containers to prevent wounding of co-workers, custodial personnel and waste handlers. If a package is apt to be punctured because of sharp-edged contents, double bagging or boxing may be necessary.

METHODS OF DECONTAMINATION

Choosing the right method to eliminate or inactivate a biohazard is not always simple. The choice depends largely on the treatment equipment available, the target organism, and the presence of interfering substances (*e.g.*, high organic content) that may protect the organism from decontamination. A variety of treatment techniques are available, but practicality and effectiveness govern which is most appropriate.

Ideally, biohazardous waste should be decontaminated before the end of each working day unless it is to be incinerated. In the latter case, the waste should be packaged and frozen until the scheduled pick-up by the Environmental Health & Safety Office (817-272-2185). Biohazardous waste should never be compacted. Ordinary lab wastes should be disposed of routinely as much as possible to reduce the amount requiring special handling.

Steam Sterilization

Decontamination is best accomplished by steam sterilization in a properly functioning autoclave that is routinely monitored with a biological indicator such as spores of *Bacillus stearothermophilus*. The tops of autoclavable biohazard bags should be opened to allow steam entry. For dry materials, it may be necessary to add water to the package prior to autoclaving.

Although we recommend autoclaving all biohazardous wastes for at least one hour, the nature of the waste in a batch should determine cycle duration. For example, if the waste contains a dense organic substrate such as animal bedding or manure, one hour may be insufficient to inactivate certain pathogens buried within. A considerably longer exposure time (8 to 12 hours) may be required to effectively decontaminate such waste. Since there is a practical limit to the time that can be spent autoclaving waste, in such a case incineration would be a more effective and economical treatment method. However, as with most generalizations, it is difficult to prescribe methods that meet every contingency. Such decisions are best left to the personnel directly involved, provided they are well informed and prepared to verify the effectiveness of the treatment.

Use extreme caution when treating waste that is co-contaminated with volatile, toxic, or carcinogenic

chemicals, radioisotopes, or explosive substances. Autoclaving this type of waste may release dangerous gases (*e.g.*, chlorine) into the air. Such waste should be chemically decontaminated, incinerated, or sent to a hazardous waste landfill. Consult the Environmental Health & Safety Office (817-272-2185) for more information.

Sewage Treatment

Most fluid waste, including human blood, can be discarded by pouring into the sanitary sewer, followed by flushing with water. Care should be taken to avoid generation of aerosols. The routine processing of municipal sewage provides chemical decontamination. However, if the fluid is contaminated with infectious agents or biological toxins, it must first be rendered safe by chemical or autoclave treatment before sewer disposal. Sewer lines should be decontaminated by flushing with hypochlorite (10%) prior to servicing.

Chemical Disinfection

Where autoclaving is not appropriate, an accepted alternative is to treat material with a chemical disinfectant, freshly prepared at a concentration known to be effective against the microorganisms in use. The disinfectant of choice should be one that quickly and effectively kills the target pathogen at the lowest concentration and with minimal risk to the user. Other considerations such as economy and shelf life are also important. Allow sufficient exposure time to ensure complete inactivation.

Halogens such as hypochlorite (household bleach) are the least expensive and are also highly effective in decontaminating large spills. Their drawbacks include short shelf life, easy binding to nontarget organic substances, and corrosiveness, even in dilute forms. Hypochlorite typically is diluted 1:10 to 1:100 such that the available halogen is 0.01–5.0%. Also be aware that using chlorine compounds to disinfect substances co-contaminated with radioiodine may cause gaseous release of the isotope.

Alcohol (ethanol or isopropanol) is effective against vegetative forms of bacteria and fungi, and hydrophobic viruses, but will not destroy spores or hydrophilic viruses. Additional characteristics limiting its usefulness are its flammability, poor penetration of protein-rich materials, and rapid evaporation making extended contact time difficult to achieve. It is important to be aware that common laboratory disinfectants can pose hazards to users. For example, ethanol and quaternary ammonium compounds may cause contact dermatitis. Further information about chemical disinfectants can be obtained from the Environmental Health & Safety Office.

Large volume areas such as fume hoods, biological safety cabinets, or rooms may be decontaminated using gases such as formaldehyde, ethylene oxide, or peracetic acid. These gases, however, must be applied with extreme care. **Only experienced personnel who have the specialized equipment and protective devices to do it effectively and safely should perform gas decontamination.**

Incineration

Incineration is the recommended method for disposal of infected animal carcasses and biohazardous wastes. Metallic items and certain plastic objects must be excluded from packages to be incinerated. Please consult the Environmental Health & Safety Office (817-272-2185) for more information.

Emergency Plans

No matter how carefully one works, laboratory accidents occur and necessitate emergency response. Emergency plans should be tailored for a given biohazardous situation. The laboratory supervisor should prepare instructions specifying immediate steps to be taken. These instructions should be displayed prominently in the laboratory and periodically reviewed with personnel. No single plan will apply to all situations but the following general principles should be considered:

< In the event of an extensive or explosive spill of virulent pathogens, everyone should leave the affected area **immediately**. Clothing, if contaminated, should be removed. Exposed skin should be washed thoroughly.
< Close the laboratory door and post a "No Entry" sign indicating the hazard. Notify the laboratory supervisor and the Environmental Health & Safety Office (817-272-2185).
< Determine the necessity and extent of medical treatment for persons exposed to infectious microorganisms. Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation of an infectious agent should be given appropriate first aid and transported to a hospital emergency room. If necessary, call UTA Campus Police (817-272–3003 or 911) for transportation at any hour.
< Do not reenter the room until aerosols have settled (30 minutes, minimum), and the extent of the hazard and its dissemination has been determined.
< Each person who enters the laboratory for clean-up should wear proper protective clothing.
< Use an appropriately concentrated disinfectant to decontaminate the area. A supply of stock disinfectants should always be available.
< Decontaminate all materials used in clean-up procedures.

In any emergency situation, attention to immediate personal danger overrides containment considerations. Currently, there is no known biohazard on the UTA campus that would prohibit properly garbed and masked fire or security personnel from entering any biological laboratory in an emergency.

Shipping Hazardous Biological Materials

Hazardous materials, capable of posing an unreasonable risk to health, safety, and property, are commonplace in University facilities. Amongst them are chemicals and solvents, cleaning agents, radionuclides, infectious agents and toxins. When hazardous materials are transported in commerce, complex federal regulations for shipping hazardous materials must be followed. Seemingly minor technical violations can result in major fines while more serious violations can endanger the public. The U.S. Department of Transportation requires all persons involved in shipping hazardous materials to be trained and certified in proper handling of these materials. Activities for which training is required include:

preparing shipping papers

loading and unloading trucks
marking and labeling packages
filling packages
supervising these activities

For more information about this training, please contact the Environmental Health & Safety Office (817-272-2185).

REFERENCES

CDC/National Institutes of Health, 1999. Biosafety in microbiological and biomedical laboratories. 4th ed. Atlanta.
http://www.cdc.gov/od/ohs/biosafety/bmbl/bmbl-1.htm

CDC/National Institutes of Health. Primary containment for biohazards: selection, installation and use of biological safety cabinets, 1st edition, 1995.
http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm

Miller, B.M., *et al.*, 1994. Laboratory safety; principles and practices, p. 185.
American Society for Microbiology, Washington, DC.

National Institutes of Health. *Guidelines for research involving recombinant DNA molecules* (NIH *Guidelines*) 64 FR 25361 May 1999
http://www.nih.gov/od/orda/toc.htm

National Research Council, 1989. Biosafety in the laboratory. Prudent practices for the handling and disposal of infectious materials. National Academy Press, Washington DC.

WHO 1993. Laboratory biosafety manual, 2d ed. Geneva.

Appendix A

Biosafety Protocol Registration Process

As a major research institution, the University of Texas at Arlington provides assurances that its sponsored research activities are in compliance with state and federal regulations and guidelines. In this context, the Institutional Biosafety Committee reviews research activities involving biologically hazardous materials and/or recombinant DNA molecules/organisms. Thus, the Office of Research requests submission of a biosafety protocol for research activities involving:

microbiological agents infectious to humans, animals or plants; provide a copy of any required federal permit.

exotic plants, animals, and microbes; provide a copy of any required federal permit.

human blood, tissues, and cell lines as well as potentially infectious animal blood, tissues and cell lines.

animals for which a reasonable potential for transmission of zoonotic agents exists, e.g., wild-trapped animals, sheep, and rhesus macaques.

carcinogens, mutagens, drugs, and toxins when administered *in vivo* to animals or *in vitro* to cell cultures to induce a biological outcome.

recombinant DNA molecules and recombinant DNA-containing organisms or cell cultures which are subject to the NIH *Guidelines for Research Involving Recombinant DNA Molecules*, including human gene therapy trials.

To insure that protocols remain current with research activities, the Office of Research (OR) requires annual certification of all protocols. We encourage project-based protocol consolidation, even if funded by multiple agencies, to facilitate a comprehensive risk assessment and to reduce "paper work."

Please call or visit the biosafety website (www.uta/edu/research - Biosafety) if you have any questions about the biosafety protocol registration process.

Office of Research
301 S. Center St. Suite 412, Box 19145
Phone: 817-272-2105
Fax: 817-272-5808

Appendix B

Classification of Human Etiologic Agents on the Basis of Hazard
(Reproduced from NIH Guidelines, May 1999)

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.

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*, *Escherichia coli*-K12, 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.

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.

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 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
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 RG3) including *M. avium* complex,
M. asiaticum, M. bovis BCG vaccine strain, *M. chelonei, M. fortuitum,*
M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis,
M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi
Mycoplasma, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
Neisseria gonorrhoeae, *N. meningitidis*
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
Rhodococcus equi
Salmonella including *S. arizonae, S. cholerasuis, 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

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
Microsporum

Paracoccidioides braziliensis
Penicillium marneffei
Sporothrix schenckii
Trichophyton

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. granulosis, E. multilocularis, E. vogeli*
Entamoeba histolytica
Enterobius
Fasciola including *F. gigantica, F. hepatica*
Giardia including *G. lamblia*
Heterophyes
Hymenolepis including *H. diminuta, H. nana*
Isospora
Leishmania including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
Loa loa filaria worms
Microsporidium
Naegleria fowleri
Necator human hookworms including *N. americanus*
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
Trypanosoma including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
Wuchereria bancrofti filaria worms

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 *Risk Group 4*) Cytomegalovirus
Epstein Barr virus
Herpes simplex types 1 and 2
Herpes zoster
Human herpesvirus types 6 and 7
Orthomyxoviruses
Influenza viruses types A, B, and C
Other tick-borne orthomyxoviruses as listed in 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 *Risk Group 3*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox
Reoviruses: all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
Rhabdoviruses
Rabies virus-all strains
Vesicular stomatitis virus-laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow
Togaviruses (see Alphaviruses and Flaviviruses)
Rubivirus (rubella)

Risk Group 3 (RG3) Agents

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

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 *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

Risk Group 3 (RG3): Fungal Agents
Coccidioides immitis (sporulating cultures; contaminated soil)
Histoplasma capsulatum, H. capsulatum var. duboisii

Risk Group 3 (RG3): Parasitic Agents
None

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 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
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 spongioform 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

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.

Risk Group 4 (RG4): Bacterial Agents
None

Risk Group 4 (RG4): Fungal Agents
None

Risk Group 4 (RG4): Parasitic Agents
None

Risk Group 4 (RG4): Viral Agents

Arenaviruses
- Guanarito virus
- Lassa virus
- Junin virus
- Machupo virus
- Sabia

Bunyaviruses (Nairovirus)
- Crimean-Congo hemorrhagic fever virus

Filoviruses
- Ebola virus
- Marburg virus

Flaviruses (Togaviruses): Group B Arboviruses
- Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
- Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
- Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

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

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

Retroviruses
- Avian leukosis virus
- Avian sarcoma virus

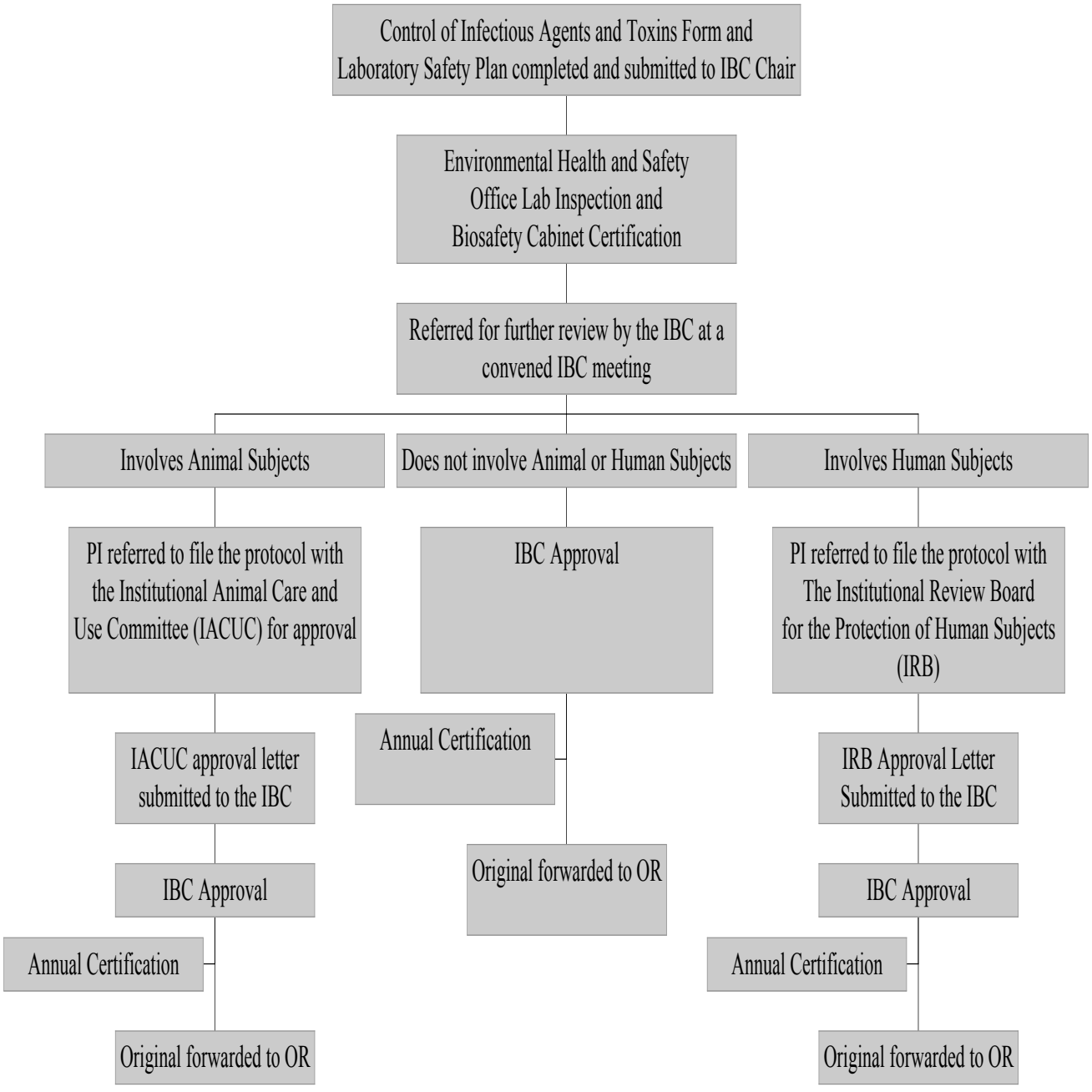
Bovine leukemia virus
Feline leukemia virus
Feline sarcoma virus
Gibbon leukemia virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine leukemia virus
Murine sarcoma virus
Rat leukemia virus

Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered under BSL1 containment.

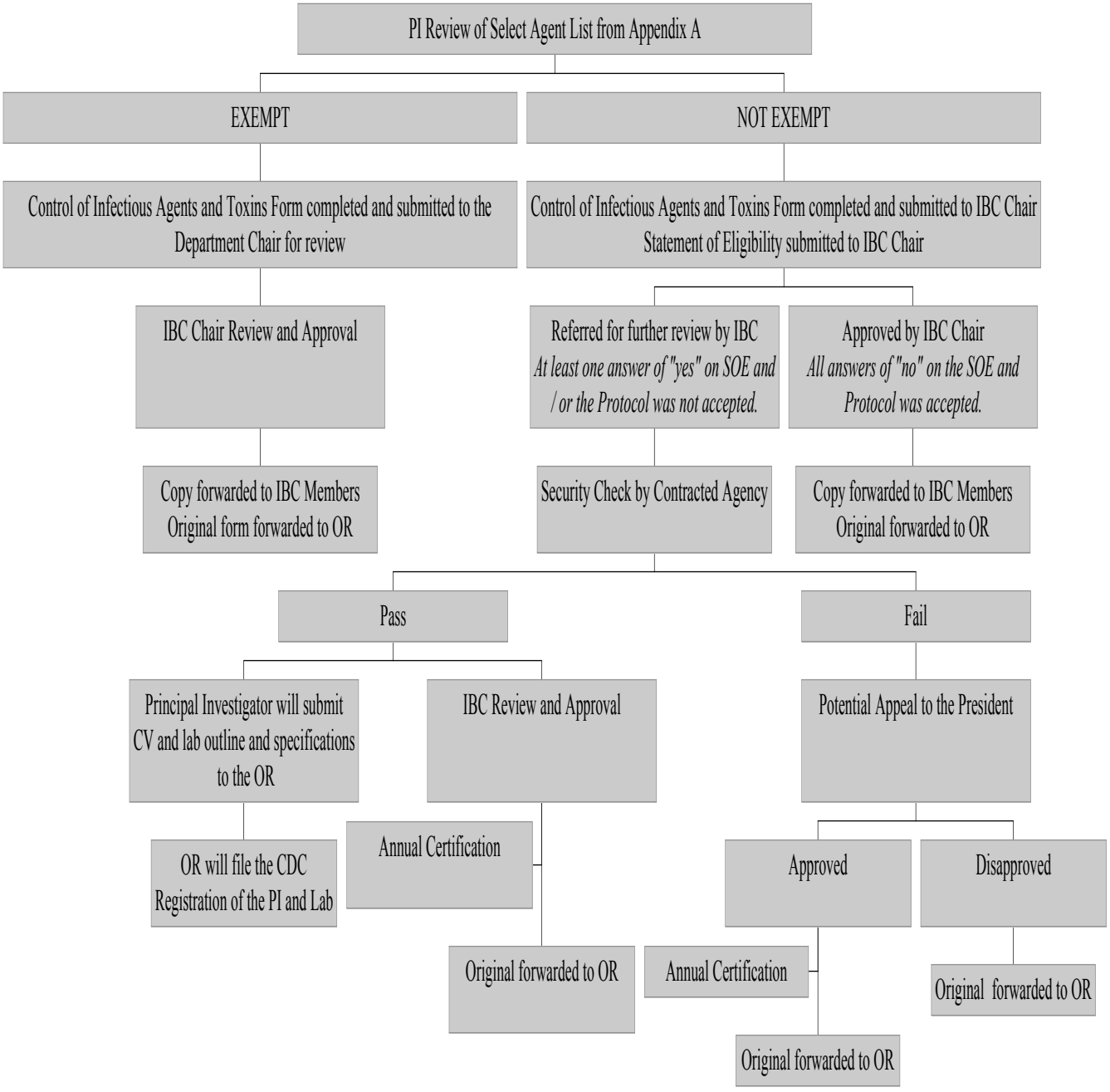
Appendix C

IBC Reveiw of the Use of Infectious Agents



Appendix D

IBC Reveiw of the Use of Select Agents



Appendix E

SELECT AGENT LIST

Viruses

( ) Crimean-Congo haemorrhagic fever virus
( ) Eastern Equine Encephalitis virus
( ) Ebola viruses
( ) Equine Morbillivirus (Hendra virus)
( ) Lassa fever virus
( ) Marburg virus
( ) Rift Valley fever virus
( ) South American Haemorrhagic fever viruses
    ( ) Junin,
    ( ) Machupo,
    ( ) Sabia,
    ( ) Flexal,
    ( ) Guanarito)
( ) Tick-borne encephalitis complex viruses
( ) Variola major virus (Smallpox virus)
( ) Venezuelan Equine Encephalitis virus
( ) Viruses causing hantavirus pulmonary syndrome
( ) Yellow fever virus

Bacteria

( ) *Bacillus anthracis*
( ) *Brucella abortus, B. melitensis, B. suis*
( ) *Burkholderia (Pseudomonas) mallei*
( ) *Burkholderia (Pseudomonas) pseudomallei*
( ) *Clostridium botulinum*
( ) *Francisella tularensis*
( ) *Yersinia pestis*

Rickettsiae

( ) *Coxiella burnetii*
( ) *Rickettsia prowazekii*
( ) *Rickettsia rickettsii*

Fungi

( ) *Coccidioides immitis*

Toxins

( ) Abrin
( ) Aflatoxins
( ) Botulinum toxins
( ) *Clostridium perfringens* epsilon toxin
( ) Conotoxins
( ) Diacetoxyscirpenol
( ) Ricin
( ) Saxitoxin
( ) Shigatoxin
( ) Staphylococcal enterotoxins
( ) Tetrodotoxin
( ) T-2 toxin

Recombinant organisms / molecules

( ) Genetically modified microorganisms or genetic elements from organisms listed above, show to produce or encode for a factor associated with disease.

( ) Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins listed above, or their toxic subunits

Appendix F

IBC Reveiw of the Use of Recombinant DNA