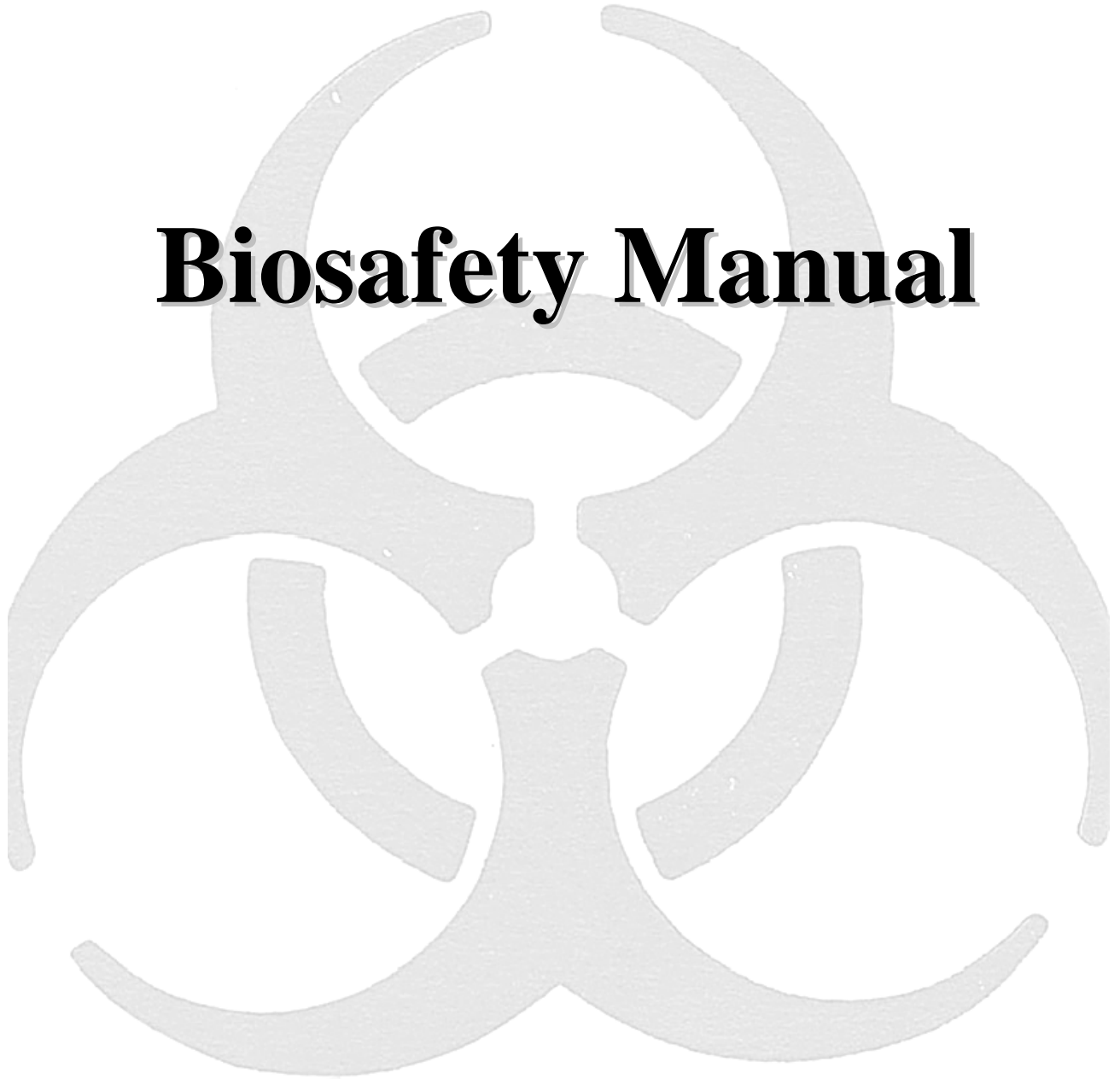


Biosafety Manual



University of Missouri – Kansas City

Contents

Table of Contents	1
Chapter 1 Biosafety program and responsibilities.....	5
1.1 Activities Covered by the Biosafety Plan and Definition of Biohazardous Materials	5
1.2 Roles and Responsibilities	5
1.2.1 Principal Investigators and Course Supervisors (Registered Users)	6
1.2.2 Research Personnel	7
1.2.3 Deans, Directors, and Department Heads	7
1.2.4 Institutional Biosafety Committee	7
1.2.5 Biological Safety Officer	8
1.2.6 Office of Research Services	8
1.2.7 Chancellor and Provost	9
1.3 Registration and Use of Biohazardous Materials.....	9
1.4 Coordination with IRB, IACUC, and/or Radiation Safety Committee.....	10
1.5 Closing a Research or Teaching Laboratory due to Non-Compliance	10
Chapter 2 Biological Risk Assessment	12
Chapter 3 Biological safety levels	14
3.1 Standard Microbiological Practices.....	14
3.2 Recommended Laboratory Practices for Biological Safety Levels	18
3.2.1 Biosafety Level 1 (BSL-1).....	18
3.2.2 Biosafety Level 2 (BSL-2).....	19
3.2.3 Biosafety Level 3 (BSL-3).....	21
3.3 Biosafety levels associated with animal and plant research.....	25
3.4 Animal Facilities	25
3.5 Plant Facilities	26
Chapter 4 National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules.....	27
4.1 NIH rDNA guidelines	27
4.2 Registration through the Submission of Research Protocols for Approval by the Institutional Biosafety Committee.....	27
4.2.1 Exempt Recombinant DNA Experiments	28
4.2.2 Initial Registration	28
4.2.3 Blood Borne Pathogens, Select Agents and Toxins, USDA Regulated Agents.....	29
4.2.4 Human Gene Transfer Experiments.....	30
4.2.5 Animal Experiments Involving rDNA Molecules or Infectious Agents.	30
4.3 Annual Renewal	30
4.4 Re-Registration.	31
4.5 Training	31
Chapter 5 Biological Risk Groups	34
5.1 Definition of Risk Groups as defined by the NIH Guidelines	34
5.2 Definition of Risk Groups as defined by the World Health Organization	35
Chapter 6 Biohazards	36
6.1 Biohazard sign information	36
Chapter 7 Biosecurity	38
7.1 Laboratory Biosecurity	38
7.2 Security at UMKC.....	39

7.2.1	Physical Security	40
7.2.2	Personal Security	40
7.2.3	Material control and accountability	40
7.2.4	Transport security	41
7.2.5	Information security	41
7.2.6	Program management.....	41
7.3	Select Agent program	41
Chapter 8 Principals and Concepts of working safely with biohazardous materials		43
8.1	Basic Biosafety Practices.....	43
8.1.1	Laboratory Practice and Technique	43
8.1.2	Safety Equipment (Primary Barriers).....	43
8.1.3	Facility Design and Construction (Secondary Barriers)	44
8.2	Laboratory practices.....	45
8.2.1	Basic personal protective equipment for the laboratory	45
8.2.2	Laboratory clothing	45
8.2.3	Eye safety	46
8.2.4	Gloves.....	46
8.2.5	Respiratory protection	46
8.3	Other protective equipment or methods.....	47
8.3.1	Footwear.....	47
8.4	Laboratory Emergency equipment.....	47
8.5	Housekeeping	47
8.6	Chemical, Radiological and mixed waste	48
8.7	Food and drink in the laboratory	48
8.8	Pipettes and Pipetting Aids	48
8.9	Syringes and Needles	49
8.10	Cryostats	49
8.11	Centrifuge Equipment	50
8.12	Blenders, Ultrasonic Disrupters, Grinders and Lyophilizes.....	50
8.13	Lyophilizer and Ampoules	51
8.14	Loop Sterilizers and Bunsen Burners	51
8.15	Transportation of infectious materials and biohazardous materials in and between laboratories on the UMKC campus	51
8.16	Hand washing	52
8.17	Laundry	52
Chapter 9 Laboratory Acquired Infections.....		53
Chapter 10 Working with Tissue Culture		55
10.1	Clinical Laboratories.....	55
10.2	Human Blood, Body Fluids, Tissue and Other Potentially Infectious Materials.....	56
10.3	Preventing the Transmission of Tuberculosis.....	57
10.4	Select Agent regulation	57
Chapter 11 Safety equipment.....		59
11.1	Biological safety cabinets (BSC).....	59
11.2	Laminar flow clean air benches	59
11.3	Biological safety cabinets.....	60
11.3.1	Biological safety cabinet work	64
11.3.2	Moving or disposal of a biological safety cabinet	65
11.4	Other Safety Equipment.....	66

Chapter 12 Biological spills	67
12.1 Spill involving recombinant DNA materials	67
12.2 Biological spill kit	67
12.3 Biological spill response plan	67
12.4 Elements of a biological spill plan risk assessment	68
12.5 Biological spill cleanup procedures	69
12.5.1 Biological spills within Biological safety cabinet	69
12.5.2 Biological spills in laboratory or common laboratory areas	70
12.5.3 Biological spills in a centrifuge	71
12.5.4 Biological spills with mixed hazards (biological /chemical, or radiological)	72
 Chapter 13 Other Safety programs	 73
13.1 Sharps policy	73
13.2 Sharps disposal	74
13.3 Emergency procedures	75
13.4 Bloodborne Pathogens Program and Exposure Control Plan	75
 Chapter 14 Infectious waste disposal and disinfection	 76
14.1 Infectious waste	76
14.1.1 Isolation waste	76
14.1.2 Contaminated surgical, dialysis and laboratory waste	76
14.1.3 Cultures and stocks of infectious agents and associated biologicals	76
14.1.4 Blood and blood products	77
14.1.5 Animal carcasses and tissues	77
14.1.6 Pathology wastes	77
14.1.7 Sharps	77
14.2 Disposal of Infectious Waste	77
14.3 Decontamination	78
14.3.1 Requirements for Recombinant DNA Disposal	79
14.3.2 Methods of Decontamination	79
14.4 Room Decontamination	81
14.5 Hand Hygiene in Research Laboratories	81
 Chapter 15 Shipping infectious materials	 83
15.1 Shipping of infectious materials via express couriers (Excluding USPS)	84
15.2 Category A infectious substances	84
15.2.1 UN 2814 Infectious substance affecting humans	84
15.2.2 UN 2900 Infectious substances affecting animals	85
15.3 Types of infectious material shipping	85
15.3.1 Packaging	86
15.3.2 Outside shipping container markings	86
15.3.3 Category A shipments	87
15.4 Category B infectious substances	89
15.4.1 Packaging for Category B infectious substances	89
15.5 Shipping documentation	91
15.6 Shipping with dry ice	91
15.7 Shipping companies	92
15.8 Training requirements	92
 Chapter 16 Resources	 93
16.1 Regulatory Agencies and Authorities	93
16.2 BMBL5	94
16.3 NIH-OBA	94
16.4 The American Biological Safety Association	94
16.4.1 ABSA Risk Group Classification for Infectious Agents	94

16.5	Centers for Disease Control and Prevention	95
16.6	U.S. Department of Labor	95
16.7	Public Health Agency of Canada	95
16.8	World Health Organization	95
16.9	Laboratory Biosafety Manual- Third Edition	95
16.10	International Air Transport Association (IATA)	95
16.11	United States Department of Transportation	95
Acknowledgments.....		96

Appendices

Appendix A

Application Form	IBC Application Form	
Application Form A-1	Recombinant DNA Registration Form (Exempt and Non-Exempt).....	
Application Form A-2	Registration of Biosafety Level 2 (BSL-2) and Biosafety Level 3 (BSL-3) Infectious Agents, Select Agents, and Biological Toxins.....	
Application Form A-3	Biosafety Level 3 (BSL-3) Containment Measures.....	
Application Form A-4	Registration of Blood Borne Pathogens and Other Potentially Infectious Material (OPIM).....	

Chapter 1

Biosafety program and responsibilities

The purpose of the UMKC Biosafety Plan is to establish and implement University policies regulating the use of Recombinant DNA molecules and other Biohazardous materials in research and teaching laboratories at UMKC. This policy does not apply to the use of biohazardous materials in patient care – unless involving a program of clinical research. The goal is to ensure that recombinant DNA molecules and Biohazards are utilized safely and in compliance with the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) and the NIH and CDC *Biosafety in Microbiological and Biomedical Laboratories*. The Biosafety Plan is designed to protect students, faculty, staff, and the public from potential adverse consequences arising from exposure to recombinant DNA molecules and biohazardous material used in UMKC research and teaching activities. While the overriding goal is to ensure safety, regulatory policies are intended to do so while at the same time facilitating the research and teaching activities that are regulated.

1.1 Activities Covered by the Biosafety Plan and Definition of Biohazardous Materials

All recombinant DNA work, whether it is exempt or non-exempt by NIH guidelines, must be registered with the UMKC Institutional Biosafety Committee, as must Biosafety Level 2 (BSL-2) or higher research activities (see Section 2.4). Currently, **BSL-4 research activities are not allowed at UMKC**. The level of detail involved in registering or regulating activities involving recombinant DNA or biohazardous materials should be dependent upon the hazard associated with the recombinant DNA molecules or biohazardous material under study. For example, the registration and regulation of research on BSL-2 level organisms might reasonably be expected to be less detailed and less involved than the registration and regulation of research on BSL-3 level organisms.

Biohazardous materials include any microorganism, or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing: 1) death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism; 2) deterioration of food, water, equipment, supplies, or material of any kind; or 3) harmful alteration of the environment. These include, but are not limited to:

- Certain bacteria, fungi, viruses, rickettsiae, protozoa, parasites
- Recombinant products
- Toxins of biological origin
- Allergens
- Cultured human or animal cells and the potentially infectious agents these cells may contain
- Viroids and prions
- Other infectious agents as outlined in laws, regulations, or guidelines.

Examples include all materials containing rDNA; transgenic animals or plants; human, animal or plant pathogens; biological toxins (such as tetanus toxin); human blood and certain human body fluids; select agents; high consequence livestock pathogens and toxins; and human or monkey cell cultures. **Specifically excluded are non-rDNA BSL-1 research activities.**

1.2 Roles and Responsibilities

Effective implementation of the UMKC Biosafety Plan requires the coordinated and cooperative actions of seven individuals or groups from within the campus community. Individuals may fall into more than one of these categories:

- Principal Investigators and Course Supervisors (Registered Users)
- Research Personnel
- Deans, Directors, Administrators, and Department Heads
- Institutional Biosafety Committee
- Biological Safety Officer
- Office of Research Services
- Chancellor and Provost

1.2.1 Principal Investigators and Course Supervisors (Registered Users)

The Principal Investigator (PI) has primary responsibility for the safe performance of all activities within his/her research laboratory. The PI must be knowledgeable of the policies and guidelines described in this plan and of the principals and procedures appropriate for the safe use of the specific recombinant DNA molecules and biohazardous materials used in his/her research. He/she must apply these principals and procedures to protect students, faculty, staff, and the general public from any undesirable consequences that might arise from the research activities in his/her laboratory. For teaching laboratories that are associated with courses, the Dean or Department Head of the academic unit that offers the course shall designate a Course Supervisor who shall have the same responsibilities for that teaching laboratory as PI's have for their research laboratories. The **minimal** responsibilities of the PI or Course Coordinator are:

- Performing a risk assessment and ascertaining the Biosafety level(s) appropriate for the recombinant DNA molecules or biohazardous materials being used in his/her laboratory. Ensuring that laboratory procedures, personal protective equipment, containment equipment, and laboratory facilities are appropriate for those Biosafety level(s), and ensuring that laboratory facilities are appropriately maintained.
- Select the appropriate section of the NIH Guidelines that the research falls under.
- Submitting research protocols and any subsequent changes to the Institutional Biosafety Committee (IBC) for review and approval prior to initiating rDNA or Biosafety Level 2 or 3 research activities or, when permitted by the NIH Guidelines, simultaneously with initiating these activities.
- Submitting any required annual renewal forms to the IBC for research activities.
- Developing or identifying appropriate training procedures or courses to ensure that all laboratory personnel are appropriately trained in the use of recombinant DNA molecules and biohazardous materials prior to commencing work in the laboratory.
- Developing or identifying appropriate procedures for refresher training of all laboratory personnel, to occur a maximum of 3 years after the date of the initial training.
- Ensuring that lab personnel (including the PI) take all required IBC administered, web based training modules and the accompanied exams, as described in Section 2.2 of this manual. This training is in addition to any lab-specific training developed and administered by the PI.
- Reporting any newly identified select agents, high consequence livestock pathogens or toxins and plant pathogens immediately to the Office of Environmental Health and Safety (EHS)
- Completing and posting appropriate biohazard signs, labels or other Notification Signage (Appendix G).
- Requesting collection of Biohazardous Unwanted Materials in a timely manner.
- Ensuring the availability of reference information on biological hazards, and ensuring that all staff understands how to use these references.

- Ensuring that all workers under his/her supervision use proper Personal Protective Equipment.
- Understanding the proper procedures to use in the event of a release or other emergency, and implementing those procedures as required.
- Working with EHS personnel to maintain safe work areas in compliance with UMKC policies and government regulations.
- Complying with shipping requirements for recombinant DNA and biohazardous materials.
- Completing proper Biohazardous Materials Laboratory Closure prior to termination of work with biohazardous materials.
- Report all significant violations, releases, spills, injuries or illnesses related to biohazardous materials use.

1.2.2 Research Personnel

Research Personnel include postdoctoral fellows, graduate students, technical staff, and undergraduate students who are doing research in the Principal Investigator's laboratory. Research Personnel are required to:

- Complete requirements for approval to work in the laboratory and ensure that all work is conducted in compliance with UMKC, NIH, CDC, OSHA and other applicable guidelines.
- Learn the operating procedures for the laboratory, the potential hazards of the infectious agents in use and emergency procedures. Help maintain the facility in good working condition.
- Report to the Principal Investigator any medical restrictions, reportable illnesses, and any event that may be an exposure or result in the creation of a potential hazard. Report all irregular conditions.
- If inexperienced in handling human pathogens or tissue cultures, receive training and demonstrate proficiency in standard microbiological practices from the Principal Investigator.
- Complete any medical surveillance requirements.
- Perform responsibilities assigned by the PI regarding the maintenance and operation of the research facility.

1.2.3 Deans, Directors, and Department Heads

The Dean, Director, or Department Head is responsible for the safe operation of all laboratories under his/her overall jurisdiction. They should demonstrate an ongoing commitment to the principles and practices of biosafety. The minimal responsibilities of the Dean, Director, or Department Head are:

- Ensuring that all individuals with exposure to biohazardous materials under his/her jurisdiction have access to a copy of the UMKC Biosafety Manual and understand the importance of compliance with the guidelines therein.
- Ensuring that the PI has the training that is commensurate with the proposed project and that the project design and monitoring methods meet institutional safety standards.
- Ensuring that appropriate facilities are available to contain biohazardous materials and to enable the PI to comply with pertinent campus policies.
- Ensuring that all work practices involving biohazardous materials are reviewed by the Institutional Biosafety Committee and that all accidents and incidents are reported to the Biosafety Officer.
- Ensuring that all eligible personnel are assigned to the appropriate occupational health care program.
- Assisting in the elimination of all known unsafe acts and practices.
- Nominating qualified members to the Institutional Biosafety Committee.

1.2.4 Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) has been charged by Federal law with the planning and implementation of the campus Biosafety Program with a purpose to ensure the health and safety of all personnel working with biohazardous materials. At UMKC, membership on the IBC is appointed by the Chancellor, upon recommendation of the Vice Provost for Research. The IBC consists of the Chairperson, faculty, and community representatives. Two community members, with no Institutional affiliation other than membership on the IBC, are required and appointed to represent the interest of the surrounding community with respect to health and the protection of the environment. The Vice Provost for Research is an *ex officio* member. The IBC as a whole represents collective expertise and research experience in biohazardous materials and biosafety in experiments that may pose potential risks to health or the environment.

The IBC is responsible for ensuring that research conducted at the Institution is in compliance with the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and the *Select Agent Rule*, drafting campus biosafety policies and procedures, overseeing the implementation of those procedures, and reviewing individual research proposals for biosafety concerns. The IBC does not oversee biosafety policies for affiliated hospitals, clinics, or clinical laboratories.

Principal Investigators (PIs), who wish to perform research using biohazardous materials, must submit an application to the IBC (see IBC web site at <http://www.umkc.edu/ors/ibc/>) for the application process and forms needed. The full Committee reviews all applications. Studies that involve work at Biological Safety Level BSL-2 or BSL-3 containment are pre-reviewed by a primary and secondary reviewer. All reviews include an assessment of the a) containment levels required by the *NIH Guidelines* for the proposed research, b) laboratory facilities procedures and practices, and c) training and expertise of personnel involved in the research activity.

The IBC is authorized by the Chancellor to limit or suspend any research that does not comply with biosafety policies and procedures set forth in this *Biosafety Manual* and in the *NIH Guidelines*. Additionally, noncompliance with basic procedures as determined during the laboratory inspection may result in the halting of research until corrective action is taken. The procedures to be followed for halting research or closing a laboratory due to non-compliance involve the IBC, Biological Safety Officer, PI, his/her Department Head and/or Dean, and Provost, and are described in Section 1.4 of this manual.

1.2.5 Biological Safety Officer

The Biological Safety Officer is a member of the Institutional Biosafety Committee, and will serve as the inspection arm to the IBC. The Biological Safety Officer works closely with Deans, Department Chairs, Principal Investigators, other academic leaders, and the IBC to ensure that biosafety policies and programs are followed throughout the University. Significant activities of the Biosafety Officer include:

- Providing technical advice and training to the IBC and researchers on laboratory containment concerning biosafety and biosecurity procedures
- Developing emergency plans for the handling of spills and personnel contamination.
- Conducting laboratory inspections to ensure that established safety standards are rigorously maintained <http://www.umkc.edu/ors/ibc/> - Appendix B.
- Investigating all accidents involving possible escape and/or exposure to potentially infectious or toxic materials and reporting findings and recommendations to prevent future occurrences to laboratory management and the IBC.
- Ensuring that decontamination procedures are followed after spills or breakage involving infectious materials and maintaining a record of accidents and incidents.
- Assisting in tracking sickness and absence among staff in cases associated with work on infectious agents.

- Ensuring the decontamination of used materials and equipment. Monitoring the safe disposal of infectious waste after treatment.
- Act as the Responsible Official to ensure that the requirements of 42 CFR Part 73 entitled, "Possession, use, and transfer of select agents and toxins rule" are met on behalf of the Institution.

1.2.6 Office of Research Services

The Office of Research Services is responsible for all official communication between UMKC and NIH, NSF, the USDA, and other Federal and State agencies. The Office of Research Services will maintain the IBC web site, oversee and administer the IBC-mandated training modules and examinations that are required of PI's and other research personnel engaged in certain types of research (described in Section 2.2 of this manual). The Office of Research Services will also be responsible for maintaining the appropriate documentation of training of research personnel.

1.2.7 Chancellor and Provost

The Chancellor is ultimately responsible for assuring that comprehensive campus-wide programs are in place for the safe handling of recombinant DNA molecules, infectious agents, and all other biohazardous materials at UMKC. As the Chief Academic Officer of the University, the Provost will be responsible for administration of UMKC Biosafety policies. This will include appointing members of the IBC and the Biological Safety Officer, and approving all IBC-recommended policies and procedures.

1.3 Registration and Use of Biohazardous Materials

The Institutional Biosafety Committee is charged with regulating two types of experiments: i.) those involving recombinant DNA molecules, and ii.) experiments involving infectious agents, biological toxins and other biohazardous materials, regardless of whether or not they involve recombinant DNA molecules.

The Principal Investigator (PI) has primary responsibility for the safe performance of all activities within his/her research laboratory. More specifically, the Principal Investigator's responsibilities fall into four general areas:

1. Registration of research involving recombinant DNA molecules and biohazardous materials through the submission of research protocols for approval by the Institutional Biosafety Committee;
2. Ensuring that all laboratory personnel (including the P.I.) are properly trained and proficient in the practices and techniques required for handling the rDNA molecules and biohazardous materials used in the laboratory;
3. Maintaining the laboratory and all equipment in a safe working condition, including posting appropriate biohazard warning signs at laboratory entrances and on freezers, refrigerators, or other containers that are used for storage of rDNA molecules or other biohazardous materials;
4. Participating in and facilitating Annual Biosafety Inspections of the laboratory by the UMKC Biosafety Officer.

Table 1 summarizes the registration, training, and inspection requirements that are the PI's responsibility.

Table 1. Summary of Principal Investigator Responsibilities

If using items below, requirements with an "X" must be followed	Required Registration							Required Training					Required Inspections			
	Form 1. Recombinant DNA and Biohazardous Materials Application	Form 2. Blood Borne Pathogens Registration	Form 3. Select Agents and Toxins Registration	Institutional Review Board	NIH, FDA	IACUC	CDC	USDA	Module 1. General Biosafety Training	Module 2. BL-3 Training	Module 3. Blood Borne Pathogens Training	Module 4. Select Agents and Toxins Training	IACUC	Annual Biosafety Inspection	CDC Select Agent Inspection	USDA Regulated Agents Inspectio
Recombinant DNA – Exempt	X															
Recombinant DNA – Non-Exempt	X							X						X		
Infectious Agents & Toxins BL-2	X							X						X		
Infectious Agents & Toxins BL-3	X							X	X					X		
Human Materials & Primary Human Origin Cell Lines	X	X		X				X		X				X		
Animal Use with BL-2 agents	X					X		X					X	X		
Animal Use with BL-3 agents	X					X		X	X				X	X		
Human Gene Therapy	X	X		X	X			X		X				X		
Select Agents -- See list in Section 12	X		X				X	X			X			X	X	
USDA Regulated Agents	X						X	X						X		X

1.4 Coordination with IRB, IACUC, and/or Radiation Safety Committee

The IBC is responsible for regulating the use of all recombinant DNA molecules, infectious agents, and other biohazardous materials at UMKC. Research that also involves human subjects, animals, or radioactivity is also subject to regulation by the appropriate UMKC Institutional Review Board, the IACUC, or the Radiation Safety Committee.

1.5 Closing a Research or Teaching Laboratory due to Non-Compliance

The Biological Safety Officer will work cooperatively with the PI to identify and discuss any ways in which work with recombinant DNA molecules or biohazardous materials is not in compliance with UMKC Biosafety policies. If problems of a non-emergency nature persist, the Biological Safety Officer may stop any work that creates an unreasonable risk to the health and safety of students, faculty, staff, or the general public, provided he/she has the written concurrence of the Chairperson of the IBC or, in his/her absence, the written concurrence of three other IBC members. As soon as possible, members of the IBC will meet and review the problem with the PI, his/her Department Head and/or Dean, and the Biological Safety Officer. The IBC will then send written recommendations to the Provost, who will make a final decision on the action to be taken.

In the case of a laboratory emergency that creates an imminent risk to the health and safety of students, faculty, staff, or the general public, the Biological Safety Officer may close the laboratory

immediately. He/she will immediately inform the Principal Investigator, his/her Department Head and/or Dean, and the Chairperson of the IBC of the problem. As soon as possible, members of the IBC will meet and review the problem with the PI, the Department Head and/or Dean, and the Biological Safety Officer to determine what actions must be taken to allow the laboratory to be reopened. If no resolution can be agreed upon, the IBC will send written recommendations to the Provost, along with any written recommendations from the PI or Department Head and/or Dean. The Provost will make a final decision on the action to be taken.

A decision by the IBC to close a research or teaching laboratory and/or an activity may be appealed.

- a. If approval of a proposal is denied by the IBC because there are serious concerns about the risk to the health and safety of students, faculty, staff, or the general public, the PI or course instructor can request an appearance before IBC to answer the questions raised by the Committee. The investigator or instructor may demonstrate, or be asked to demonstrate to the IBC, the procedures to be used in the research/teaching.
- b. If the first appeal to the Committee does not resolve matters to IBC's satisfaction, the investigator or instructor may, after consultation with the Vice Provost for Research and Provost, request a second meeting with the IBC. At this meeting the investigator or instructor may present expert witnesses or elsewhere to testify to the adequacy or necessity of the research/teaching being conducted. The decision of the Committee following this meeting will be final.

Chapter 2

Biological Risk Assessment

The biological risk assessment is the cornerstone of any safety program. A thorough risk assessment should be the starting point of any research with biological materials. The ability to define the risk of an activity and mitigate those risks is the core of biosafety. By performing a risk assessment the risk can be identified before actual work or procedures are performed. The risk may then be communicated and mitigated or reduced to an acceptable level. All of the risk identified cannot be reduced to zero risk. But, the foreseen risk is reduced to where potential of the risk is reduced or the outcome of that risk is greatly reduced. A risk assessment is not necessarily a straight forward process. Many variables have to be investigated and the effects of one variable on another can then influence the overall risk. A risk assessment should continually be reviewed and modified to meet changes in policy, procedures, personnel and technology.

A risk assessment is divided into groups to be able to appropriately assess the risk. These groups can be divided into the following:

Risk in relation to the organism:

- What is the organism
- Does the organism cause disease in an immunocompetent adult human
- What are the symptoms of the disease
- What is the infective dose
- What is the route of infection
- What is the survivability in the environment
- What is an appropriate disinfectant
- Is the organism genetically modified
- What is the nature of the donor gene or sequence
- What is the BSL of the donor organism

Risk in relation to the procedures:

- Will the agent be concentrated, How
- What is the volume being manipulated, over 10 liters
- Are there any procedures that have the potential for the production of aerosols
 - Grinding
 - Pipetting
 - Sonication
 - Large droplets
 - Centrifugation
 - Splashes
- Manipulations using sharps
- Use of animals

Risk in relation to the laboratory worker

- Biosafety training
- Bloodborne pathogen training
- Training of laboratory procedures
- Training documented with specific pieces of equipment
- Immune status of worker
- Training of emergency procedures and equipment
- Has the laboratory equipment been maintained, annually certified where required and documented

With the above items in mind an informed risk assessment can be made. The consultation of content experts, biosafety professionals and others can aid in preparing a sound risk assessment. The above list of items to cover in a risk assessment is not fully comprehensive. These items are just the basics to cover in a risk assessment. Specialized techniques, new technology or equipment and individual circumstances will require a varied approach with individualized sets of questions.

Chapter 3

Biological safety levels

In the following text are descriptions of biosafety level one through biosafety level three. The descriptions are only a part of what constitutes a biosafety level. Each of the biosafety levels build upon the previous level. The level of safety equipment, training and supervision increases as the biosafety level increase. A description of biosafety level three has been included; no BSL-3 facilities are available at this time at UMKC. For a further description refer to the U.S. Department of Health and Human Services publication jointly written by the Centers for Disease Control and Prevention and the National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th edition. The following descriptions of biosafety levels are direct quotes from the 5th edition of the BMBL.

The CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* lists a number of specific laboratory practices and procedures. The practices are divided into four biosafety levels. Biosafety levels (BSL) are a combination of practice and procedures, safety equipment and the facilities used. BSL-3 and BSL-4 experiments are currently not permitted at UMKC. BSL-3 and BSL-4 laboratory work require very specialized facilities to work safely.

3.1 Standard Microbiological Practices

Below are the Standard Laboratory Practices from the 5th edition of the BMBL. All eleven of the practices are identical in the four biosafety levels. These practices are the foundation for safe work at any biosafety level.

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items.

These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

- d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required.
 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Table 2

Biosafety Level 1 (BSL-1)	
Agents:	Agents not ordinarily associated with disease processes in humans, however, opportunistic pathogens and may cause infections in the young, the aged and the immunodeficient or immunosuppressed individuals.
Practices:	Containment relies on standard microbiological practices.
Safety Equipment: (Primary barriers)	No special primary or secondary barriers recommended. Sink available for hand washing.
Facilities: (Secondary barriers)	
Biosafety Level 2 (BSL-2)	
Agents:	Indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity.
Practices:	With good microbiological techniques, these agents can be used safely in activities conducted on the open bench top, provided the potential for producing aerosols or splashes is low. Primary hazards to personnel working with these agents relate to the accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Caution should be used when manipulating contaminated sharps. Procedures that have the possibility to produce splashes or aerosols should be conducted in a BSC.
Safety Equipment: (Primary barriers)	Class I or II Biological Safety Cabinet (BSC) or other physical containment devices used for the manipulations that could create splashes or aerosols of materials. Centrifuges should have aerosol containing safety cups. Personal Protective Equipment (PPEs) including laboratory coats, gloves, splash shields, face and eye protection as needed.
Facilities: (Secondary barriers)	Hand washing facilities and waste decontamination facilities must be available to reduce potential environmental contamination.

Biosafety Level 3 (BSL-3)

Agents:	Work is done with indigenous or exotic agents with a potential for respiratory transmission; and which may cause serious or potentially lethal infection. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.
Practices:	BSL-2 practice plus controlled access, decontamination of all waste, and decontamination of lab clothing before laundering. Laboratory staff receive specific training in handling pathogenic and potentially lethal agents. They must be supervised by scientist competent in handling infectious agents and associated procedures.
Safety Equipment: (Primary barriers)	Primary barriers include use of Class II BSC or other physical containment devices to protect personnel in contiguous areas, the community and the environment from exposure to potentially infectious aerosols. PPE includes the use of protective lab clothing, gloves, face and eye protection, and respiratory protection as determined by risk assessment.
Facilities: (Secondary barriers)	Laboratory access is controlled. Specialized ventilation requirements minimize the release of infectious aerosols from the laboratory.

3.2 Recommended Laboratory Practices for Biological Safety Levels

The CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) lists a number of specific laboratory practices and procedures that are recommended for BSL-1 through BSL-4 experiments. BSL-4 experiments are currently not permitted at UMKC. The recommended practices for BSL-1 through BSL-3 experiments are reprinted below from the BMBL.

3.2.1 Biosafety Level 1 (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

Special Practices

None required.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
 - a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratories windows that open to the exterior should be fitted with screens.

3.2.2 Biosafety Level 2 (BSL-2)

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that

- 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures
- 2) access to the laboratory is restricted when work is being conducted
- 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment. The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. When appropriate, a baseline serum sample should be stored.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a) Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b) Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
 - a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Chairs used in laboratory work must be covered with a non-

porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available.
9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

3.2.3 Biosafety Level 3 (BSL-3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment. A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
 - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. See Appendix G of the 5th edition of the BMBL.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:
 - a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
 - a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
 - b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
 - c. Ceilings should be constructed, sealed, and finished in the same general manner as walls. Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
 - a. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
 - b. The laboratory exhaust air must not re-circulate to any other area of the building.
 - c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
12. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and
15. supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
16. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

3.3 Biosafety levels associated with animal and plant research

3.3.1 Animal Facilities

The IACUC should be contacted for questions regarding the use of animals for teaching and research. Principal Investigators planning to use animals for any UMKC activity must submit an application to the IACUC for review prior to the start of the project, regardless of the source of funding for the project. A copy of the application can be obtained from the IACUC. (The completed form will include descriptions of experimental protocols, plans for animal care, available facilities, and information on the use of hazardous materials including infectious agents. All animal protocols involving the use of rDNA and infectious or transmissible agents must be submitted to the IBC for review prior to final approval by the IACUC.

Use of Animals in Research and Teaching

The use of animals in research and teaching is subject to state and federal laws and guidelines. Policy specifies that:

- All animals under the sponsorship of the Institution will be treated humanely;
- Prior to their inception, all animal projects receive approval by the Institutional Animal Care and Use Committee (IACUC).
- Researchers will comply with state and federal regulations regarding the use and care of animals.

Four biosafety levels are also described for activities involving infectious disease work with experimental animals. These four combinations of practices, safety equipment, and facilities are designated *Animal Biosafety Levels 1, 2, 3, and 4*, and provide increasing levels of protection to personnel and the environment. A full description of the requirements for Animal Biosafety Levels can be found in the 5th edition of the BMBL and also in Appendix Q of the NIH rDNA guidelines. Another resource for animal biosafety is the World Health Organization's *Laboratory Biosafety Manual*.

3.3.2 Plant Facilities

As with animal facilities there is specific guideline for biosafety levels for research involving plants. The NIN rDNA guidelines Appendix P outlines requirements for the four biological safety levels associated with plant research.

Chapter 4

National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules

4.1 NIH rDNA guidelines

The manipulation, use and research involving recombinant DNA that is federally funded through the National Institutes of Health are required to follow the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Lack of compliance with the NIH rDNA guidelines can result in **suspended, limited or termination of funds** for research. Recombinant DNA is defined in the guidelines as: (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, or (ii) molecules that result from the replication of those described in (i) above.

The University of Missouri-Kansas City is a NIH sponsored research institution and must comply with the NIH rDNA guidelines. All research at UMKC must comply with the NIH rDNA guidelines as a requirement of funding (Section I-D-1 and Section I-D-2). UMKC has convened an Institutional Biosafety Committee (IBC) to oversee the research of recombinant DNA as part of the agreement of receiving NIH funding. The UMKC IBC reviews and approves research activities based on meeting the requirements of the NIH rDNA guidelines and other federal guidelines and regulations. There are four levels of research experiments that are covered by the NIH rDNA guidelines. The four levels are 1) Exempt research that does not require approval 2) Research that requires IBC notification on initiation of the research 3) Research that requires IBC review and approval and 4) Research that requires NIH, the NIH Recombinant DNA Advisory Committee (RAC), IBC and other approval. All of the above levels of approval are described in detail in the NIH rDNA guidelines. The guidelines are set forth to provide oversight of recombinant DNA experiments and the appropriate assignment of biological safety containment of the research. The guidelines have been put in place to protect the use of funds and for the safety of the research into recombinant DNA activities.

All recombinant DNA activities at UMKC are under the oversight of the Office of Research Services. Applications for approval of research involved in recombinant DNA may be accessed through the Office of Research Services web site. The Office of Research Services web site is <http://www.umkc.edu/ors/>

Useful NIH links:

National Institutes of Health, Office of Biotechnology Activities:
<http://oba.od.nih.gov/oba/index.html>

NIH rDNA guidelines complete text: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

4.2 Registration through the Submission of Research Protocols for Approval by the Institutional Biosafety Committee.

The first step in registering research with the Institutional Biosafety Committee is to perform a risk assessment of the rDNA molecules and biohazardous materials that are used in the laboratory. The Risk Assessment first involves classifying infectious agents into one of four risk groups (RG-1 through RG-4) with **Risk Group 1 (RG-1)** of low or no hazard and **Risk Group 4 (RG-4)** representing highly infectious agents. A discussion of the risk groups along with a comprehensive list of Risk Group 1, 2, 3, and 4 agents can be found in Section 2.5 and *Appendix B* of the *NIH Guidelines*.

The research is next classified into one of four **Biosafety Levels (BSL-1 through BSL-4)** with regard to the level of containment and the laboratory practices that are required for safe performance of the research. Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Levels 2 and 3 involve increasingly dangerous infectious agents and require increasingly restrictive levels of containment and laboratory practices. A more complete discussion of the Biosafety Levels along with a list of BSL-1 through BSL-4 agents can be found in Section 2.6 and *Appendix C* of the *NIH Guidelines*. Also, The 5th edition of the BMBL can be consulted for the most current standard for the four biosafety levels.

At present, only BSL-1 through BSL-3 research is allowed at UMKC. Biosafety Level 4 (BSL-4) experiments require a very specialized containment facility, which is not available on the UMKC campuses. **BSL-4 experiments are, therefore, not allowed at UMKC.**

The risk assessment also requires the PI to determine whether research involving rDNA molecules is “Exempt” or “Non-Exempt” under the NIH Guidelines and that it is the responsibility of the PI to select the Section of the NIH Guidelines that their research falls under. The criteria by which research can be declared “Exempt” from the NIH Guidelines are discussed in Section 2 *Section III-F* of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*.

4.2.1 Exempt Recombinant DNA Experiments

Experiments using the following DNA molecules are exempt from the NIH Guidelines (adapted from Section III-F of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*). Nevertheless, all recombinant DNA research (both exempt and non-exempt) must be registered with the UMKC Institutional Biosafety Committee.

- Recombinant DNA molecules that are not in organisms or viruses.
- Recombinant DNA molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Recombinant DNA molecules that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- Recombinant DNA molecules that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- Recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such natural exchangers can be found in *Appendix A* of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, which is reprinted as E of this manual.
- Recombinant DNA molecules that do not present a significant risk to health or the environment, as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. A list of the classes of experiments that are exempt under this criterion can be found in *Appendix C* of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, which is reprinted as Appendix E of this manual.

4.2.2 Initial Registration

All recombinant DNA work must be registered with the Institutional Biosafety Committee, regardless of whether it is Exempt or Non-Exempt according to the NIH guidelines. In addition, all Biosafety Level 2 and 3 research must be registered with the IBC regardless of whether or not it involves recombinant DNA. Biosafety Level 1 (BSL-1) experiments that do not use recombinant DNA molecules do not need to be registered with the Institutional Biosafety Committee, nor do research protocols need be submitted to the IBC for these experiments. Laboratories that conduct exclusively BSL-1 experiments that do not utilize recombinant DNA are not subject to an Annual Biosafety Inspection.

To register the PI should:

- Download a copy of Form 1 “Recombinant DNA and Biohazardous Materials Application Form” from the Institutional Biosafety Committee web site (<http://www.umkc.edu/ors/ibc/>) A copy of this form is included as Appendix A of this manual.
- Complete the Application according to the instructions included on the Application form, save an electronic copy on his/her computer, and submit an electronic copy of the completed Application (without signatures) as an email attachment addressed to the IBC.
- Submit a hard copy of the completed Application, including the signatures of the PI, Department Head, and Dean, to the IBC by campus mail.
- Following review and approval of Applications for BSL-2 and BSL-3 research by the IBC, the PI will be contacted by the Biosafety Officer to schedule an inspection of the laboratory.

Biosafety Level 1 (BSL-1) experiments that fall within sections III-E or III-F of the NIH Guidelines may be initiated coincident with the submission of the Recombinant DNA and Biohazardous Materials Application Form to the IBC. BSL-2 and BSL-3 experiments and experiments that fall within sections III-A through III-D may not be initiated until all required approvals, including approval of the application submitted to the IBC, have been obtained. The IBC must give formal approval of such applications before research may begin.

IBC approval to work with a biological agent or recombinant DNA project is for a maximum period of three years, subject to annual renewals 1 and 2 years after the date of initial approval as described. After three years, the principal investigator must submit a new application if the program is to continue. If the program will not continue, the PI needs to notify the IBC.

4.2.3 Blood Borne Pathogens, Select Agents and Toxins, USDA Regulated Agents

Research with some agents requires completion of additional registration forms besides Appendix A “Recombinant DNA and Biohazardous Materials Application Form.”

Work with known blood borne pathogens, human blood, body fluids, or tissues requires registration by submission to the IBC of a completed Form which can be downloaded from the IBC web site (<http://www.umkc.edu/ors/ibc/>) and is included in Appendix A of this manual.

Work with “Select Agents” requires submission to the IBC of a completed the appropriate form which can be downloaded from the IBC web site, and is included as Appendix A of this manual. In addition, the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires that all persons in possession of any “Select Agent” notify the Secretary of the Department of Health and Human Services (DHHS). The Center for Disease Control and Prevention (CDC) has been designated as the agency responsible for providing guidance on this notification to the Secretary, DHHS. A list of “Select Agents” is maintained by the CDC and USDA at <http://www.selectagents.gov/>. PI's working with Select Agents should contact the IBC for more information regarding how to register with DHHS.

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 also requires all persons in possession of any “High Consequence Livestock Pathogens and Toxins” and Plant Pathogens notify the Secretary of the Department of Agriculture (USDA). The Animal and Plant Health Inspection Service (APHIS) has been designated as the agency responsible for providing guidance on this notification to the Secretary, USDA. A list of these USDA regulated agents is maintained on-line at <http://www.aphis.usda.gov/>. PI's working with USDA regulated agents should contact the IBC for more information regarding how to register with the USDA.

4.2.4 Human Gene Transfer Experiments

Before initiation, proposed clinical trials involving human gene transfer require registration and approval by the IBC, the Institutional Review Board (IRB), administrative committees of any participating hospitals, as well as federal agencies. NIH defines human gene transfer as the “the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects.” Federal requirements (NIH and FDA) for these experiments are described in detail in *Appendix M* of the NIH Guidelines for Research Involving Recombinant DNA, May, 1999, and in the Code of Federal Regulations, 21 CFR, Part 312 (FDA Points to Consider). PI's contemplating human gene transfer research should contact the IBC for more information regarding registration with the appropriate University and hospital committees and appropriate federal agencies.

4.2.5 Animal Experiments Involving rDNA Molecules or Infectious Agents.

Animal experiments involving recombinant DNA molecules and/or infectious agents require registration with both the IBC and IACUC. PI's should contact the IBC for guidance on the required registration procedures.

4.3 Annual Renewal

Annual renewal of IBC approved protocols is required at 1 and 2 years after the date of initial approval. Approximately 60 and 30 days before the end of the approval period, the IBC will send the PI, by email attachment, a copy of an Annual Renewal Form for each IBC approved protocol. A copy of this form can also be downloaded from the Institutional Biosafety Committee web site site (<http://www.umkc.edu/ors/ibc/>) and is also included as Appendix A of his manual.

To complete the Annual Renewal the PI should:

- Complete the Annual Renewal form according to the instructions included with the form, save an electronic copy on his/her computer, and submit an electronic copy of the completed form as an email attachment addressed to the IBC.
- Submit a hard copy of the completed form, including the PI's signature to the IBC by campus mail.
- Minor changes in laboratory protocols should be reported on the Annual Renewal Form. The Annual Renewal should also reflect any changes in personnel participating in the research since the time of last annual renewal or the initial registration, whichever was more recent. However, the PI must not wait until the next annual renewal to inform the IBC of any new personnel participating in the project. New personnel must be properly trained (see below) before commencing work and proper documentation should be submitted to the IBC at that time.
- The PI will be informed of IBC approval of the Annual Renewal by email and campus mail.

- Major changes in laboratory protocols require the submission of a new “Recombinant DNA and Biohazardous Materials Application Form” as described above for the Initial Registration.
- Changes in the location where BSL-2 or BSL-3 research is performed will require inspection of any new laboratory space by the Biosafety Officer as described for the Initial Registration, or decommissioning of any vacated space as described in Section.

4.4 Re-Registration.

Any IBC approved protocol may be subject to annual renewal a maximum of two times. Three years after initial approval of the protocol the PI must submit a new “Recombinant DNA and Biohazardous Materials Application Form” as described in Section 2.1.1 for the Initial Registration. At approximately 60, and again at 30 days before the deadline for re-registration, the IBC will remind the PI of the need to submit a new “Recombinant DNA and Biohazardous Materials Application Form”. However, PI must file a revised registration form at any time that significant changes are made in the approved protocol.

4.5 Training

All persons working with non-exempt recombinant DNA molecules or biohazardous materials must have appropriate training. The *NIH Guidelines* state that it is the responsibility of the Institution "to ensure appropriate training for principal investigators and laboratory staff regarding safety and implementation of the *NIH Guidelines*" (*Section IV-B-1-h*). In many cases, the individual most knowledgeable about the rDNA molecules and biohazardous materials used in the laboratory and best qualified to train others is the P.I. The Principal Investigator is responsible for training all personnel in his/her laboratory on the hazards of the agents that they will be working with and the appropriate laboratory procedures to safely use these agents. The PI will determine the type of training for every individual, based on their contact with the project.

In addition to training directed by the PI, all individuals (including the PI) working with BSL-2 or BSL-3 materials or with non-exempt recombinant DNA molecules are required to take an appropriate web-based Biosafety course, administered through the IBC web site, before they commence working in the laboratory. Once the initial training has been received, refresher training is required every 3 years. Where applicable, the PI and laboratory personnel must also take an IBC-administered web-based course on Blood Borne Pathogens, Select Agents and Toxins, or Agricultural Pathogens and Toxins. These courses must be taken at the time of initial protocol review followed by annual refresher training. The IBC Administrator will inform the PI at the time of initial protocol submission if any of these additional training courses is required.

The training courses required of individuals working in particular classes of research is summarized in Table 1 and also below.

Biosafety Level 1 (BSL-1) Experiments that Do Not Use Recombinant DNA

Individuals involved in Biosafety Level 1 (BSL-1) experiments that do not use recombinant DNA molecules are not required to take any training other than that specified and administered by the PI. Specifically, they are not required to take any of the Biosafety courses administered by the IBC.

Exempt Recombinant DNA Experiments at Biosafety Level 1 (BSL-1)

Individuals involved in Biosafety Level 1 (BSL-1) research that involves rDNA molecules that are “Exempt” under the NIH Guidelines are not required to take any training other than that specified and administered by the PI. Specifically, they are not required to take any of the Biosafety courses administered by the IBC.

Non-Exempt Recombinant DNA Experiments and Biosafety Level 2 (BSL-2) Research

Individuals engaged in Non-Exempt Recombinant DNA experiments and/or Biosafety Level 2 research are required to take the web-based “Training Module 1 General Biosafety Training” and the accompanying examination administered through the IBC web site. Investigators are referred to Appendix C of this manual “Biosafety Training Program,” and the IBC for further information.

Biosafety Level 3 (BSL-3) Research

Individuals engaged in Biosafety Level 3 research are required to take the web-based “Training Module 1: General Biosafety Training” and “Training Module 2: Biosafety Level 3 Training,” as well as the accompanying examinations administered through the IBC web site. Investigators are referred to Appendix C of this manual “Biosafety Training Program,” and the IBC for further information.

Research Involving Human Materials, Primary Human Origin Cell Lines, or Known Blood Borne Pathogens

Individuals engaged in research involving Human Materials, Primary Human Origin Cell Lines, or known Blood Borne Pathogens are required to take the web-based “Training Module 1: General Biosafety Training” and “Training Module 3: Blood Borne Pathogens Training,” as well as the accompanying examinations administered through the IBC web site. Investigators are referred to Appendix C of this manual “Biosafety Training Program,” and the IBC for further information.

Research Involving Select Agents and Toxins

Individuals engaged in research involving Select Agents and Toxins are required to take the web-based “Training Module 1: General Biosafety Training” and “Training Module 4: Select Agents and Toxins Training,” as well as the accompanying examinations administered through the IBC web site. Investigators are referred to Appendix C of this manual “Biosafety Training Program,” and the IBC for further information.

Laboratory Courses

Instructors in laboratory courses involving recombinant DNA molecules, BSL-1 or BSL-2 infectious agents are required to undergo the same training as PI's and research personnel using those items in research laboratories. However, since the purpose of these courses is to train students in the use of these agents, students enrolled in the courses are not required to taking any IBC administered training programs. Training of the students is the sole responsibility of the course instructor.

Documentation of Training

The University is required by Federal statute to maintain documentation that personnel using rDNA molecules or biohazardous materials have the appropriate training. The UMKC Office of Research Services will maintain such documentation. When PI's and other research personnel take an IBC administered training module and pass the accompanying web-based exam they will be automatically entered into the Office of Research Administration Training Data Base. This will result in the required documentation of training.

Biosafety Inspections

The Biological Safety Officer will conduct an Annual Biosafety Inspection of all registered laboratories conducting BSL-2 or BSL-3 experiments and/or research involving Non-Exempt Recombinant DNA molecules. These inspections will be based on the Annual Biosafety Inspection Checklist created and maintained by the BSO of each institution for which the UMKC IBC is the IBC-of-record.

Chapter 5

Biological Risk Groups

The following are NIH requirements for Campus Research with Recombinant DNA in humans, animals, and plants. As part of the registration process for experiments with recombinant DNA molecules, the PI must make a determination of the Risk Group (RG) that is most appropriate for the experiments in his/her laboratory. A detailed description of the NIH Risk Groups can be found in the NIH Guidelines for Research Involving Recombinant DNA Molecules http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

A risk groups is a system of classification given to microorganisms that assesses the relative risk to cause disease in a healthy adult. The Biosafety Microbiological and Biomedical Laboratories, 5th edition, uses risk assessments to assign a risk group to an organism. Other agencies and institution from around the world do describe Biological Risk Groups. Below are examples of descriptions of risk group descriptions from National Institutes of Health *Guidelines for Research Involving Recombinant DNA molecules* and the Third edition of the World Health Organization, *Laboratory Biosafety Manual*. Those involved in recombinant DNA activities in the US will have responsibilities to the NIH risk group classifications. Risk group classifications differ slightly between the NIH and thw WHO. The risk groups for WHO are based on the “principal characteristics and the route of the natural disease” (BMBL, 5th edition). The NIH guidelines base the risk groups on the basis of hazard, primarily from aerosol exposure. Risk group four (RG-4) organisms are not allowed at UMKC. Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria:

5.1 Definition of Risk Groups as defined by the *NIH Guidelines*

Risk Group 1 (RG1)

Agents are not associated with disease in healthy adult humans.

Risk Group 2 (RG2)

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

Risk Group 3 (RG3)

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

Risk Group 4 (RG4)

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

5.2 Definition of Risk Groups as defined by the World Health Organization (Laboratory Biosafety Manual, 3rd edition, 2004)

Risk Group 1 (no or low individual and community risk)

- A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 (moderate individual risk, low community risk)

- A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

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

- A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk)

- A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Chapter 6

Biohazards

6.1 Biohazard sign information

Biohazard symbol was originated in 1967 by Charles L. Baldwin (Dow Biohazards Research & Development) and Robert Runkle (National Cancer Institute). They stated that the symbol “shall be used to signify the actual or potential presence of a biohazard and shall identify equipment, containers, rooms, materials, experimental animals or combinations thereof which contain or are contaminated with viable hazardous agents”. What is a biohazard? One good definition is “those infectious agents presenting a risk or potential risk to the well being of man, either directly through his infection or indirectly through disruption of his environment” Examples of biohazardous materials are; bacteria, viruses, infected animals, blood, protozoan and fungi. The biohazard sign is posted when Biosafety level two, BSL-2, organisms are present.

The Occupational and Safety Hazards Administration describes the use and description of the biohazard symbol in 29 Codified Federal Regulation §1910.145. OSHA defines biological hazard or biohazard “means those infectious agents presenting risk of death, injury or illness to employees.” Below is an excerpt directly from 29CFR 1910.145.

“The biological hazard warning shall be used to signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals, or combinations thereof, which contain, or are contaminated with, viable hazardous agents. For the purpose of this subparagraph the term "biological hazard," or "biohazard," shall include only those infectious agents presenting a risk or potential risk to the well-being of man.” “Biological hazard tags shall be used to identify the actual or potential presence of a biological hazard and to identify equipment, containers, rooms, experimental animals, or combinations thereof, that contains or is contaminated with hazardous biological agents.”

The current fifth edition of the *Biosafety in Microbiological and Biomedical laboratories*” published by the Department of Health and Human Services states in the description of Standard Microbial Practices for all biosafety levels “ A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.”

The symbol design for biological hazard tags shall conform to the design shown below:

BIOLOGICAL HAZARD SYMBOL CONFIGURATION

Examples:





EHS office 816-235-5241
Biosafety office 816-235-1844
UMKC Police 816-235-1515

2008-05-14



EHS office 816-235-5241
Biosafety office 816-235-1844
UMKC Police 816-235-1515

2008-05-14

Biohazard signs that can be seen on the UMKC campus:

The biohazard signs are generally the biohazard symbol in black printed on a fluorescent orange to red-orange background. Lettering is a contrasting color, generally black. **Note:** These Biohazard Warning Signs are in addition to the Emergency Notification Sign posting required for all laboratories on the UMKC Campus.

The BIOHAZARD sign can be found on any of the following:

- Refrigerators, freezers
- Incubators
- Waste containers, BIOHAZARDOUS material may be inside
- Containers (storage, transportation, specimen etc.)
- Entrance doors to laboratories
- Sharps containers
- Animals and/or animal caging

This is only a partial list and the biohazard symbol can be placed anywhere based on the research being performed in the lab.

Biohazard signage can be acquired through the Office of Research Services or the Biosafety Officer.

If you have any questions please feel free in contacting the Office of Research Services at 816-235-5600, UMKC EHS office at 816-235-5242 or the Biosafety Office at 816-235-1844.

Chapter 7

Biosecurity

Biosecurity is defined **as protection of high-consequence microbial agents and toxins, or critical relevant information against theft or diversion by those who intend to pursue intentional misuse.** The following biosecurity issues should be considered by all laboratories handling biohazardous agents:

- risk and threat assessment
- facility security plans
- physical security
- data and electronic technology systems
- security policies for personnel
- policies regarding accessing the laboratory and animal areas
- specimen accountability
- receipt of agents into the laboratory
- transfer or shipping of biohazardous agents from the laboratory
- emergency response plans
- reporting of incidents, unintentional injuries, and security breaches

As part of a biosecurity program and to comply with Federal legislation, special procedures are required for the possession and transfer of specific biohazardous agents included in lists of CDC/USDA Select Agents or APHIS Regulated Agents (see above).

As part of the Institutional biosecurity program, the PI should address the following issues in the conduct of research activities:

- a. personnel suitability and reliability (including student access)
- b. pathogen accountability (both on-site and through the transfer process)
- c. response to biosecurity incidences.

7.1 Laboratory Biosecurity

What is Laboratory Biosecurity? What does it provide?

Laboratory Biosecurity is a set of preventative measures designed to reduce the risk of intentional removal (theft).

What is “intentionally removed”?

The natural thought would be a pathogen of high consequence. But this is not necessarily true. Other valuable assets can be stolen. These include toxins, genetic sequence data, rare samples, one of a kind equipment, experimental data and computer data to name a few. While the theft of an organism can have the potential for catastrophic results, other assets can be as damaging to an institution. The institution’s image can be damaged; funding removed and public outcry can all have negative effects.

What can be done to prevent an intentional removal of an asset?

A fully developed and functioning biological safety program is a benefit to a biological security program. Both programs can complement the other. As with a good biological safety program, a good biological security program begins with the risk assessment. An institution has to have an understanding of what is an acceptable and what is not an acceptable risk. The first step of the risk assessment is to determine what the assets are and where are they located. Are these assets of interest to a person or group? Is there an inventory of those assets? Who are the responsible

individuals for those assets? Do the inventory and the physical inventory match? Does the asset pose a threat to be easily used for malicious activities?

The second step is to determine: Who would have cause to steal the asset?

An institution must look at internal and external entities when determining who would steal the asset. An individual would have to have three things to be able to steal an asset. Those three things are; motive, means and opportunity. An “insider” who has access can be more of a threat than an external threat. The insider can have access to the materials and knowledge of the details of those materials, means and opportunity. Many times the insider threat is an upset employee, motive. An external threat for theft can be motivated by nationalistic or extremist activities, animal rights, revenge or protest. The means and opportunity for the external threat can be the limiting factor to discourage a theft.

What are ways to discourage the theft of biological material and associated assets?

Salerno and Gaudioso describe six components to Biosecurity. The components are:

- 1) Physical security
- 2) Personal security
- 3) Material control and accountability
- 4) Transport security
- 5) Information security
- 6) Program management.

(Salerno, R. and Gaudioso, J. 2007, *Laboratory Biosecurity Handbook*, Boca Raton, FL, CRC Press)

Physical security can include such things as fences, locked doors and restricted access by the use of security systems.

Personal security looks at the individuals that have access to the asset. Routinely before being hired an institution will perform a routine background check, verify references and academic and/or work histories. If an individual will work with a high consequence pathogen the level of investigation may be increased. Another area commonly investigated is credit histories. Foreign nationals may be excluded because of the nation of origin or affiliations. In the United States any workers involved in a Select Agent program must pass a Department of Justice background check.

The development of an inventory and tracking program is key to meeting the Material Control and Accountability. An institution or laboratory must know what they have, how much of the asset they have, where it is, who has it, who is the responsible individual for the asset to determine if something is even missing. If you do not know what you have then you will never know if it has been stolen. Just having an inventory is just the beginning. A well organized laboratory, freezer, refrigerator or storage area is essential. If something seems out of place it is easy to recognize and investigate. If the area is in disarray a theft could go unnoticed for a considerable amount of time or not at all. Remember that the asset that is the target of theft may not be just an organism but can be data, computer files and the like. Many of these items may not easily be noticed if missing, copied or altered.

7.2 Security at UMKC

Most laboratories working with biological materials at the University of Missouri-Kansas City do not work with high consequence pathogens. So, the level of security will be less involved. Individual laboratory biosecurity plans at the University of Missouri-Kansas City should include the applicable components for biosecurity identified above.

7.2.1 Physical Security

Securing of assets

All research is valuable. The results of the research can be a valuable organism, toxin, protein, animal model, assay or combination. The materials in all phases of research should be secured when not being manipulated. These assets have value beyond the time and efforts that have went in creating them. They should be secured. For most research materials and products locking the door to the laboratory and the freezer or refrigerator is all that would be necessary.

Laboratory access

Access to any laboratory should be limited and controlled. Access to the laboratory should be accessible to only those involved in the research of that lab. Laboratory access includes animal areas, freezer/refrigerator areas, data, note books and other areas where organisms or toxins are located. Most labs at UMKC are access limited by issuing keys to the individuals involved in the lab. Also, the Chemical and Biological science building limits access to the fourth and fifth floors by requiring a key in the elevator for those floors. Other areas that can limit access are to secure freezers and refrigerators with locks.

7.2.2 Personal security

Personal reliability

Each laboratory should carefully determine who has access to the laboratory, who has access to the biological materials in the laboratory and any sensitive information that may be in the laboratory. Any individual that has been granted access must be reliable in their activities and be trustworthy in the information that they acquire through participation in the laboratory research. The Select Agent program requires background checks to insure that an individual meets personal reliability requirements to be able to work with select agents.

7.2.3 Material control and accountability

Inventory

How would one know if anything was stolen or misplaced or even what is in the laboratory?

A laboratory inventory of biological agents and toxins should be generated for all laboratories. This is the only way to have a complete description of the items held in the laboratory. This document needs to be a living document. This means that the inventory is verified at least once a year. Uses, transfers and reconciliations of the inventory need to be documented.

Items that should be included in the inventory are:

- 1) What is the organism or toxin?
- 2) Where did it come from?
- 3) Where is it stored?
- 4) Disposition (Has it been used completely, transferred to another lab or destroyed)
- 5) Is it a genetically modified organism?
- 6) What was the donor organism?
- 7) What is the host organism?

These are just a few items that should be included. Depending on the nature of the research and the safety requirements of the organism the list may be expanded.

These inventories should include the microorganisms as well as any laboratory animals used in the course of research. Simply having a list is not the complete story. An inventory should describe the source of the materials, location stored, if the stock has been used, when, by who and the final

disposition. Was the materials used completely in a procedure, autoclaved or transferred to another laboratory. This type of information will be useful when the laboratory is audited both internally and by external entities.

7.2.4 Transport security

Transportation security can pose an area of exposure to theft. A good chain of custody system with transporting an item within an institution or externally is needed. A chain of custody will provide documentation of who had access and where the asset was. The ability to back track in the case lost asset will help determine if it was a true loss or a theft. The documentation will provide the most likely point of loss. This may aid authorities in the investigation in certain circumstances. The chain of custody documentation will also be useful in the reconciliation of the inventories of the shipping and receiving laboratories.

7.2.5 Information security

All forms of research, manufacturing and investigation create a considerable amount of data. The data can be in many varied forms. The data in many cases can be as if not more important than the organism that is being manipulated. The information may be the process of developing new pharmaceuticals, agent pathogenicity, genetic information or production processes. The loss of this information can have economic, environmental and public impact. The information can be used to gain a competitive advantage in the market place, first to publish or aid in the malicious use of an organism. The information can be saved in many varied form. The information can be held in laboratory notebooks, CDs, flash memory, institutional servers or personal computer. All of these items need to be safe guarded against theft of the information. This can be accomplished by the use of secure servers, limiting access to computer systems with access codes and physically securing electronic data storage devices.

7.2.6 Program management

The Biosecurity program at UMKC is a joint responsibility between the individual Principal Investigator of Professor and the Biological safety program. The programs should be reviewed on an annual basis. The Biosecurity program should be updated to reflect any changes in the regulatory climate. The Biosecurity should be tested throughout the year to determine if there are any weaknesses.

7.3 Select Agent program

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 required the HHS Secretary by regulation to establish a list of each biological agents and toxins that has the potential to pose a severe threat to public health and safety. The list is updated regularly. The Centers for Disease Control and the United States Department of Agriculture maintain a combined on-line registry (<http://www.selectagents.gov/>) of Select Agents and the USDA/APHIS maintains an on-line list of Regulated Agents (<http://www.aphis.usda.gov/>) that have potential to affect human and animals. Some agents are common to both list and are called overlap agents.

The Select Agent program requires an increased level of risk assessment, security procedures and personal reliability. Codified Federal regulation 7 CFR Part 331.11, 9 CFR Part 121.11 and 42 CFR Part 73.11 describe the procedures and requirements for being secured to possess select agents. Possessing and working with select agents requires federal security clearances, application with the agency that has oversight for the agent in question providing security assessments of the laboratory and the appointment of a Responsible Official for the institution.

If Select Agents are considered for research, University officials and Environmental Health and Safety should be consulted before the agent is acquired. There are very stiff penalties and possible jail time for violating the select agent laws. The specifics of the select agent requirements can be found at <http://www.selectagents.gov/>.

Chapter 8

Principals and Concepts of working safely with biohazardous materials

8.1 Basic Biosafety Practices

Biosafety practices can be divided into three areas. The three areas are practices and techniques, primary barriers and secondary barriers. Persons working with biohazardous materials must be aware of potential hazards and must be trained and proficient in specific policies and procedure, primary barriers and secondary barriers.

8.1.1 Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required to handle such material safely. The director or person in charge of the laboratory is responsible for providing or arranging the appropriate training of personnel. Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or material. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment. When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

8.1.2 Safety Equipment (Primary Barriers)

Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Open-fronted Class I and Class II biological safety cabinets are primary barriers which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment. An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this hazard, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents that can be transmitted through the aerosol route of exposure. Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with biological safety cabinets and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in biological safety cabinets, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal

studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

8.1.3 Facility Design and Construction (Secondary Barriers)

The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents which may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities. When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the *Applications Handbook for Heating, Ventilation, and Air-Conditioning (HVAC)* published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).

Common biosafety practices:

- Identify and evaluate risks to develop a laboratory specific exposure control plan.
- Develop procedures for the specific biohazards present. Microbiological procedures or techniques to handle unknown biohazardous material, such as diagnostic use, should be designed to assume the worst-case risk scenario. Knowing how infectious organisms are transmitted and what their infectious dose is can help in evaluating the risk and avoiding infection. Information about the organism(s) should be gathered prior to commencing work with them.
- Know where information resources for biohazardous materials can be found.
- Collect and communicate all the facts and information resources for biohazardous materials to appropriate personnel to minimize exposure risk.
- Make sure all biohazard signs and labels are present.
- Post appropriate biohazard signs and labels to assure only authorized personnel, informed of potential risks, enter areas where biohazardous material are used.
- Utilize appropriate safety equipment and facility design for the Biosafety level.
- Primary containment safety equipment, such as biological safety cabinets, is designed to reduce or eliminate exposure to biohazardous materials. Secondary containment facility design is intended to contain biohazardous materials in the laboratory so that they cannot cause harm to the general public or the environment.
- Maintain good housekeeping and personal hygiene.

- Good housekeeping is the most important step to improve safety. Good housekeeping also leaves a good impression upon visitors. Floors, laboratory benches, equipment, and other surfaces should be disinfected routinely. All biohazardous material waste should be autoclaved, sterilized, or placed in a biohazard Unwanted Materials container for disposal. Personal hygiene, such as frequent hand and laboratory clothes washing, should be observed at all times.

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

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

8.2 Laboratory practices

8.2.1 Basic personal protective equipment for the laboratory

"Safe science is great science", said Jim Welch, Executive Director, Elizabeth R. Griffen Research Foundation. Safety and good science goes hand in hand. To be safe in the laboratory, basic personal protective equipment needs to be provided and used properly. PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. Personal protective devices and safety equipment must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. For any laboratory where research is being conducted the basic personal protective equipment (PPE) is a laboratory coat, safety glasses and gloves. Other forms of PPE include but not limited to foot protection, hearing protection and respiratory protection. The PPE protects both the researcher and the research materials being manipulated. The contamination to the researcher can create possible health problems short and long term. Contamination of the research can create delays in research, increased cost and misleading results. A sign should be posted on the entry door of the laboratory to indicate the required PPE to enter the laboratory.

8.2.2 Laboratory clothing

Laboratories at UMKC should require the use of a lab coat, smock, scrub or jump suit for personal protection. Each laboratory should have a supply of laboratory coats or clothing that is specific to the situation. These coats can be reusable or disposable. The laboratory coat should be sized correctly, have long sleeves and be long enough to cover past the waist. The reusable laboratory coats can be supplied for the lab staff. Reusable laboratory garments should be capable of with standing autoclaving in the case of contamination biological or infectious materials. The laboratory coats should be laundered on a regular basis. The laboratory coats should not be taken home to be laundered. Visitors should be supplied with disposable paper coats or designated visitor reusable lab coats. Disposable lab coats can be a viable alternative for certain laboratory procedures.

Teaching laboratories should require the students to have a laboratory coat in any laboratory section along with safety glasses or goggles. Gloves should also be provided in the laboratory, the type will be based on an assessment of the materials and manipulations being performed.

Laboratory coats, smocks or gowns etc. should remain in the laboratory and should be removed when leaving the laboratory. A PPE specific sign or including required PPE on the biohazard sign on the entry door to the laboratory should be posted.

8.2.3 Eye safety

Eye and or face protection must be worn at all times when there is a reasonable possibility of contact with hazardous materials (infectious materials, chemical hazards, radiation etc.) in any laboratory at UMKC. Protective eyewear is required while in laboratories at UMKC to meet Missouri State law and UMKC campus policy. The type of eye protection is dependent on the potential hazard in the laboratory. All safety glasses need to have side shields. Safety goggles are also acceptable. If an UV light source is present then the safety glasses need to also protect against UV. Full face shield are also an option for eye protection, they also provide protection to the face in case of a splash.

Contact lenses **DO NOT** provide a level of protection to the eye. Materials can become trapped between the contact lens and the eye and cause increased injury to the eye. This is true when working around chemical fumes, gasses and dust. Goggles should be worn over contact lenses at all times in the laboratory. Prescription safety glasses are an alternative that can replace contact lenses in the laboratory. At **NO TIME** should contact lenses be handled in the laboratory.

A sign or notation on the biohazard sign on the entry door to the laboratory should include that safety glasses are required for entry to the laboratory.

8.2.4 Gloves

When working in the laboratory at UMKC gloves should be worn when performing any manipulations involving micro-organisms, potentially infectious materials or unknown materials. The use of gloves is essential in the laboratory. The type of glove should be carefully considered. Not all types of gloves work in all situations. Latex gloves are very common but have limitations. Nitrile gloves are a good alternative for general use. The determination of glove type for a specific procedure or chemical compatibility can be found in many catalogs and on many glove manufacturer web sites. The use of gloves provides a barrier layer of protection to the hands. Gloves will prevent skin absorption, infection, contamination and injury to the hands from various laboratory hazards. Some good glove guidelines are:

- Change gloves frequently
- Check gloves for tears or punctures
- DO NOT reuse disposable gloves
- Discard contaminated gloves as soon as possible
- Wash hands after removing gloves

Gloves should be removed before exiting the laboratory. Dispose of the gloves in the appropriate waste stream, infectious, chemical or general waste. After removing any glove it is good laboratory practice to wash your hands. A sign or notation on the biohazard sign on the entry door to the laboratory should include that gloves may be required for entry to the laboratory or when research is being conducted.

8.2.5 Respiratory protection

For certain protocols and projects, additional PPE like respiratory protection may be required. Respiratory protection should be chosen carefully after a proper risk assessment. A respirator is protection against the inhalation of potentially infectious materials or hazardous chemicals. If after

a risk assessment a respirator is required consult the UMKC Environmental Health and Safety Office for guidance. N95, N100, partial face and full face respirators require a medical evaluation and a respirator fit test be preformed before use. All personnel utilizing a Biosafety Level 3 containment facility must have a passing "Fit Test Report" on file.

8.3 Other protective equipment or methods

8.3.1 Footwear

Every person in a laboratory at UMKC is required to wear footwear while in the laboratory. All footwear should cover the entire foot. **At no time** should open toed-shoes, sandals, flip flops and shoes that expose the bare foot in any way should be allowed in the laboratory. These types of shoes **DO NOT** provide suitable protection for the foot. In specific instances or after a risk assessment steel toed shoes, treated shoes or other safety shoes may be required in specific situations. Anyone not wearing adequate footwear in a laboratory should not be allowed to enter or work in the laboratory. That person should be instructed that they will not be allowed into the laboratory until proper footwear is changed into.

8.4 Laboratory Emergency equipment

Each laboratory on the UMKC campuses should have at least in the general vicinity an eye wash station, safety shower, spill response kit (biological and/or chemical), first aid kit and a close by fire extinguisher. It should be common practice to give any new individual to the laboratory or the first lecture of a semester a description as to the location, function and operation of these pieces of emergency equipment.

8.5 Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination and clutter. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keep the laboratory neat and free of clutter.
- Benches, counter tops, equipment and any used work area should be cleaned and or disinfected every day and or after every shift.
- Door knobs, phones, key boards and any common item should be cleaned or disinfected regularly.
- Surfaces should be clean and free of infrequently used chemicals, glassware and equipment.
- Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must never be blocked.
- Proper disposal of chemicals and wastes. Old and unused chemicals should be disposed of promptly and properly. Contact Enviromental Health and Safety for disposal.
- Proper storage or disposal of any animal carcasses and/or tissues from animals. This should be in compliance with the Laboratory Animal Research Core SOP entitled: "Process and Disposal of Animal Carcasses." (<http://www.umkc.edu/ors/larc/>)
- Providing a workplace that is free of physical hazards. Aisles and corridors should be free of tripping hazards.

- Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment and avoidance of the creation of electrical hazards in wet areas. Extension cords are a temporary means of power and should be minimized as much as possible.
- All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance. Some equipment may need or be required to be full decontaminated before being moved or repaired (e.g. Biological safety cabinets, clean air benches, incubators).
- Gas cylinders must be securely fastened to a wall or bench to prevent falling over.

8.6 Chemical, Radiological and mixed waste

In all laboratories at the University of Missouri-Kansas City it is the responsibility of the generator of the waste to dispose of it properly. In many biological laboratories chemical, radiological and mixed waste may be generated in the course of biological research. Infectious waste disposal is described in further detail in the Infectious waste section of this manual. When a waste of chemical, radiological or mixed composition is created it needs to be disposed of in the proper means to meet local, state and federal regulations. If there are questions about the disposal of these types of waste the laboratory should contact the Environmental Health and Safety office on how to proceed. Prison time and or large fines can be levied against parties that improperly dispose of these types of waste. Also, the public image and credibility of the individual and the University can be permanently damaged.

8.7 Food and drink in the laboratory

At **NO** time should eating and drinking be allowed at any point in a laboratory at the University of Missouri-Kansas City. The 5th edition of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) states "Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose". The BMBL is the **minimum** standard of practice for biosafety in the United States. The consumption of food and drink in any laboratory other than those involved in that type of research is not permitted. The School, Department, Principal Investigator and Laboratory director should make available to the laboratory staff areas to store and consume food outside of the laboratory areas. Refrigerators and freezers in the laboratories must also be labeled to indicate that they are not for the purpose of food storage. If food is part of a research project it should be labeled in a way to distinguish it so that it is easily identifiable as research materials. Laboratory microwaves and or any other equipment should **never** be used for the preparation of food that is consumed.

8.8 Pipettes and Pipetting Aids

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids inside a BSC if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench.

The following precautions should be followed:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Biohazardous materials should not be forcibly discharged from pipettes. Use "to deliver pipettes rather than those requiring "blowout".
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.

- Place contaminated reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a biosafety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet as well while in use.

8.9 Syringes and Needles

Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. Do not use a syringe and needle as a substitute for a pipette.

Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe. When using syringes and needles with biohazardous or potentially infectious agents:

- Work in a BSC whenever possible.
- Wear gloves.
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid and bubbles from the syringe vertically into a cotton pad moistened with a disinfectant.

Needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe following use. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one-handed scoop method must be used. Always dispose of needle and syringe unit promptly into an approved sharps container. Do not overfill sharps containers (2/3 filled = full) before discarding.

8.10 Cryostats

Frozen sections of unfixed human or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections.

When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination,
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

8.11 Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions including safety precautions should be prominently posted on the unit. Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and resuspending sediment pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation.

To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in case of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when resuspending sediment material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

8.12 Blenders, Ultrasonic Disrupters, Grinders and Lyophilizes

The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.

Safety Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. If blender containers are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

8.13 Lyophilizer and Ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized and are fitted with polyethylene caps with silicone washers.

8.14 Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended.

Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence that disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter and/or the seal of the HEPA filter.

8.15 Transportation of infectious materials and biohazardous materials in and between laboratories on the UMKC campus

The transportation of any infectious material or biohazardous substance between laboratories, buildings or large laboratory areas must be in secondary containment. In some instances, transportation within a large laboratory suite should be in secondary containment as good practice. The secondary container is to provide a means of containment of the material in the event of an accident. It is much easier to decontaminate a plastic secondary container than decontaminating a public area such as a corridor or stairway. The secondary container may be as simple as a plastic container with a lid that can be securely closed. These types of containers can be purchased from many local retail stores for a minor cost. For small items a 50ml plastic centrifuge tube can be utilized. Also, some sort of absorbent like paper towels or a bench pad should be included. Any secondary container MUST have a biohazard label and a label or marking to identify what is in the container, who it belongs to and any other relevant information.

8.16 Hand washing

The best way to prevent contamination of oneself or their research is to wash your hands on a regular basis. One should wash their hands in the laboratory after removing their gloves, after finishing their shift or before leaving the laboratory. It is good practice to wash your hands on a regular basis during the day. The following instructions are taken directly from the CDC website (<http://www.cdc.gov/cleanhands/>).

When washing hands with soap and water:

- Wet your hands with clean running water and apply soap. Use warm water if it is available
- Rub hands together to make a lather and scrub all surfaces
- Continue rubbing hands for 20 seconds. Singing "Happy Birthday" twice is about 20 seconds
- Rinse hands well under running water
- Dry your hands using a paper towel or air dryer. If possible, use your paper towel to turn off the faucet

When using an alcohol-based hand sanitizer:

- Apply product to the palm of one hand
- Rub hands together
- Rub the product over all surfaces of hands and fingers until hands are dry.

8.17 Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be collected in special hamper (labeled or color coded) or in biohazard bags. Materials containing a drippable biohazardous agent or those contaminated with a RG-3 agent should be decontaminated by steam sterilization.

Chapter 9

Laboratory Acquired Infections

Early in the history of laboratory activities involving microorganisms it was recognized that laboratory staff were contracting infections from the work being performed in the lab. And in some cases individuals not directly associated with laboratory activities were also being infected in an unknown manner. Sulkin and Pike began research in the 1950's investigating the process of laboratory acquired infections and the activities that presented the most risk. This research continues today. The research has identified that the production of aerosols produced during ordinary laboratory operations present a very identifiable risk.

Laboratory acquired infections are preventable when working in a laboratory that manipulates infectious materials, biological toxins and other biologically active materials. The use of laboratory techniques, procedure and safety equipment can prevent laboratory acquired infections.

Factors involved in laboratory acquired infections

Pathogenicity or Virulence

Pathogenicity or virulence is the ability of a biohazardous material to produce or develop a rapid, severe, or deadly disease. Some materials are highly pathogenic, even in healthy adults, whereas others are opportunistic pathogens able to infect only hosts with lowered immunity or sites other than their normal habitat. Some biohazardous materials are attenuated, or weakened, and do not produce significant disease. The more severe the potentially acquired disease, the higher the risks.

Routes of Entry

An infection occurs when pathogenic microorganisms enter the human body in sufficient numbers and by a particular route which overcomes the body's defense system. By understanding the mode of transmission (pathway from source to you) and route of entry (entry route into body), procedures or controls to prevent exposure and infection can be developed.

Inhalation hazards

Inhalation of aerosolized biohazardous materials is the most common route of entry into the body. Inhalation of aerosols involves microscopic solid or liquid particles small enough to remain dispersed and suspended in air for long periods of time. Sources of aerosols include:

- Aerosolized solid material (spores, dust, particulate, etc.).
- Liquid material (mists and sprays, coughing, spittle, sputum, etc.).
- Technical process (blending, grinding, sonicating, lyophilizing, sawing, centrifuging, etc).

Ingestion hazards

Ingestion of biohazardous materials occurs frequently as the result of poor personal hygiene and poor laboratory practice. Proper hand washing minimizes the opportunity for mouth and eye exposures. Examples of how ingestion occurs include:

- Eating, drinking, and smoking in laboratory
- Mouth pipetting and suction techniques
- Transfer of microbes to mouth by contaminated fingers or articles

Direct (Skin/Eye) Contact hazards

Direct contact to biohazardous materials occurs through cross-contamination and mucous membrane exposure including the skin, eyes, inside of the mouth, nose, and the genitals. The main avenues by which biohazardous materials enter the body through the skin are hair follicles, sebaceous glands, sweat glands, and cuts or abrasions. Examples of how ingestion occurs include:

- Splash or spray of biohazardous material onto skin, eye, mouth, or nose
- Handling contaminated equipment with unprotected non-intact skin
- Transfer or rubbing by contaminated fingers or gloved hand
- Applying cosmetics or contact lens in laboratory

Injection or inoculation hazards

Inoculation or injection occurs when biohazardous material is accidentally introduced into the body with contaminated objects through the intact skin barrier. Inadequate control of sharp instruments and infected animals or arthropod vectors usually results in accidental inoculation or injection. Examples of injection and inoculation hazards include:

- Inoculation with a hypodermic needle, broken glassware, scalpels, or other sharp instruments
- Sharps injuries (needle sticks, glass pipettes, syringes, etc.)
- Animal bites, scratches, kicks, abrasions, punctures

Agent Stability or Viability

Stability and viability refer to the ability of a biohazardous material to retain its biohazardous characteristics such as aerosol infectivity and survival time in environment. Factors such as temperature, humidity, pH, oxygen, sunlight or ultraviolet light, chemical disinfectants, growth factors (food reservoir or media), and competition with endemic organisms must be considered.

Infectious Dose

The infectious dose is the number of microorganisms required to initiate an infection. This dose can range from one to hundreds of thousands of units depending on agent, exposure route, virulence, and host immune status or susceptibility for the disease.

Concentration (Amount of Agent)

Concentration is the number of infectious organisms per unit volume. As the viable agent concentration and volume increases, the risk potential gets higher. The media/reservoir, laboratory activity, volume (especially >10 liters) need to be considered in risk determination.

Immune Status

Immune status is the current condition of a living organism to resist and overcome infection or disease. The primary function of the immune system is to protect the body from foreign substances by an acquired ability to distinguish self from non-self. Host susceptibility or immune status helps determine the level of risk of acquiring a disease upon exposure. CDC and NIH guidelines presume a population of immuno-competent individuals.

Chapter 10

Working with Tissue Culture

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same RG level as that recommended for the agent. The Centers for Disease Control and Prevention (CDC) and OSHA recommend that all cell lines of human origin as well as non-human primate cell lines should be handled at BSL-2 level.

Cell lines which are non-primate which are not contaminated with bacteria or fungi and which are well established, may be considered Class I cell lines and handled at a Biosafety Level 1. Appropriate tests should confirm this assessment.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory and all virus and *Mycoplasma*-containing primate cell lines are classified as RG-2 and should be handled at a Biosafety Level 2.

10.1 Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g. sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory which realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g. suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at Biosafety Level 2, the recommended level for work with bloodborne pathogens such as hepatitis B virus and HIV. The containment elements described in Biosafety Level 2 are consistent with the OSHA standard, "*Occupational Exposure to Bloodborne Pathogens*" from the Occupational Safety and Health Administration. This requires the use of specific precautions with **all** clinical specimens of blood or other potentially infectious material (Universal or Standard Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g. *Micobacterium tuberculosis*), or when the use of a biological safety cabinet (Class II) is indicated to protect the integrity of the specimen. The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

10.2 Human Blood, Body Fluids, Tissue and Other Potentially Infectious Materials

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or other potentially infectious materials. The Bloodborne Pathogens Standard applies to all employers having employees that are “occupationally exposed” to human blood or other potentially infectious materials. An employee is considered occupationally exposed if there is “reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of an employee’s duties”.

Other potentially infectious materials include:

- Human cell or tissue cultures.
- Organ cultures
- Any unfixed tissue or organ, other than intact skin, from a human being (living or dead)
- HIV- or HBV- containing culture media or other solutions
- Human body fluids, except urine, feces, saliva or tears (unless visibly contaminated with blood)
- Blood, organs or other tissues from experimental animals infected with HIV or HBV or other bloodborne pathogens
- Animal infected with human bloodborne pathogens that could transfer the human bloodborne pathogen

An individual is also considered occupationally exposed if they do not have direct contact with blood or other potentially infectious material in case the employee uses equipment that is used to process or store blood, other potentially infectious materials or bloodborne pathogens.

All occupationally exposed employees are required by OSHA to attend a Bloodborne Pathogens training session prior to beginning work and annually thereafter. There are additional requirements for research laboratories and production facilities engaged in the culture, production, concentration and manipulation of HIV and HBV.

OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens such as:

- *Babesia* species
- Human Immunodeficiency virus (HIV)
- *Borrelia* species
- Colorado Tick Fever viruses
- Hepatitis B virus (HBV)
- *Brucella* species
- Arboviruses
- Hepatitis D virus
- *Leptospira* species
- *Spirillum minus*
- Hepatitis C virus
- *Francisella* species
- Creutzfeldt-Jakob virus
- *Plasmodium* species
- *Treponema* species
- *Streptobacillus moniliformis*
- Human T-lymphotropic Virus Type I
- Hemorrhagic Fever viruses

Consult the *Bloodborne Pathogen Training Manual for Clinical and Laboratory Personnel* for additional information on the exposure control plan, training requirements, work practices, housekeeping, engineering controls, personal protective equipment, signs/label requirements, Hepatitis B vaccination, emergency actions, exposure incident procedures, post-exposure evaluation and follow-up, and recordkeeping. Contact the Office of Environmental Health and Safety for assistance with exposure determination and for training information.

10.3 Preventing the Transmission of Tuberculosis

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30-year downward trend. Recently, drug resistant strains of *Mycobacterium tuberculosis* have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of healthcare workers have died. In October 1994, CDC published its *Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities*. The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at risk for exposure to tuberculosis. Propagation and/or manipulation of *Mycobacterium tuberculosis* and *M. bovis* cultures in the laboratory or animal room must be performed at BSL-3 and require IBC approval.

Laboratories that are engaged or plan research with *Mycobacterium tuberculosis* need to consult OSHA standard 29 CFR 1910.139, *Respiratory protection for M. tuberculosis*.

10.4 Select Agent regulation

Recent events have brought to light the ability of microorganisms or toxins to produce great harm to humans and animal health and safety. SARs, Ebola, and the Anthrax letters all have been in the headlines of television and newspaper regarding the affects to human health around the world. Bovine Spongiform Encephalitis has been a threat to animal health and the food chain in Great Britain. Because of the great potential for nefarious acts that individuals or terroristic groups could inflict with biological agents or toxins the United States Government have passed legislation to help protect against such acts. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the US Government to prepare and be able to respond to acts of biological terrorism and other emergencies that could pose significant threats to public and agricultural health and safety. Human health agents are addressed in the *Possession, Use and Transfer of Select agent and Toxins Act* (42 CFR Part 72 and 73) (42 CFR Part 1003), Animal and plant agents are covered under the *Agricultural Bioterrorism Protection Act of 2002: Possession, Use and Transfer of Biological Agents and Toxins* (7 CFR Part 331) and (9 CFR Part 121) The select agent list is part of this legislation. The select agent list is composed of microorganisms and biologically derived toxins. The agents that pose harm to human health are overseen by the Centers for Disease Control and Prevention; animal threats are handled by the United States Department of Agriculture, Animal and Plant Health Inspection Service. Some organisms may be both animal and human pathogens. These agents are overseen by the agency that the institution chooses to register with. For an institution or individual to possess, manipulate, transfer or even attempt to procure, many approvals have to be secured well in advance. An Institution must appoint a Responsible Official and an Alternate Official, individuals with access to the agents must successfully pass a Security Risk Assessment conducted by the Department of Justice, the laboratories must meet safety requirements, a fully developed security plan must be in place to safeguard the agents and personnel, the institution must meet all aspects of 42 CFR part 73 and then pass a safety and security inspection by either the USDA or the CDC or both. The process of registration is lengthy and takes many months. Any individual who has research interest in any of the select agents should begin the registration process as soon as feasibly possible. The application process, criminal background check and onsite inspections are detailed and lengthy. The securing of approval from the CDC or the USDA will take several months to complete. All

approvals must be in place **BEFORE** attempting to acquire any agent on the select agent list. Fines and jail time can be quite significant for violation of any part of the regulations to both the individual and the institution involved. The links below are for the CDC select agent program and the USDA select agent program websites. The process of approval forms and select agent list can be found on these websites.

- Select Agent list website:
 - <http://www.selectagents.gov/>
 - http://www.aphis.usda.gov/programs/ag_selectagent/
- Permissible toxin list (<http://www.selectagents.gov/agentToxinList.htm>) and amounts (<http://www.selectagents.gov/toxinLimits.htm>)

The select agent and toxin list is reviewed on a regular basis. This list should be referred to verify any new acquisitions and purchases are not select agents.

Any person or laboratory at UMKC that may have interest in working with select agents should contact the UMKC Environmental Health and Safety office for guidance on how to proceed. The ability to start the application well in advance of any work is a necessity in order not to delay any research. The process will take several months.

Chapter 11

Safety equipment

11.1 Biological safety cabinets (BSC)

A biological safety cabinet is a means of primary containment. Primary containment can either be a piece of equipment, BSC, or a technique that provides a primary means to protect personnel, product and or the environment against contamination. There are three types of biological safety cabinets. These are the only types of cabinet or hood that provide protection. The three types of cabinets each provides varying degrees of protection. BSCs provide safety protection based on the design features incorporated into the cabinet. The central feature of a BSC is the incorporation of a HEPA, High Efficiency Particulate Air Filter. A HEPA filter is a specific type of filter that is very good at removing particles from the air stream of 0.3 µm in size. A HEPA filter can remove at least 99.97% of the particles of 0.3 µm in size. Particles of greater or smaller size will be removed at a greater efficiency. A HEPA filter is able to do this by using four ways to remove particles 1) impaction 2) diffusion 3) Interception 4) Electrostatic. A HEPA filter will NOT remove vapors or gases. The HEPA filter is composed of a cellulose/borosilicate media that has a very large surface area. The filter is folded into pleats and is separated by dividers to achieve the large surface area. The filter is sealed to the frame to achieve a complete seal. If the BSC is moved the seal or the filter media may be compromised. **Recertification must be performed on a yearly** basis by a qualified service technician. A biological safety cabinet or a clean air bench **MUST** be properly decontaminated, including the HEPA filter, prior to removing from the laboratory for disposal. Contact HSE or the Biosafety office for guidance for decontamination and disposal. Also, if a BSC is to be repositioned in a lab is required to be recertified. A biological safety cabinet should be certified at least yearly.

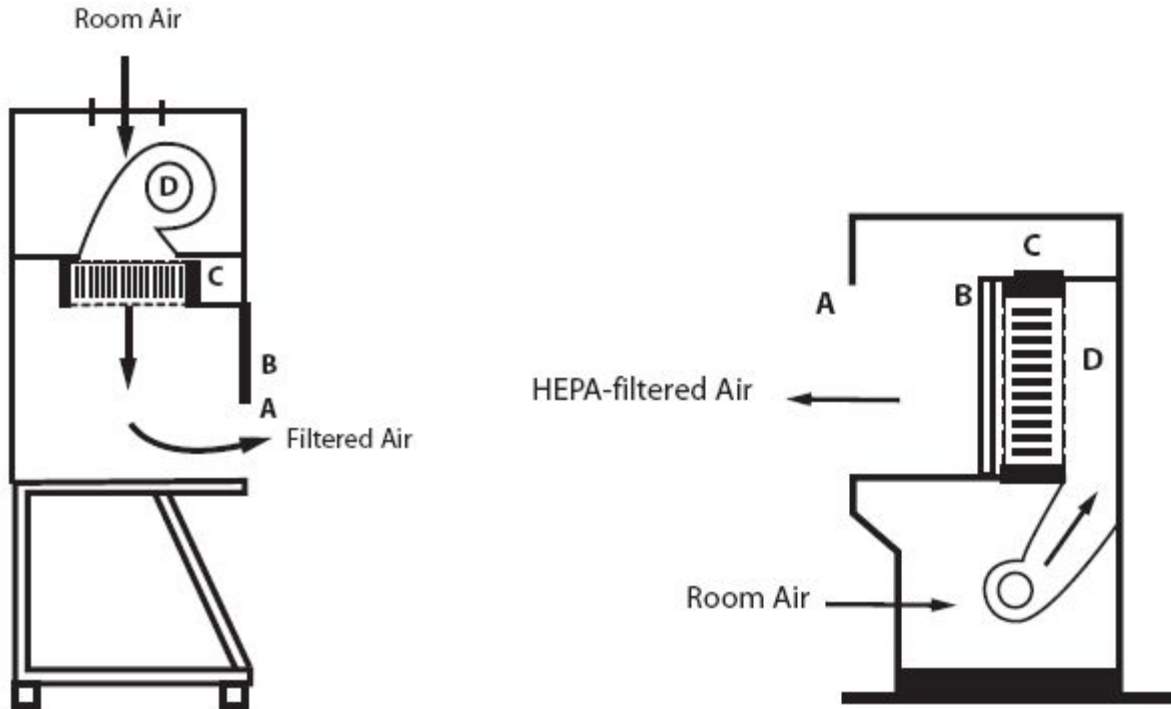
The National Sanitation Foundation (NSF/ANSI 49) sets the standard for the United States and the certification must be at least yearly.

“To assure that all cabinet operating criteria contained in this annex continue to be met, each cabinet should be field tested at the time of installation and at least annually thereafter. In addition, recertification should be performed whenever HEPA filters are changed, maintenance repairs are made to internal parts, or a cabinet is relocated.³⁶ More frequent recertification should be considered for particularly hazardous or critical applications or workloads.” (NSF/ANSI 49-2002 Annex F)

Both the *NIH Guidelines for recombinant DNA research* and the Centers for Disease Control and Prevention’s *Biosafety in Microbiological and Biomedical Laboratories* states that certification should be yearly.

11.2 Laminar flow clean air benches

Laminar flow benches are **NOT** biological safety cabinets. Laminar flow benches should not be used as a substitute for a biological safety cabinet. Laminar flow benches only provide protection to the product or item in the sterile air stream. The operator is **NOT** protected from any possible infectious material, chemical fume or toxin. The laminar flow clean air benches provide a continuous stream of HEPA purified air in laminar sheets. Any object placed in the path of the air stream creates an area of turbulence in its wake where the sterility may have been compromised by that object.



Left: Vertical flow clean air bench (Primary containment of Biohazards, 3rd ed., CDC)

Right: Horizontal flow clean air bench (Primary containment of Biohazards, 3rd ed., CDC)

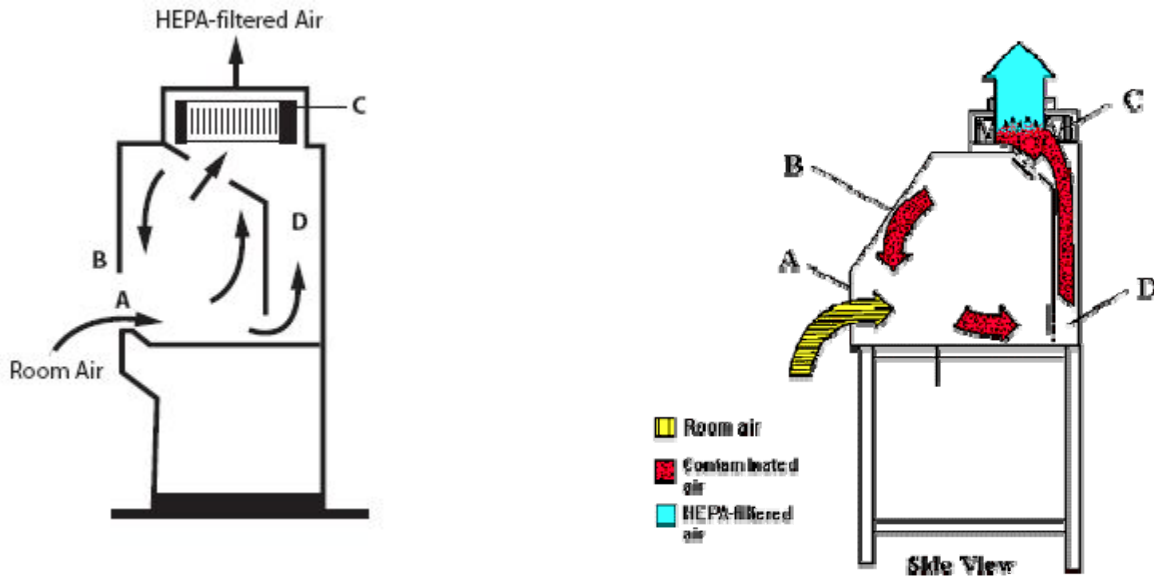
11.3 Biological safety cabinets

Biological safety cabinets, BSCs, are a primary means of containment of potentially infectious materials. A biological safety cabinet is able to protect the operator personal protection, environmental protection and protection of the materials being manipulated from contamination. A BSC uses a High Efficiency Purifying Air (HEPA) filter to remove particles from the air. The HEPA is able to remove at least 99.97% of all particles 0.03 microns in diameter. Particle of larger and smaller size can be removed at a higher efficiency.

Biological safety cabinets can be classified into three main classes. The classes are I, II and III. Each class has its own specific features.

Class I

A Class I biological safety cabinet provides only protection to the operator and the environment. There is little to no protection to the product being manipulated. The HEPA filter in a Class I biological safety cabinet filters the air as it exits the cabinet. The air is drawn in through the opening in front access opening. The air flow into the cabinet is the “dirty room” air and flows across the work surface. The personal protection of a Class I cabinet is provided by the air flow in to the cabinet and away from the operator. A Class I BSC is suitable for work with low risk materials.



Left: Example: Class I BSC (Primary containment of Biohazards, 3rd ed., CDC)

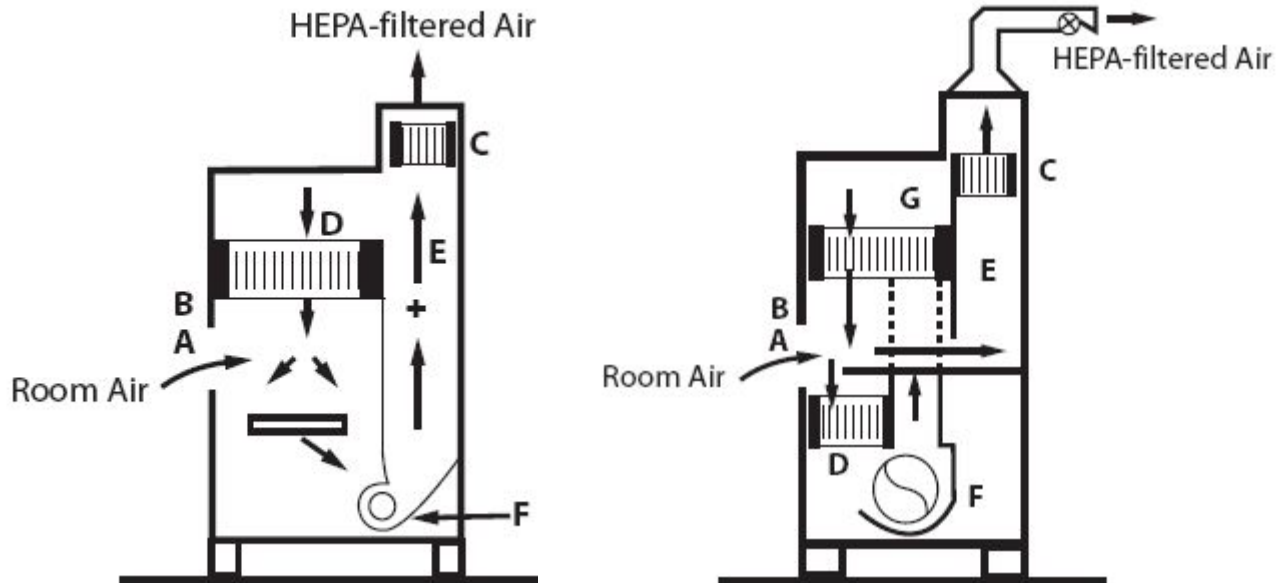
- A** Front opening
- B** Sash
- C** Exhaust HEPA filter
- D** Exhaust plenum

Right: From the CDC, Primary containment 2nd ed.

Class II

A Biological Safety Cabinet Class II must provide protection to the operator, the environment and the product being used. Class II BSCs are further divided up into two categories, types A and B. The types are then further divided. The knowledge of what the different types of Class II BSCs will aid in the selection of the proper cabinet for the work being performed. Type A cabinets have a 70%/30% split in the amount of air recirculated within the cabinet and what is exhausted. In this case 70% of the air entering the cabinet would be recirculated and 30% would be exhausted through the HEPA system. Class II B1 has a 60%/40% split and the Class II B2 has a 100% exhaust with no recirculation.

Chemicals and radiologics should be manipulated in a fume hood. Biological safety cabinets have limitations when manipulating chemicals and radiologics. Also, BSC's are not spark-proof. A build up of vapors may cause an explosive atmosphere to accumulate inside the BSC. Some BSC's exhaust to the laboratory environment and the fumes or vapors may pose a health hazard to the individuals in the laboratory. Again, for the use of chemical and radiologics a fume hood is the



best practice.

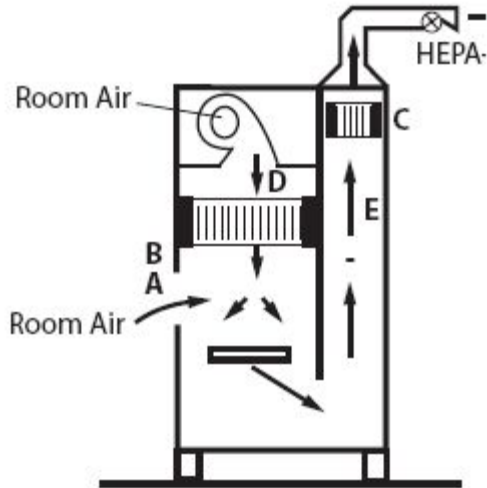
Left: Example: Class II Type A1 BSC (Primary containment of Biohazards, 3rd ed., CDC)

- A Front opening
- B Sash
- C Exhaust HEPA filter
- D Supply HEPA filter
- E Common plenum
- F Blower

Right: Example: Class II Type B1 BSC (Primary containment of Biohazards, 3rd ed., CDC)

- A Front opening
- B Sash
- C Exhaust HEPA filter
- D Exhaust HEPA filter
- E Negative pressure dedicated exhaust plenum
- F Blower
- G Additional HEPA filter for supply air

Exhaust needs to be hard connected to building exhaust system



Example: Class II B2 BSC (Primary containment of Biohazards, 3rd ed., CDC)

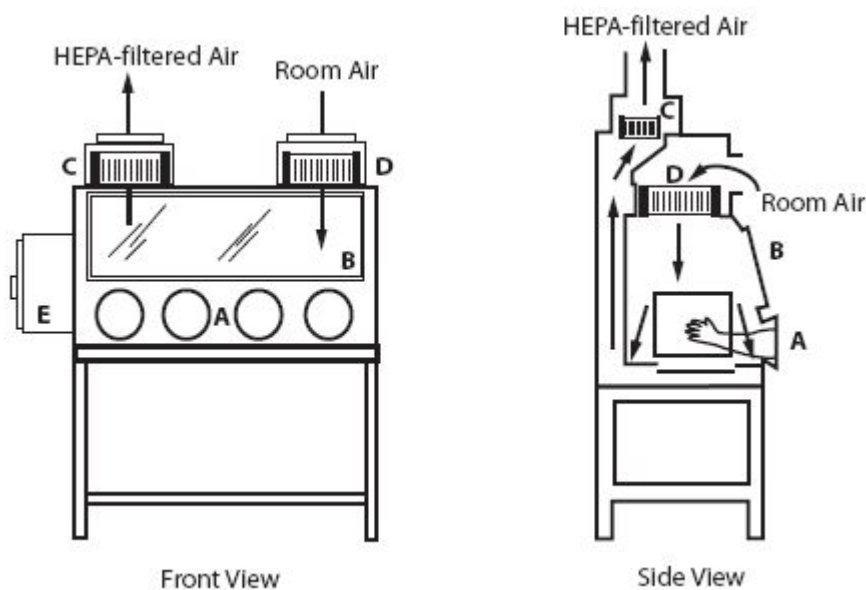
- A Front opening
- B Sash
- C Exhaust HEPA filter
- D Supply HEPA filter
- E Negative pressure exhausts plenum

Carbon filter in the exhaust system is not shown

The cabinet needs to be hard connected to the building exhaust

Class III

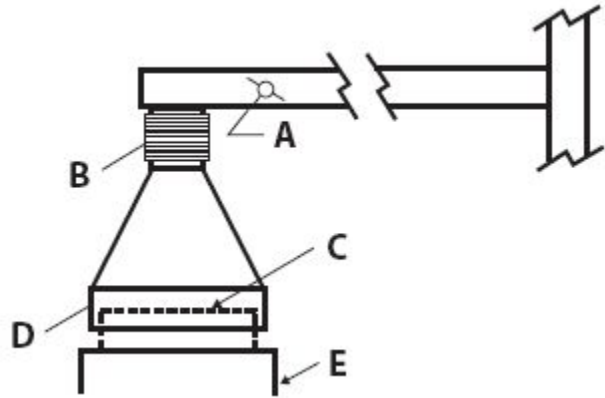
Class III BSCs provide the highest level of protection of all. The Class III cabinets are a fully contained cabinet. To access the inside of the cabinet there are full length glove ports attached to the cabinet. The use of HEPA filters is employed for the air entering and the air exiting the cabinet. The exit HEPA must be a double HEPA or can be a single and be associated with an incineration system.



Example: Class III BSC (Primary containment of Biohazards, 3rd ed., CDC)



Left: Example: Class III BSC (National Institute of Infectious Disease, Japan)



Right: Example: Thimble unit (Canopy connection) (Primary containment of Biohazards, 3rd ed., CDC)

A more thorough description of the requirements and uses of biological safety cabinets can be found on the CDC website following the below link to the Third edition of “*The primary containment of Biohazards.*”



http://www.cdc.gov/od/ohs/biosfty/primary_containment_for_biohazards.pdf

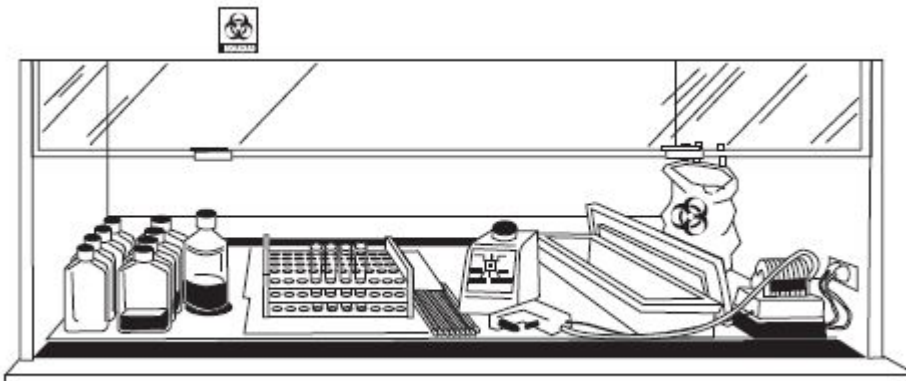
Also, additional information can be found in Appendix A of the 5th edition of the BMBL, *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.*

11.3.1 Biological safety cabinet work

Understanding the limitations and strengths of a BSC can help in the ability to perform work safely in a BSC. Work to be performed in a biological safety cabinet should be planned out in advanced. This will aid in the gathering materials and eliminate unnecessary entry and exiting from the cabinet. The first step in working in a BSC is to make sure the cabinet is on and is functioning properly. A check of the monitor or gauge on the BSC will help determine if the cabinet is working properly. Verify that the window is at the correct height to work safely. Adjust the stool in front of the cabinet to a comfortable height. Verify that if the BSC has a drain valve and that it is closed. Before beginning any work the cabinet needs to be running for at least 15 minutes. This time allows for any particles that have found their way into the interior of the cabinet to be removed by the HEPA filters. Turn off the UV light if used. Be sure to adjust the sash to the correct operating height and verify that the sash alarm is on. Don all appropriate PPE, Generally the required PPE is a lab coat, gloves and safety glasses but this can vary based on the agent and work being carried out. The entire surface area of the interior of the cabinet should be disinfected with an appropriate disinfectant. If bleach was used as the disinfectant then the surfaces need to be wiped down with sterile water. The bleach over time will corrode or pit the stainless steel surface of the cabinet. All materials that will be used in the BSC should be wiped down with disinfectant before being loaded into the cabinet. The cabinet can then be loaded with the equipment and materials that will be used. Do not block the grill in the front of the workspace or the grills in the back or on the sides of the interior. Any blocking of the grill will affect the air flow within the cabinet and containment of the

cabinet. Allow an additional two or three minutes after loading the cabinet to remove any airborne contaminants introduced while loading the BSC. The cabinet should be set up to follow a work flow of clean to dirty. Absolutely **DO NOT** at any time use an open flame in a biological safety cabinet. The use of an open flame has the possibility to harm the HEPA filters, the seals around the HEPA and disrupt the air flow within the cabinet. There are suitable incinerators or other equipment that can replace a flame in the BSC. If the BSC is operating correctly there should be no need to use an open flame to treat the neck of culture flask and bottles for example. Place all clean items on one side of the cabinet and work towards the opposite side, the dirty side. When entering the cabinet it is best to enter directly into the cabinet at ninety degrees to the front of the cabinet instead of a sweeping motion from the side. A sweeping motion can allow aerosols within the BSC to escape the containment of the BSC. This disruption of the air curtain will expose the operator and near by persons to the aerosols.

A small biohazard container should be located within the BSC for any waste generated. A horizontal pan placed in the BSC and partially filled with an appropriate disinfectant should be used when working with serological pipettes. Any item that is removed from the BSC should be surface disinfected with an appropriate disinfectant before being removed from the BSC. The entire interior surface of the BSC should be disinfected after the work is finished. One should not place their head inside of the biological safety cabinet at any time. A weekly disinfecting of the plenum below the work surface should also be performed. All cleaning of the BSC should be documented. If UV light are being utilized for disinfection they need to be regularly tested to assure that they are performing to standards. The UV bulbs in BSCs tend to lose effectiveness quickly and need to be replaced often and can be expensive. The bulbs need to be kept clean and free from dust to ensure the best performance from the bulbs. Generally the use of UV in not recommended in BSCs. Allow the BSC to run for an additional three to five minutes before shutting down.



CLEAN → → → → → → **CONTAMINATED**

11.3.2 Moving or disposal of a biological safety cabinet

A biological safety cabinet **MUST** be decontaminated inside and out before it can be moved. Generally formaldehyde gas, vaporized hydrogen peroxide or chlorine dioxide is used to decontaminate a BSC. The use of these gasses will decontaminate the HEPA filters and all spaces within the BSC. A certified company must perform the decontamination. A BSC or a clean air bench **MUST** be recertified after any moving. Moving a cabinet may harm the HEPA filter, seals around the filter etc. that may decrease the effectiveness of the BSC and lead to an unsafe situation. The UMKC Biosafety office can assist with the moving and certification of BSCs and clean air benches.

11.5 Other Safety Equipment

Safety Showers

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are available from Facilities Maintenance and from the Environmental Health and Safety Industrial Hygienist.

Eyewash Stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially infectious materials.

Ventilation Controls

Ventilation controls are those controls intended to minimize employee exposure to hazardous chemicals and infectious or toxic substances by removing air contaminants from the work site. There are two main types of ventilation controls:

General (Dilution) Exhaust

Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work. General exhaust systems are inadequate for RG-3 agents or BSL-3 work.

Chapter 12

Biological spills

12.1 Spill involving recombinant DNA materials

The National Institutes of Health Guidelines for research involving recombinant DNA molecules (NIH Guidelines) states that "...any significant problems with or violations of the NIH Guidelines, or any significant research-related accident and illness to the appropriate institutional official and the NIH/OBA within 30 days..." (Section IV-B-2-b-(7)). Spills and accidents that result in an exposures or loss of containment involving materials in BL 2 require immediate reporting. Spills, overt or potential exposures and loss of containment involving materials in BL 3 and BL 4 require immediate reporting. It is important to contact the UMKC Office of Research Services and Environmental Health & Safety as soon as possible after an incident to meet the reporting requirements. The UMKC Office of Research Services along with Environmental Health & Safety can help in determining if an event requires reporting. The UMKC IBC, Environmental Health & Safety and the UMKC Office of Research Services must be notified of any and all spills, accident or breaches of containment involving recombinant DNA materials. The Principal Investigator of the laboratory or a designated individual of that laboratory is responsible for reporting the above outlined incidents to the UMKC IBC, Environmental Health & Safety and the Office of Research Services. The Responsible Official for UMKC will report any incidents to NIH OBA. The UMKC Office of Research Services will assist in the capture of information and reporting to the National Institutes of Health Office of Biotechnology Activities. The UMKC IBC will direct any follow up investigation as warranted. The UMKC IBC web page: <http://www.umkc.edu/ors/ibc/> has links to the appropriate NIH OBA web pages for more information.

12.2 Biological spill kit

A biological spill kit should be located in each laboratory handling biological agents. A biological spill kit is the responsibility of the Principal Investigator or Laboratory Director to prepare. The Biosafety Professional in the EH&S office can provide guidance as to the contents of the spill kit. The spill kit should be located in a central location that is easily accessible. One common location is under the sink in the laboratory. The spill kit should be checked on a monthly basis to ensure that the contents are complete. Most items to be included in a spill kit are commonly found laboratories. A sample of the items to include in the biological spill kit includes the following. (Note that this is an example and is not inclusive for all laboratories, procedures and organisms.)

- Plastic container with closable lid to contain the contents of the spill kit
- Absorbent towels
- Forceps to pick up sharps or glass
- Biohazard bags
- Household bleach or appropriate disinfectant for the organism being manipulated
- Container to mix dilute disinfectant solution, one gallon jug
- Appropriate PPE (gloves, goggles, tyvek jump suit, booties, specific PPE to protocols)
- Comet®, Ajax® or the like. Can be used to contain small spills. The chlorine in these products can be corrosive.
- Plastic scoop, plastic scraper and or autoclavable/disposable broom and dust pan

12.3 Biological spill response plan

Every effort should be taken to minimize the possibility of accidental spills of any biological materials. But, spills do happen. The risk of an exposure from such an incident can be greatly reduced with pre-planning. For this reason, it is important that each laboratory has a biological spill

plan in place. A biological spill plan for a laboratory is the responsibility of the Principal Investigator. The plan must be tailored to each particular lab's research and the organism(s) that they are manipulating. A biological spill response plan should be available to the individuals working in the laboratory and those who may be visiting. A response plan should be posted in the laboratory for all to see. The risk assessment(s) should also be included in the spill response plan. A good location to post the plan is near the exit to the laboratory. A biological spill response plan should include:

- The name of the Principal investigator and/or laboratory director and current contact information
- A list of the current lab staff with contact information
- Contact information for the EHS office
- The organisms being manipulated in the lab. Exceptions can be made due to select agent requirements.
- Location and inventory of the spill kit(s)
- Location of next nearest spill kit outside of laboratory
- Descriptions of decontamination plans
- Data sheets with information regarding the biological material/organisms and procedures to be followed if the potential for exposure exists.

The BSO can provide an appropriate and up-to-date template for a biological spill response plan.

12.4 Elements of a biological spill risk assessment

The risk assessment is an evaluation of what factors could come into play if a spill should occur. The risk assessment has to be performed before an incident occurs. The risk assessment and prior planning can positively affect the outcome of an incident or possibly prevent an accident. However, it should be noted that performing a risk assessment is only the start. After the risks have been identified the next in the process is Risk management. Risk management is what can be done to reduce the identified risk. Can procedures, equipment, organism etc. be changed to reduce the risk but also allow completion of the research? The risks must then be communicated to those involved in the laboratory. This risk communication will better prepare all individuals involved with the work as to what the risks are. Factors to consider when performing a risk assessment are:

- What is the organism?
- What is the Risk group classification of the organism? (RG-1, RG-2, RG-3)
- What is the route of infection? (aerosol, ingestion, mucus membrane exposure etc.)
- What is the volume being manipulated?
- What is an appropriate disinfectant for that organism? Appropriate contact time? Equipment considerations with the disinfectant?
- Will the spill be contained/uncontained? In a biological safety cabinet, in the open lab or possibly in open common spaces?
- Will there be the potential for release into the environment?
- Any glass or sharp objects used in the manipulations?
- What are the procedures being used in the laboratory? Can they be changed or modified to reduce the risk?
- Where is the work being performed?
- Will there be transportation of the biologic material outside the lab?

The risk assessment for incidents involving material containing recombinant DNA are to consider the following guidance:

- those organisms or viruses that are human pathogens, animal pathogens, or plant pests are to be responded to as etiologic agents regardless of whether or not they contain recombinant DNA;
- organisms or viruses that do not present pathogenic hazards are to be responded to as etiologic agents
 - if they contain recombinant DNA that encodes the complete genome of an etiologic agent,
 - if they possess recombinant DNA sequence that may confer upon the host the factor involved in the etiology of an agent, with or without additional elements provided by the host, or,
 - if they contain recombinant DNA that has not been adequately characterized as to rule out the ability to code for the factor of an organism, virus, or toxin involved in eliciting human, animal, or plant disease.

Recommendation from the NIH Guidelines for recombinant DNA, 2009.

More detailed information on how to perform a biological risk assessment can be found in the *Biosafety in Biomedical and Microbiological Laboratories* guidelines 5th edition (<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>).

12.5 Biological spill cleanup procedures

12.5.1 Biological spills within Biological safety cabinet

1. Alert the laboratory staff.
2. Leave Biological Safety cabinet running. Under no circumstance turn off the BSC.
3. If the spill has overflowed the work surface and flowed into the drain pan contact the EHS Biosafety Professional for assistance on clean up. The BSC may need more extensive decontamination. Make sure the drain is closed.
4. While wearing appropriate PPE (Safety glasses, gloves, lab coat and any other necessary PPE required by agent or procedures being utilized) begin to clean up the spill using the following procedures.
5. Cover the spilled materials and a margin around the spill with absorbent towels. This may require that the complete work surface and equipment be covered with paper towels to contain the spill. If there is any doubt to whether material has overflowed into the drain pan or catch basin, flood the work surface and the drain pan with disinfectant. Be sure that the drain is closed on the catch basin first.
6. Pour disinfectant onto the paper towels/absorbent towels working from the margins to the center of the affected area until saturated with the disinfectant. A fresh 1:10 dilution of household bleach is sufficient to use with most biological materials.
7. Allow the disinfectant soaked paper towels to sit for at least 30 minutes. You may need to add additional disinfectant if the toweling begins to dry out during the required contact time. Wipe down the walls and front sash of the BSC with a disinfectant soaked towel.
8. After the 30 minutes collect the materials and place them into a biohazardous waste container. Use tongs or forceps to pick up any broken glass or sharp objects. Ideally this would be done inside of the BSC. If necessary, use a plastic container with a small biohazard bag inside to help contain any liquids.

9. After all of the materials are collected, disinfect all of the exposed surfaces of the BSC with a disinfectant. Dispose of the clean up materials into a biohazardous waste container.
10. Wipe down the inside of the BSC with water. This will prevent the bleach (which is corrosive) from pitting of the stainless steel surface of the BSC. If another disinfectant is used refer to the MSDS or the product insert for recommendations.
11. Dispose of all waste according to established procedures.
12. Wash hands and any exposed skin with soap and water.
13. Allow the biological safety cabinet to run for 15 minutes before resuming work.
14. Document the spill and the procedure, disinfectant and agents involved. Direct this report this to the laboratory director.

12.5.2 Biological spills in laboratory or common laboratory areas

1. Alert the laboratory staff and leave the room. While exiting avoid inhaling any of the possible aerosolized materials.
2. Remove any contaminated PPE or clothing. Turn contaminated clothing inside out while removing to reduce further contamination. Place PPE/clothing into a bag for autoclaving. Wash any contaminated skin with antimicrobial soap and water. Use the nearest eye wash station to flush the eyes, if needed.
3. Post a biological spill sign on any access doors to the lab.
4. Inform the laboratory director/principal investigator of the spill. Contact the EHS staff.
5. Prepare for the clean up of the spill. Collect all materials needed for the spill clean up (disinfectants, spill kits from other labs if necessary, personal protective equipment, etc.)
6. Collect as much information about the spill as possible. (Organism/toxin, mode of transmission of the organism, concentration, volume, area of spill, any equipment contaminated etc.)
7. Allow at least 30 minutes for any aerosols generated to settle before re-entering the laboratory to begin clean-up.
8. Don appropriate PPE (Safety glasses, gloves, lab coat and any other necessary PPE required by agent or procedures being utilized.)
9. Cover the spilled materials and a margin around the spill with absorbent towels. This may require that the complete work surface and equipment be covered with paper towels to contain the spill. Pour disinfectant onto the paper towels/absorbent towels working from the margins to the center of the affected area until saturated with the disinfectant. A 1:10 dilution of household bleach is sufficient to use with most biological materials. Towels can also be soaked in disinfectant and placed onto the spill area.
10. Allow the disinfectant soaked paper towels to sit for at least 30 minutes. You may need to add additional disinfectant if the toweling begins to dry out during the required contact time.

11. After 30 minutes carefully collect the materials and place into a double bagged biohazardous waste container. Use tongs or forceps to pick up any broken glass or sharp objects. If necessary use a plastic container with a small biohazardous bag inside to help contain any liquids.
12. After all of the materials are collected, disinfect the area again, using 10% household bleach solution or a similar disinfectant. Dispose of these materials into a double bagged biohazardous waste container.
13. Doff and dispose of all PPE in a biohazard bag.
14. Dispose of all waste according to established procedures.
15. Document and report the spill and the clean-up to the laboratory director and the EH&S Biosafety professional.

12.5.3 Biological spills in a centrifuge

1. Always use sealed rotors, safety tubes or centrifuge cups with seals. Check the o-rings of the equipment regularly to ensure they provide a good seal.
2. If upon opening the centrifuge is discovered that there has been a release of material, carefully close the lid on the centrifuge. Notify anyone else in the lab and evacuate the laboratory. If the incident happens while the centrifuge is running, shut down the centrifuge and leave the lid closed and evacuate the lab.
3. Allow at least 30 minutes for any aerosols generated to settle.
4. Remove any contaminated PPE or clothing. Turn contaminated clothing inside out while removing to reduce further contamination. Place PPE/clothing into a bag for autoclaving. Use the nearest eye wash station to flush the eyes if needed. Wash hands and any contaminated areas with antimicrobial soap and water.
5. Post a biological spill sign on any access doors to the lab.
6. Inform the laboratory director/principal investigator of the spill. Contact the EHS staff.
7. Prepare for the clean up of the spill. Collect all materials needed for the spill clean up (disinfectants, spill kits from other labs if necessary, personal protective equipment, etc.).
8. Collect as much information about the spill as possible. (Organism/toxin, mode of transmission of the organism, concentration, volume, area of spill, any other equipment contaminated etc.).
9. Don appropriate PPE (Safety glasses, gloves, lab coat and any other necessary PPE required by agent or procedures being utilized.) Respiratory protection may be necessary depending on the organism and the type of incident in the centrifuge.
10. If possible, transfer the centrifuge rotor or buckets to a biological safety cabinet. It may be necessary to move the centrifuge next to the biological safety cabinet. Place the rotor or buckets in to a disinfectant solution in the BSC. Be sure to use a disinfectant effective against the organism in use, as well as one not corrosive to the centrifuge equipment. A one hour contact time is recommended for the disinfection of the rotor/buckets. Handle any broken tubes etc. with forceps or tongs. Place materials in biohazard bags after the decontamination contact time has elapsed.

11. Carefully remove any broken materials from the centrifuge using forceps or tongs.
12. Carefully wipe down the inside of the centrifuge with towels soaked in an appropriate disinfectant and allow to air dry. Repeat the decontamination of the centrifuge. Be sure not to forget the lid of the centrifuge. Also, decontaminate the outside of the centrifuge and the surrounding area.
13. Doff and dispose of all PPE in a biohazard bag.
14. Dispose of all waste according to established procedures.
15. Document and report the spill and clean-up to the Principal Investigator/laboratory director and the HSE Biosafety professional.

12.5.4 Biological spills with mixed hazards (biological /chemical, or radiological)

1. Evacuate the area.
2. Post a sign that a biological/mixed hazard spill has occurred.
3. Secure the lab or area.
4. Contact the Principal Investigator or Laboratory Director.
5. Contact the Environmental Health and Safety office for guidance on how to proceed.

Chapter 13

Other Safety programs

13.1 Sharps policy

The University of Missouri-Kansas City has in place a policy for the handling of sharps. This information is in addition to that policy for the manipulation of biological organisms or biological derived materials. The following is directly related to the handling of sharps that have come in contact with microorganisms, potentially infectious materials or human fluids or tissues. Sharps can be defined as any object or material that contains a point or sharp edge that are capable of puncturing or cutting the skin. Examples of sharps are needles, pipette tips, microscope slides or broken glass. This is just a set of examples and many other items can be considered sharps.

Gloves should be worn while working with sharps at all times. In specific instances very specialized glove, metal chain mail, maybe required. Proper personal protective equipment should be worn at all times when handling sharps. Gloves, safety glasses and a laboratory coat are the basic minimum PPE requirements that should be worn. Depending on the situation and the procedures other more specific PPE may be required.

Safety considerations that should be observed while handling sharps would be the following:

- Always use a high degree of precaution with any contaminated sharp items (i.e., needles and syringes, slides, pipettes, capillary tubes, scalpels, scissors, etc.). They should be restricted for use in the laboratory and animal facilities only when there is no alternative.
- Only trained personnel perform procedures involving needles and other sharps.
- Use extreme caution when handling needles and syringes to avoid self-inoculation and the generation of aerosols during their use and disposal.
- Use safety devices with Engineered Sharps Injury Protection or alternatives to needles when available.
- Substitute plastic ware for glassware whenever possible to avoid potential injuries with broken glass.
- Attach needles to syringes while the needles are capped.
- Hold and carry syringes and needles with needles capped and pointing away from the body. Do not point needles at other personnel.
- Uncap needles by pulling cap off with the hands – Do Not place needle cap in mouth in order to remove the cap from the needle.
- Uncap needles by pulling cap off with the hands – do NOT uncap needles by pulling the cap off with the mouth. Do Not place needle cap in mouth in order to remove the cap from the needle.
- Place needles and sharps in secondary containment (i.e., plastic tray, test tube rack, etc.) for transport.
- Do NOT leave sharps unattended.

13.2 Sharps disposal

Sharps should be disposed of into an approved sharps container. These containers can be purchased from any laboratory supply or medical supply company. They should be red in color, have the biohazard symbol on it, lid that fits tightly, rigid and puncture resistant. The sharps containers should be placed as near to the area where it will be used as possible. There may be situations that the traditional red sharps container is not appropriate. In this instance, consult with the Environmental Health and Safety Office for more information. A sharps container should never be filled over three fourths full. Bellow is more guidelines that should be followed for sharps disposal.

- NEVER reach into the sharps container.
- Do not bend, shear, clip, recap, or separate needles from the syringe prior to disposal.
- Dispose of needles and syringes directly into a puncture-proof container which must be decontaminated, preferably by autoclaving, prior to disposal.
- Do NOT place needles or sharps in office waste containers.
- Place sharps containers in convenient locations that are easily accessible in all places where sharps are used.
- Do NOT empty the contents of a sharps container into another container.
- Do NOT remove the lid from the sharps container.
- Do NOT attempt to retrieve items from a sharps container.
- Do NOT overfill a sharps container – no materials should be sticking out of the top. Fill only to $\frac{3}{4}$ full to avoid over filling.
- Do NOT force materials into a sharps container.
- Securely close filled sharps containers and transport to the kitchen for autoclaving prior to disposal. Use tape to secure the lid closed, autoclave tape if possible.
- Contaminated micropipettes and pipette tips are discarded in a puncture-resistant container or sharps container for disposal.
- Dispose of broken glass in a puncture-proof container or sharps container.
- Do NOT pick up broken glass with fingers. Use utensils (i.e., hand sweep and tray, tongs and or forceps) to clean up broken glass. Refer to appropriate spill clean-up and emergency SOPs when spills and potential exposures occur prior to cleaning up broken glass.
- Dispose of intact glass blood tubes in a puncture-proof container or sharps container.
- Dispose of glass ampoules in a puncture-proof container or sharps container.
- Dispose of the sharps container, the lid should be taped shut. The sharps container should then be autoclaved. The container should then be placed in a red biohazard waste container for final disposal.

13.3 Emergency procedures

- Report any injuries (i.e., needle sticks, cuts, etc.) to the Principal Investigator or Laboratory Director as soon as possible.
- Initiate first aid as needed (i.e., wash wounds for no less than 5 minutes with germicidal soap and copious amounts of water, apply tourniquet, etc.).
- Complete an accident report form as soon as possible.
- Seek medical attention as soon as possible, if warranted.

13.4 Bloodborne Pathogens Program and Exposure Control Plan

The US Department of Department of Labor, Occupational Safety and Health Administration (OSHA) have created federally enforced regulations for the protection of workers who may have exposures to blood and other potentially infectious materials (OPIM). The work practices covered include procedures with needles, medical equipment or devices, research with HIV or Hepatitis B and many other procedures where there would be a reasonable expectation of coming into contact with blood or body fluids. The Blood borne pathogen standard, 29 CFR 1910.1030, is the Codified Federal Regulation (CFR) that describes what an employer is responsible to provide to those workers who might have a work related exposure from contact with blood or OPIM. The employer is required by federal law to provide to their employees training, personal protective equipment, access to Hepatitis B vaccination at no cost, safer devices, access to an exposure control plan and appropriate follow up care after an exposure. Each year the employer must review the exposure control plan, provide refresher training and review many other aspects of the Blood borne pathogen program.

All campuses are committed to protecting its employees from risks associated with exposure to bloodborne pathogens. The requirements established by the U.S. Occupational Safety and Health Administration in December 1991 (29 CFR 1910.1030). All employees that have a reasonable anticipated risk for exposure to bloodborne pathogens need to be aware of the requirements established by the U.S. Occupational Safety and Health Administration in December 1991 (29 CFR 1910.1030). The following principles must be followed when employees are potentially exposed to bloodborne pathogens:

- Minimize all exposure to bloodborne pathogens.
- Institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to bloodborne pathogens.
- Routinely employ "Universal Precautions" when exposure to blood or potentially infectious materials is anticipated.

All employees working with Bloodborne Pathogens need to attend an initial training class on bloodborne pathogens as well as an annual refresher course. In addition, employees have to be provided with Hepatitis B vaccination free of charge.

Chapter 14

Infectious waste disposal and disinfection

Infectious waste disposal is governed by regulations set forth by the state of Missouri. Infectious waste and biohazardous waste may be considered the same at UMKC. The following guidance describes the standards for the disposal of infectious waste and meet the requirement for the state of Missouri outlined in the Code of State of Regulations, Title 10-Department of Natural Resources, Division 89-Solid Waste Management, Chapter 7-Infectious waste management. Anyone who generates an infectious waste as defined by the definition found in the Missouri Code of State Regulations is responsible for the safe disposal of that waste. The following sections are an effort to assist with the disposal of infectious waste. Ultimately the generator of the infectious waste is responsible for the disposal.

Infectious waste can mean a variety of things in different laboratories and to different people. In the state of Missouri infectious waste is a regulated waste that is covered under the Code of State Regulations, the state of Missouri defines infectious waste as the following.

14.1 Infectious waste

Means waste capable of producing an infectious disease because it contains pathogens of sufficient virulence and quantity so that exposure to the waste by a susceptible human host could result in an infectious disease. These wastes include isolation wastes, cultures and stocks of etiologic agents, blood and blood products, pathological wastes, other contaminated wastes from surgery and autopsy, contaminated laboratory wastes, sharps, dialysis unit wastes, discarded biological materials known or suspected to be infectious; provided, however, that infectious waste does not mean waste treated to department specifications.” (10 CSR 80-7.010 (1)(A)).

Infectious waste at UMKC can also be considered biohazardous waste. Waste that is generated in any laboratory at UMKC that could be included in the above definition will be considered infectious/biohazardous waste. The state of Missouri further defines various types of infectious waste. Below, the six different types of waste are described, the definitions are directly from (10 CSR 80-7.010).

14.1.1 Isolation waste

Wastes generated by patients who have communicable diseases which are capable of being transmitted to others via those waste

14.1.2 Contaminated surgical, dialysis and laboratory waste

Wastes generated by surgery, dialysis and laboratory departments in the process of caring for patients who have communicable diseases which are capable of being transmitted to others via those wastes.

14.1.3 Cultures and stocks of infectious agents and associated biologicals

Cultures and stocks of infectious agents shall be designated as infectious waste when discarded because of the high concentrations of pathogenic organisms typically present in these materials. Included in this category are all cultures and stocks of infectious organisms as well as culture dishes and devices used to transfer, inoculate and mix cultures

14.1.4 Blood and blood products

All discarded human blood and blood products, including serum, plasma and other components known or suspected to be contaminated with a transmissible infectious agent; except that the term blood products does not include patient care waste such as bandages or disposable gowns that are lightly soiled with blood or other body fluids, unless such wastes are soiled to the extent that the generator of the wastes determines that they should be managed as infectious wastes.

14.1.5 Animal carcasses and tissues

All discarded or stored animal carcasses and/or tissues must comply with the Laboratory Animal Research Core SOP entitled “Process and Disposal of Animal Carcasses” (<http://www.umkc.edu/ors/larc/>).

14.1.6 Pathology waste

These wastes include tissues, organs, body parts and body fluids that are removed during surgery and autopsy. All such wastes shall be considered infectious waste. Also included are animal carcasses, body parts and bedding from animals contaminated with infectious agents capable of being transmitted to a human host. Nothing in this section shall supersede the disposal requirements for dead animals as set forth in Chapter 269, RSMo.

14.1.7 Sharps

Discarded sharps, including hypodermic needles, syringes and scalpel blades. Broken glass or other sharp items that have come in contact with material considered infectious by definition are also included.

Any infectious waste generated has to be disposed of in a manner consistent with the regulations that have been promulgated by the state of Missouri. The disposal of infectious waste is described below and is from the Missouri Code of State Regulations.

14.2 Disposal of Infectious Waste

“All sharps shall be packaged in rigid, leak-resistant and puncture-resistant containers and sealed prior to disposal. Infectious waste treated to render it innocuous may be disposed as a solid waste provided the treater certifies to the transporter, if other than the generator, and certifies to the sanitary landfill operator or processing facility operator that the waste has been rendered innocuous as required by section 260.203, RSMo. (Note: Treated infectious waste is not required to be transported in accordance with the requirements of section (4) of this rule.)”

“Certification of treated infectious waste, at a minimum, shall contain the following information: the name, mailing address, location (when different from the mailing address) and phone number of the office/facility treating the infectious waste; the printed name and the signature of the facility/office manager or person responsible for the treatment process; a brief description of the treated waste (sharps in metal containers, sharps in heavy gauge plastic containers, incinerator ash, laboratory wastes in autoclave bags); and a brief description of the method(s) of treatment (for example, steam sterilization, incineration, disinfection with bleach solution). In addition to these minimum requirements, the generator need only include a statement that the waste has been managed in accordance with the Missouri Solid Waste Management Law and rules and may legally be placed in a sanitary landfill. The certification shall be revised when changes in the operation of the office/facility result in a change to the information required by this paragraph.”

Infectious waste disposal at UMKC can be accomplished in two ways to meet the Missouri regulations. First, the waste may be inactivated and then disposed of in regular solid waste

disposal. Secondly the waste may be disposed of by an outside company contracted to dispose of infectious waste. At UMKC the contracted infectious waste disposal company is Stericycle. The red biohazard tubs are stored in a designated area in most buildings on campus. The disposal of waste by Stericycle requires that an account be put into place. The Biosafety office can assist in the establishment of an account. The cost associated with the account will be the responsibility of the waste generator.

Inactivation of infectious/biohazardous waste at UMKC can be accomplished in a variety of methods. The accumulation, storage and transportation of any infectious waste must be in a secondary container. The container must be rigid and be puncture resistant. The use of a biohazard waste barrel, tub or other suitable container should be used while transporting waste for decontamination. The container should be labeled with the biohazard symbol. For solid and liquid waste the materials can be autoclaved. The length of the autoclave cycle is determined by the type of autoclave, the volume of materials, type of materials etc. The autoclave run must be recorded with the minimum of the following information; Type of materials autoclaved, weight or volume, autoclave cycle parameters, indicator results, name of autoclave operator, lab number, and phone number. This information should be retained by the individual or laboratory that generated the waste for documentation of inactivation of that waste. After sterilization the solid waste may be disposed of in the solid waste stream. The red biohazard bags **MUST** be placed into a black bag or opaque bag or container prior to final disposal. Liquid waste may be disposed of into the sanitary sewer unless chemical or radiological hazards are present. Contact the EH&S office for guidance on final disposal if other hazards are present.

Sharps containers **MUST NOT** be disposed of in the solid waste stream. Sharps and sharps containers must be disposed of by Stericycle. The sharps containers may be autoclaved prior to being deposited into the red bins that are disposed of by Stericycle as a best practice. The sharps containers must be securely sealed shut before disposal. Laboratory tape works well.

Many liquid infectious/biohazardous wastes may be inactivated by chemical disinfection. The use effective of a liquid disinfectant must follow the directions of the disinfectant. The products information sheet will provide information on amount of disinfectant to use, contact time and final disposal will be outlined. The use of some disinfectants may produce a hazardous chemical waste that may need to be disposed of in the hazardous waste stream. Inactivated liquid waste can be then disposed of in the sanitary sewer system.

Any person or lab that generates infectious waste at the University of Missouri-Kansas City has a responsibility to dispose of the waste in a way that is safe to anyone that will come in contact with the waste down stream. The waste must be disposed of in accordance with any and all state of Missouri requirements. Contact the UMKC EH&S office or the Biosafety office with any questions concerning waste disposal.

14.3 Decontamination

Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfecting or sterilization. Disinfecting is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means that viable microorganisms are present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave or by incineration.

14.3.1 Requirements for Recombinant DNA Disposal

The *NIH Guidelines* require that all recombinant DNA materials be appropriately decontaminated before disposal. Any and all rDNA materials must be decontaminated by autoclave treatment, chemical treatment, incineration or by any other acceptable means specific to those materials. The UMKC IBC must be notified in the event that the decontamination of rDNA materials cannot be met.

To select the proper method and tools, it is important to consider, for example, the following aspects:

- Type of biohazardous agents, concentration and potential for exposure
- Physical and chemical hazards to products, materials, environment and personnel.

Physical and chemical means of decontamination fall into five main categories to include heat, liquid, chemical vapors, gases, and radiation.

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time. The following paragraphs will focus on some of these methods.

14.3.1 Methods of Decontamination

Heat

In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in overall shorter exposure *time* and lower temperature. Steam sterilization uses pressurized steam at 121-132°C (250-270°F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170°C (320-338°F) for periods of 2 to 4 hours.

Liquid Chemicals

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to consider when disinfecting:

- Nature of surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to be in contact with the surface to be effective.
- Number of microorganism present - Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms - Microbial agents can be classified according to increasing resistance to disinfectants and heat (See Table 2 below).
- Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow the activity requiring more exposure time.

Table 2. Increasing Resistance to Chemical Disinfectants

		Examples
Least Resistant	Lipid or Medium-Size Viruses	Herpes simplex virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus Human Immunodeficiency virus
	Vegetative Bacteria	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Salmonella choleraesuis</i>
	Fungi	<i>Trichophyton</i> sp. <i>Cryptococcus</i> sp. <i>Candida</i> sp.
	Non-lipid or Small Viruses	Poliovirus Coxsackievirus Rhinovirus
	Mycobacteria	<i>Mycobacterium tuberculosis</i> <i>M. bovis</i>
Most Resistant	Bacterial Spores	<i>Bacillus subtilis</i> <i>Clostridium sporogenes</i>

There are many different liquid disinfectants available under a variety of trade names; *in general*, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are discussed below:

- **Alcohols**

Ethyl or isopropyl alcohols in concentration of 70% to 90% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores. Concentrations above 90% are less effective.

- **Formalin**

Formalin is a 37% solution of formaldehyde in water. Dilution of Formalin to 5%, results in an effective disinfectant. Formaldehyde is a human carcinogen and creates respirator problems at low levels of concentration.

- **Glutaraldehyde**

This compound although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehydes are irritating to the eyes, nasal passages and upper respiratory tract. They should be used always in accordance with the instructions on the label and the appropriate personal protective equipment.

- **Phenol and Phenol Derivatives**

Phenol based disinfectants come in various concentrations ranging mostly from 5% to 10 %. These derivatives including phenol have an odor, which can be somewhat unpleasant. Phenol itself is toxic and appropriate personal protective equipment is necessary during application. The

phenol disinfectants are used frequently for disinfecting contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including *Mycobacterium tuberculosis*, fungi and lipid-containing viruses. They are not active against spores or non-lipid viruses.

- **Quaternary Ammonium Compounds (Quats)**

Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against gram-positive bacteria and lipid-containing viruses. They are less active against gram-negative bacteria and are not active against non-lipid-containing viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general cleaning.

- **Halogens (Chlorine and Iodine)**

Chlorine-containing solutions have broad-spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Diluted solutions may be kept for extended periods if kept in a closed container and protected from light. However, it is recommended to use freshly prepared solutions for spill clean-up purposes. Chlorine-containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilants since they inactivate bacterial spores. Iodine has similar properties to chlorine, iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

- **Vapors and Gases**

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility. Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like BSCs. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde. Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

- **Radiation**

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices. Ultraviolet (UV) radiation is a practical method for inactivating viruses, *Mycoplasma*, bacteria and fungi. UV radiation is successfully used in the destruction of airborne microorganisms, UV light sterilizing capabilities are limited on surfaces because of its lack of penetrating power.

14.3.3 Room Decontamination

Containment laboratories periodically undergo routine decontamination procedures using a disinfectant gas. Additionally, room decontamination may be required in an area where overt biohazardous agent contamination has occurred.

14.3.4 Hand Hygiene in Research Laboratories

Hand-washing with soap and water is considered an important measure of personal hygiene whether working within the confines of a research laboratory or within the everyday private environment. Washing of hands when handling biohazardous agents, is the major method for the prevention of disease transmission and contamination. In the research and health care setting, a number of developments have lead to new guidelines designed to improve hand hygiene practices in the research laboratory. Most of the reports describe hand-washing practices in the healthcare setting; however, these guidelines are also applicable to the research laboratory.

For an in-depth review of hand hygiene practices, refer to the recently published report by the CDC. (Center for Disease Control and Prevention, Guidelines for Hand Hygiene in Health Care Settings: Recommendation of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. *MMWR* 2002; 51 [No. RR-16]).

Chapter 15

Shipping infectious materials

The shipping of infectious materials is governed by the United State Department of Transportation and international agencies and associations. In the United States the United States Postal Service, the Department of Transportation, Department of Agriculture, Federal Aviation Administration, Centers for Disease Control and Prevention and various other agencies have some regulatory administration over the transport of infectious materials. Internationally the International Air Transport Association (IATA) and the International Civil Aviation Organization (ICAO) as well as the World Health Organization and the United Nations have input on the transportation of infectious substances and biological specimens. Because of the numerous and varied organizations involved a person who ships infectious materials should stay aware of the annual updates and changes made in the requirements to avoid shipping delays and fines.

The codified regulations in the United States falls under 49 CFR parts 171, 172, 173 and 175. 49 CFR is the codified federal regulation that regulated the transportation of any hazardous material in the United States. These regulations cover all forms of transportation. Fines can be leveled against an individual or organization that violates any part. Fines can range up to \$500,000 and imprisonment up to five years. Many federal agencies can level fines against a violator. Universities and their employees are not immune.

Below is a description of the procedure that may be required in the shipment of an infectious material or biological specimen. These guidelines are not all inclusive to all situations and shipments. The shipper should consult with the relevant guidelines for updates and additions to the applicable regulation to ensure full compliance. International shipments entering and leaving the United States will require import export documentation and further approvals. The approval process may be lengthy. Agents that have been included in the select agents list may require approval from CDC/APHIS (APHIS / CDC Form 2 - Request to Transfer Select Agents and Toxins) and multiple other agencies **BEFORE** shipment. Plan ahead, the approval process can be lengthy.

Below are some useful definitions from IATA (Dangerous Goods Regulations, 49th edition)

Infectious substance

Infectious substances are defined as substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganism (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans and animals.

Biological products

Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines.

Category A

Category A is an infectious substance which is transported in a form that, when an exposure to it occurs, is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals.

Category B

An infectious substance which does not meet the criteria for inclusion in Category A. Category B must be assigned to UN 3733

Cultures

Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include patient specimens as defined below.

Patient specimens

Patient specimens are those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Medical or clinical waste

Medical or clinical waste is waste derived from the medical treatment of animals or humans or from bio-research.

15.1 Shipping of infectious materials via express couriers (Excluding USPS)

Infectious substances are assigned to Classification 6.2. In this classification there are two categories, A and B. Category A is defined above. The organisms that are listed in Category A are below. The list is not an exhaustive list, new and emerging pathogens that meet the definition of a Category A organism must be treated in the same manner. An organism that may be doubtful in meeting the criteria must be included as a Category A organism. The proper shipping name for Category A UN 2814 *Infectious substance, affecting humans* and UN 2900 is *Infectious substance, affecting animals*. If the material affects both humans and animals then the UN 2900 *Infectious substance, affecting humans* will be used.

15.2 Category A infectious substances

15.2.1 UN 2814 Infectious substance affecting humans

Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei– *Pseudomonas mallei*– Glanders cultures
Burkholderia pseudomallei– *Pseudomonas*
Chlamydia psittaci– avian strains
Clostridium botulinum
Coccidioides immitis
Coxiella burnetii
Crimean-Congo hemorrhagic fever virus
Dengue virus
Eastern equine encephalitis virus
Escherichia coli, verotoxigenic
Ebola virus
Flexal virus
Francisella tularensis
Guanarito virus
Hantaan virus
Hantavirus causing hemorrhagic fever with renal syndrome
Hendra virus
Hepatitis B virus
Herpes B virus
Human immunodeficiency virus

Highly pathogenic avian influenza virus
Japanese Encephalitis virus
Junin virus
Kyasanur Forest disease virus
Lassa virus
Machupo virus
Marburg virus
Monkeypox virus
Mycobacterium tuberculosis
Nipah virus
Omsk hemorrhagic fever virus
Poliovirus
Rabies virus
Rickettsia prowazekii
Rickettsia rickettsii
Rift Valley fever virus
Russian spring-summer encephalitis virus
Sabia virus
Shigella dysenteriae type 1
Tick-borne encephalitis virus
Variola virus
Venezuelan equine encephalitis virus
West Nile virus
Yellow fever virus
Yersinia pestis

15.2.2 UN 2900 Infectious substances affecting animals

African swine fever virus
Avian paramyxovirus Type 1 – Velogenic Newcastle disease virus
Classical swine fever virus
Foot and mouth disease virus
Lumpy skin disease virus
Mycoplasma mycoides– Contagious bovine pleuropneumonia
Peste des petits ruminants virus
Rinderpest virus
Sheep-pox virus
Goatpox virus
Swine vesicular disease virus
Vesicular stomatitis virus

Category B is an infectious substance which does not meet the criteria for inclusion in Category A. Category B must be assigned to UN 3373. The proper shipping name of UN3373 is *Biological substance Category B*. Any biological specimen that may have a likelihood of causing a possible infection must be assigned to one of the two categories. A level of judgment must be used in these instances.

15.3 Types of infectious material shipping

There are several categories that a infectious substance may fall into.

- Division 6.2 Category A
- Division 6.2 Category B
- Non regulated biological material
- Patient specimen or sample
- Genetically modified organism

The first objective in shipping any biological material is to determine which category above the material best fits.

15.3.1 Packaging

The packaging of a shipment of an infectious material or biological material should follow the triple packaging method. The material that is to be shipped is held in a vessel that will not leak its contents. Preventing leaking may be accomplished with a self-sealing container or by additional means like taping or using wax film to create a leak-proof seal. This primary container is then placed into a secondary container that also contains absorbent material. The absorbent material should be of adequate amount to absorb the entire contents from the primary container in the event of lost of containment of the primary container. See graphic below.



The third layer is that of the outside packaging. This outer packaging should be durable, rigid and able to withstand transportation. Depending on the category of material being shipped there may be specific requirements to the individual components. For example the secondary container for the Category A infectious substances must be able to withstand an internal pressure of 95 kPa.

15.3.2 Outside shipping container markings

The outside of any container used to ship infectious substances must be marked in accordance with the pertinent guidelines. There are differences in the requirements for a Category A, category B and other packages.

Category A shipments must be labeled with the following:

- UN Packing instruction 602
- Inner packaging:
 - Water tight primary container
 - Water tight secondary container
 - Primary or secondary container must be able to withstand without leaking, internal pressure differential of no less than 95kPa (0.95 bar) and a temperature range of -40F to 130F.
 - Absorbent material able to absorb the contents of the primary container(s), can be used to separate individual primary containers

- Itemized list of contents, inserted between secondary container and outer packaging
- Rigid outer packaging, smallest external dimension can not be less than 100mm (4in).
- Only same type materials are to be shipped together. E.g. Category A and chemical hazard are not permitted to be shipped together.
- If the infectious substance being shipped are unknown but suspected of being included into category A the words “Suspected Category A Infectious Substance” must be shown in parenthesis following the proper shipping name on the documents inside the package on the list of contents.
- The package must be marked on the outside with a name and telephone number of a responsible person.
- If solid carbon dioxide is used as a refrigerant internal supports must keep the secondary container in place as the dry sublimates away.
- If wet ice is used the packaging or over-pack must be leak proof.

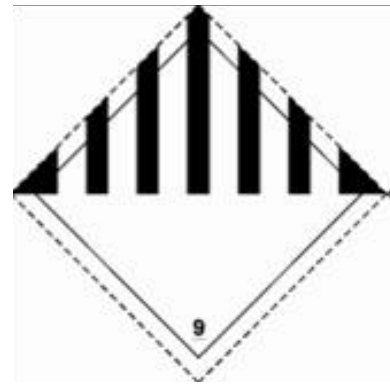
Examples of labels on a Category A package:



Infectious substance



Cargo aircraft only



Dry Ice, UN1845

Note, The weight in Kg of dry ice must be noted on the outside of the packaging.

15.3.3 Category A shipments

The shipping of Category A infectious substances requires a high level of packaging. The packaging must meet specific requirements that are outlined in the UN packing instruction 602.

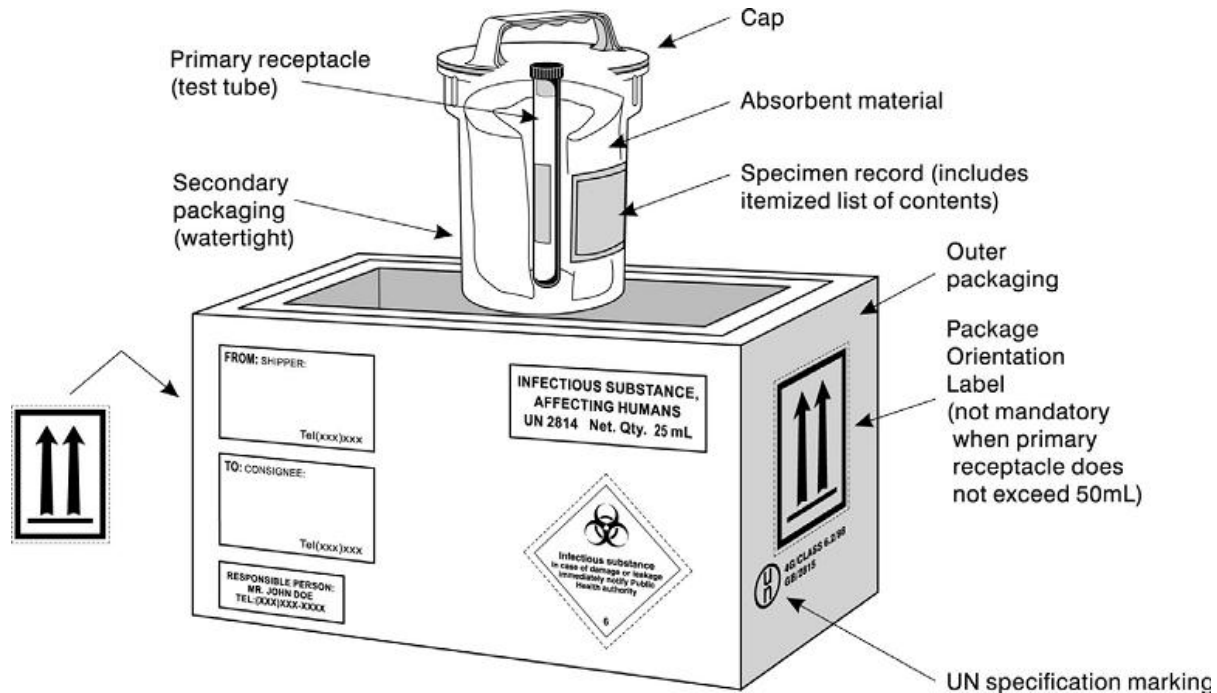
The packaging must include: (From IATA Dangerous Goods Regulations, 49th edition)

- Inner packaging comprising of:
 - Watertight primary receptacle(s)
 - A watertight secondary packaging
 Other than for solid infectious substances, absorbent material, such as cotton wool, insufficient quantity to absorb the entire contents placed between the primary receptacle(s) and secondary packaging; if multiple fragile primary receptacles are placed in a single secondary packaging, they must either be individually wrapped or separated so as to prevent contact between them.
- An itemized list of contents, enclosed between the secondary packaging and the outer packaging; and
- A rigid outer packaging of adequate strength for its capacity, weight and intended use. The smallest external dimension must be not less than 100mm (4in).

Whatever the intended temperature of the consignment, the primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95kPa (0.95 bar, 13.8 lb/in²) and temperatures in the range of

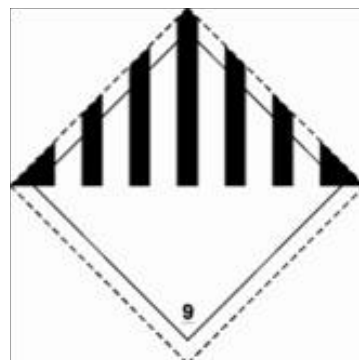
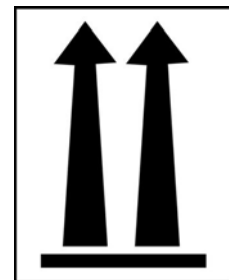
-40°C to 55°C (-40°F to 130°F). All packages containing infectious substances must be marked durably and legibly on the outside of the package with the NAME and TELEPHONE NUMBER OF A PERSON RESPONSIBLE.

Below is an example of a Category A package:



The outside markings for a Category A shipment need to include the following:

1. Infectious substance diamond
2. Proper shipping name, *Infectious substance, affecting humans UN 2814* or *Infectious substance, affecting animals, UN2900*.
3. To and from labels, include responsible person with telephone number
4. Package orientation label, if volume is over 50ml.
5. If using dry ice, use the Class 9 Miscellaneous Dangerous goods diamond. Include the weight of the dry ice on the outside of the package.



15.4 Category B infectious substances

Category B (UN3733) infectious substances are materials that are not included on the Category A list and do not meet exceptions. Category B infectious substances also are packaged under the triple packaging system. The outer packaging must be of suitable construction to withstand normal shipping. The packaging must be composed of the triple packaging model (primary container, secondary container and rigid outer packaging). The secondary packaging must contain enough absorbent material to contain the contents of the primary container in the event of loss of containment by the primary container. The secondary container must have ample packaging material to prevent movement within the outer packaging. For packages containing liquids the following must be followed:

From IATA Dangerous Goods Regulations, 49th edition:

Liquid substances

- The primary receptacle(s) must be leak proof, can not contain more than 1L
- The secondary container must be leak proof
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must either individually wrapped or separated to prevent contact between them.
- Absorbent material must be placed between primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of liquid substance will not compromise the integrity of the cushioning material or of the outer packaging.
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95kPa in the range of -40°C to 55°C (-40°F to 130°F)
- The outer packaging must not contain more than 4L. This quantity excludes ice, dry ice or liquid nitrogen when used to keep the specimens cold.

Solid substances

- The primary receptacle(s) must be siftproof and must not exceed the outer packaging weight limit.
- The secondary packaging must be siftproof
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them.
- Except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4Kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.
- If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a package suitable for liquids, including absorbent materials must be used.

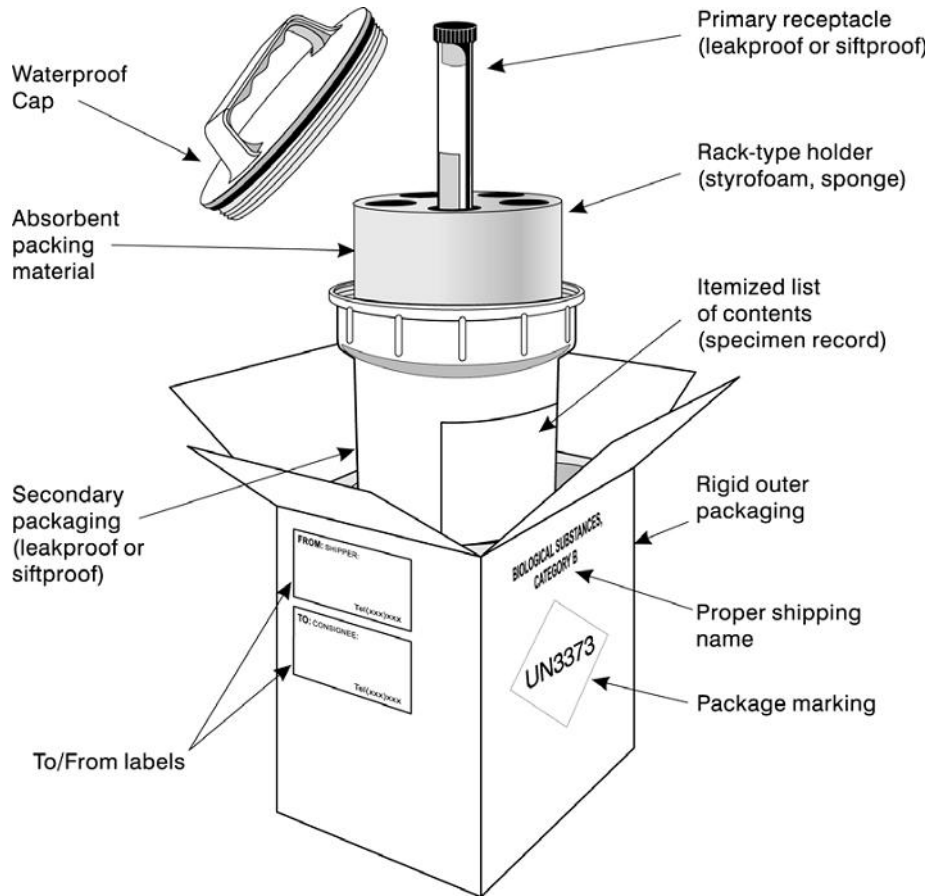
An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

At least one surface of the outer packaging must have a minimum dimension of 100mm X 100mm (4in X 4 in).

15.4.1 Packaging for Category B infectious substances

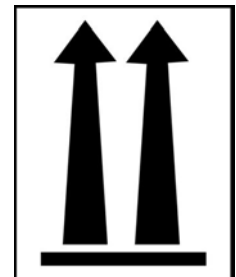
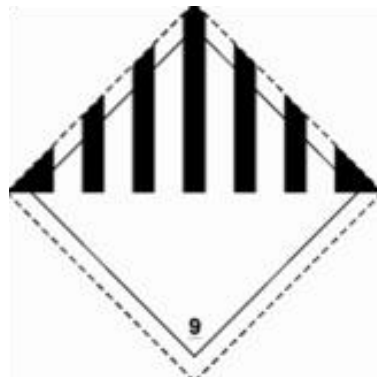
The shipping of a Category B substance will need to comply with the UN 650 packing instructions. Packagings meeting the UN 650 instructions are available from many suppliers. The packaging follows the triple packaging standard. The maximum for a single container is 1L of liquid or 1 Kg of solid material. An over pack can contain a maximum of 4L of liquid and 4 Kg of solid materials.

These quantities don not include the addition of ice, dry ice or liquid nitrogen as refrigerant for the materials. Below is an example of a Category B package.



The outside markings for a Category B shipment need to include the following:

1. UN 3733 diamond
2. Proper shipping name, *Biological substance, Category B*
3. To and from labels, include responsible person
4. Package orientation label, if volume is over 50ml
5. If using dry ice, use the Class 9 Miscellaneous Dangerous goods diamond. Include the weight of the dry ice on the outside of the package.



15.5 Shipping documentation

A Declaration of Dangerous goods form must be completed for any shipment of infectious substances. The finished printed copy must be in color. At least four copies must be with the package when shipped. One copy is retained for the shipper's documentation and archiving. The remaining copies are usually attached to the outside of the packaging and are accessible for inspection. The following areas of the Dangerous form must be completed for the package to be shipped properly. Incomplete and or incorrect forms are the most common reason for refusal of the shipment.

- **Shipper**, name, address and phone number of the person shipping the package
- **Consignee**, Full name and address of the person who will be receiving the package
- **Transport details**, mark if the package is restricted to cargo aircraft only or both passenger and cargo aircraft.
- **Shipment type**, mark out the non-applicable radiological category.
- **UN or ID number**, enter the appropriate UN or ID number, for example UN 2814.
- **Proper shipping name**, fill in the proper shipping name as determined by the UN number.
- **Class or Division**, enter the appropriate hazard class (6.2 infectious substances, 9 dry ice or GMOs)
- **Packaging group**, Biological materials do not have a packaging group, dry ice will be III
- **Quantity and Type of Packaging**, enter the net amount of the materials, use metric units. Towards the bottom of this column indicate "All packed in one fiberboard box" or "Overpack used".
- **Packaging instructions**, enter the correct packaging instructions (Eg. 602 for Category A, 650 for Category B and 904 for dry ice).
- **Authorization**, used for special quantity limitations, special packaging, any other relevant information or documents
- **Additional handling instructions**, this can be a good place to include any emergency contact information for the package.
- **Signature box**, sign and date the document. Signing the document verifies that the signee has met all applicable national and international requirements to ship that package.

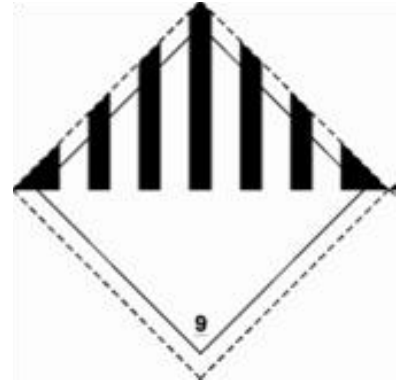
15.6 Shipping with dry ice

Many shipments of infectious materials require that the materials being shipped be kept cold. Dry ice is a common material used to keep shipments cold. Dry ice is considered a hazardous material based on the suffocation hazard from the CO₂ that is given off, the possibility of a contact hazard because of the extreme cold and the possibility of explosion if kept in a sealed container with no venting. Because of these hazards the United States Department of Transportation and the International Air Transport Association considers dry ice a hazardous material. As with any hazardous material being shipped training must be met with associated records.

The UN identification number for dry ice is 1845. Packaging instruction 904

To ship a package with dry ice one should meet the following:

The package must be able to vent the carbon dioxide that is released as the dry ice sublimates. The packaging must not seal tightly this may develop an explosive situation as pressure will build up inside a sealed container. Because of the extreme low temperature of dry ice the containers and packaging should be chosen carefully. Many plastics become brittle at low temperature. Use only approved containers for shipments using dry ice. Approved containers are part of approved shipping packages for Category A & B infectious substances. The package that is being shipped must be labeled with a miscellaneous hazard class 9 label.



The net quantity in Kilograms of the dry ice must be marked on the outside of the package. Information for the Dangerous goods declaration are UN or ID number, UN 1845; Proper shipping name, Dry ice or Carbon dioxide, solid; Class or Division, 9; Quantity, Enter the amount of dry ice used in Kg; Packing instructions, 904. Remember when shipping with dry ice not to seal the package completely to prevent venting of the evolved CO₂. Do not completely tape every seam fully on the package.

15.7 Shipping companies

United States Postal Service

The United States Postal Service does accept infectious materials for shipment. But, the Postal regulations are much more restrictive in the materials shipped. The USPS will not accept Category A infectious materials for shipment. Information describing the Postal Services handling of hazardous material shipping can be found in USPS Publication 52.

United Parcel service (UPS)

The UPS will mail packages falling under the UN3373 class and patient specimens. No category A class shipments will be accepted.

DHL

DHL will accept all shipments that are compliant with the IATA and DOT regulations.

Federal Express

FedEx will not accept shipments involving Risk Group 4 organisms. FedEx Express and Ground will accept shipments that are compliant with the IATA and DOT regulations.

Be sure to check with the specific carrier for any restrictions. The policies can and will change over time.

15.8 Training requirements

1. General awareness with aspects of shipping dangerous goods
2. IATA & DOT regulations
3. Specific training to duties
4. Safety training
5. Test of material
6. Successful completion, certificate issuance

The training frequency is every three years for DOT and every two years for IATA.

Chapter 16

Resources

The following links and information have been put together as a resource. Researchers, staff and students will be able to better inform themselves about topics in the area of biosafety by taking advantage of this material.

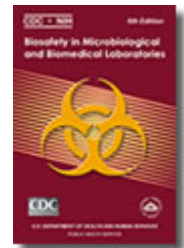
16.1 Regulatory Agencies and Authorities

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and recombinant DNA molecules. Copies of these documents are available by access to the appropriate website.

1. *National Institute of Health (NIH): Guidelines for Research Involving Recombinant DNA Molecules*, April 2002 (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA (rDNA) molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. Because of the committee's activity, the initial version of the *NIH Guidelines* was published in 1976. It has been amended and revised many times since then. Included in the *NIH Guidelines* is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed rDNA research using the *NIH Guidelines* as a minimum standard.
2. *Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Guidelines on Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, 2007 (BMBL manual) (<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>). In 1984, the CDC/NIH published the first edition of the BMBL Manual. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. This manual has been revised several times and is the standard for biosafety.
3. *Occupational Safety and Health Administration: Bloodborne Pathogens Standard* (<http://www.osha.gov/SLTC/bloodborne pathogens/standards.html>). In 1992, the Occupational Safety and Health Administration (OSHA) promulgated a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions
4. *Department of Health and Human Services (HHS): Additional Requirements for Facilities Transferring or Receiving Select Agents*. In 1996, HHS published a set of rules that require facilities and institutions to be registered and approved in order to transfer or receive certain biological agents and toxins. HHS requires UNMC to comply with the BMBL manual (see above), OSHA's Laboratory Safety Standard 29 CFR 1910.1450, and 42 CFR Part 73.
5. Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens and biological products are addressed in the following rules and guidelines:
 - United Nations - *Recommendations of the Committee of Experts on the Transportation of Dangerous Goods*
 - International Civil Aviation Organization (ICAO) - *Technical Instructions for the Safe Transport of Dangerous Goods by Air*

- International Air Transport Association (IATA) - *Dangerous Goods Regulations* <http://www.iata.org>
 - U.S. Department of Transportation - 49 CFR Part 72 <http://www.dot.gov.rules.html>
 - U.S. Public Health Service - 42 CFR Part 72
 - U.S. Postal Service - 39 CFR Part 111
 - U.S. Department of Labor, OSHA - 29 CFR 1910.1030
6. Importation permits are required for infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, *Foreign Quarantine*. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.
7. Possession, Use and Transfer of Select agent and Toxins (42 CFR Part 72 and 73) (42 CFR Part 1003) - This regulation became effective on February 7, 2003. This document establishes a final rule regarding the possession, use, and transfer of select agents and toxins. The final rule implements provisions of the Public Health Security and Bioterrorism Preparedness and response Act of 2002 and is designed to protect public health and safety. The select agent list is reviewed every two years. A list of the organisms and toxins included in the Select Agent list can be found at <http://www.selectagents.gov/>
8. Agricultural Bioterrorism Protection Act of 2002: Possession, Use and Transfer of Biological Agents and Toxins (7 CFR Part 331) and (9 CFR Part 121) These rules establish the regulations governing the possession, use, and transfer of biological agents and toxins that have been determined to have the potential to pose a severe threat to public health and safety, to animal health, to plant health, or to animal and plant products. This rule is also reviewed every two years. Many of the agents on this list overlap with the agents from 42 CFR, they are known as overlap agents. The list of APHIS Regulated Agents can be found at <http://www.aphis.usda.gov/>

16.2 BMBL5: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition
<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>



16.3 NIH-OBA: National Institutes of Health, Office of Biotechnology Activities
<http://www4.od.nih.gov/oba/>

16.4 The American Biological Safety Association

The American Biological Safety Association is an association of academic, governmental and privately held institutions biological safety professionals and individuals related to the safety of biological materials. (<http://absa.org/>)



16.4.1 ABSA Risk group classification of organisms

In many countries, including the United States, infectious agents are categorized in risk groups based on their relative risk. Depending on the country and/or organization, this classification system might take the following factors into consideration:

- Pathogenicity of the organism
- Mode of transmission and host range
- Availability of effective preventive measures (e.g., vaccines)

- Availability of effective treatment (e.g., antibiotics)
- Other factors

Website database: Search Bacteria, Search Viruses, Search Fungi, Search Parasites

16.5 Centers for Disease Control and Prevention
(<http://www.cdc.gov/od/ohs/>)



16.6 U.S. Department of Labor

Blood Borne Fact Sheets by Number:

http://www.osha.gov/OshDoc/data_BloodborneFacts/index.html

16.7 Public Health Agency of Canada

Material Safety Data Sheets for Infectious Agents

The MSDS are organized to contain health hazard information such as infectious dose, viability (including decontamination), medical information, laboratory hazard, recommended precautions, handling information and spill procedures. The intent of these documents is to provide a safety resource for laboratory personnel working with these infectious substances. Because these workers are usually working in a scientific setting and are potentially exposed to much higher concentrations of these human pathogens than the general public, the terminology in these MSDS is technical and detailed, containing information that is relevant specifically to the laboratory setting. It is hoped along with good laboratory practices, these MSDS will help provide a safer, healthier environment for everyone working with infectious substances. (From the website)

<http://www.phac-aspc.gc.ca/msds-ftss/index.html>

16.8 World Health Organization

Biorisk management: Laboratory biosecurity guidance

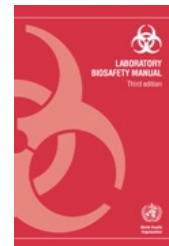
http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6/en/index.html

Guidance on regulations for the Transport of Infectious Substances 2007-2008

http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2/en/index.html

16.9 Laboratory Biosafety Manual- Third Edition

http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/index.html



16.10 International Air Transport Association (IATA)

<http://www.iata.org/index.htm>

Infectious substances shipping

http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm

16.11 United States Department of Transportation: Pipeline and Materials Safety Administration

<http://phmsa.dot.gov/>

Transport of hazardous materials

Title 49CFR parts 100-179 (172.323 Infectious substances)

http://www.access.gpo.gov/nara/cfr/waisidx_07/49cfrv2_07.html

Acknowledgments

Portions of this manual were adapted or quotes from the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, the NIH and CDC *Biosafety in Microbiological and Biomedical Laboratories*, the University of Missouri-Columbia Biosafety Manual, the Biosafety Manual of the University of Nebraska Medical Center, the Yale University Biosafety Manual, and the University of Pittsburgh Biosafety Manual.