University of Kentucky

Biosafety Manual

(rev. 2017-0324)

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1.0 Preface

The policy of the University of Kentucky is to provide a safe and healthful work environment for employees, students and visitors. It is the intent of the University of Kentucky to minimize, to the extent practicable, all recognizable hazards and to comply with all Federal and State laws and regulations. The implementation of this policy is the responsibility of all employees of the University. Supervisors at all levels shall be accountable for the health and safety of employees engaged in activities under their supervision. Supervisors shall insist that employees comply with health and safety rules and work in a safe manner.
The Division of Environmental Health and Safety has responsibility for assisting the University to meet the intent of this policy and to achieve overall safety compliance. Specifically, biosafety is under the direction of the Institutional Biosafety Committee (IBC) and the Biological Safety Officer (BSO).

2.0 Introduction

Microbiological, molecular biology, and biomedical research laboratories pose a number of special circumstances that set them apart from other types of laboratories. Most of these circumstances exist because the work conducted within them often involves organisms that are potentially infectious to humans. The purpose of this manual is to define the appropriate kinds of facilities and work practices for microbiological, molecular biology, and biomedical research laboratories to reduce the probability of infection to laboratory personnel and to mitigate any additional hazards associated with release of genetically modified plants, animals, or organisms or agents potentially infectious to plants and animals. All principal investigators, faculty, staff, and students working with potentially infectious agents or recombinant and synthetic nucleic acids are expected to abide by the provisions of this manual.

The Institutional Biosafety Committee (IBC) has adopted the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Ed as the University's official biosafety guidelines for the use of potentially infectious agents. In addition, University of Kentucky follows the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) for work with human source materials, as well as the Practical Guide for Containment for plant research with transgenic plants or plant microbes. Recombinant DNA research is regulated by NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Additional information regarding safe practice, procedures, and equipment is available at the Department of Biological Safety website, http://ehs.uky.edu/biosafety/ or can be obtained by emailing the department at biosafety@uky.edu.

Research involving human source materials (OPIM), infectious agents, or recombinant or synthetic nucleic acids shall be registered via the online registration system http://topaz.uky.edu and reviewed by the Biological Safety Officer and the IBC. All proposals, regardless of funding source, are subject to this review. The IBC ensures the proper safety precautions and procedures have been met and recommends approval or disapproval of the protocol. Additional information regarding IBC registration can be found at http://ehs.uky.edu/docs/pdf/bio_ibc_registration_0001.pdf .

2.1 Department of Biological Safety Contact Information

University of Kentucky Department of Biological Safety
505 Oldham Court
Lexington, KY 40502-0473
Phone: (859) 257-1049
Fax: (859) 323-3838
Email: biosafety@uky.edu
Web: http://ehs.uky.edu/biosafety/

Personnel	Office Phone	Cell Phone	Email
Brandy Nelson, Biological Safety Officer, Responsible Official	(859) 257-1049	(859) 550-0333	bnels3@uky.edu
Holley Trucks, Asst. Biological Safety Officer, Alternate Responsible Official	(859) 257-8655	(859) 699-6082	hmtr222@uky.edu
Eric Rouse, Senior Safety Specialist	(859) 323-5728	(859) 797-8431	ejrous0@uky.edu
Dee Webb Mazzetti, Senior Safety Specialist	(859) 257-1073	(859) 539-4140	d.mazzetti@uky.edu

2.2 Emergency Telephone Numbers

Name	Office Phone
UK Police Department (UKPD)	911 from campus phone (859) 257-5770
UK Police Dispatch	(859) 257-1616
Med Center Security	(859) 323-6946
PPD Dispatch	(859) 323-6281
EMS	911
Chandler Emergency Department	(859) 323-5901
UK Worker's Care Plan	1-800-440-6285

2.3 Division of Environmental Health & Safety Information

The EHS Division supports the University's teaching, research, and public service mission by promoting a safe, healthful, clean, and accessible campus environment.

The Division's programs are intended to provide safe and healthy conditions for work and study, protect the environment, and comply with applicable laws and regulations. The Division serves the University community by providing technical services, education and training, periodic audits, and compliance assistance.

Environmental Health & Safety
252 E. Maxwell St.
Lexington, KY, 40506-0314
Phone: (859)257-3845
Fax: (859) 257-8787
Web: http://ehs.uky.edu/

Name	Office Phone	Cell Phone
David Hibbard, Director EH&S, Alternate Responsible Official	(859) 257-3845	(859) 699-6083
Lee Poore, Director Occupational Health and Safety	(859) 257-2924	(859) 227-7499
Robert Kjelland, Director Environmental Management	(859) 257-3285	(859) 509-2238
Greg Williamson, UK Fire Marshal	(859) 257-8590	(859) 338-1055
Gerald Schlenker, Radiation Safety Officer	(859) 323-6308	(859) 699-6084

3.0 Responsibilities

3.1 Department Chairperson

The Department Chairperson is responsible for the implementation and maintenance of safe practices and procedures in the department. The Chairperson shall ensure compliance among researchers and lab personnel with safe practices and procedures in the laboratories within the department. The Department Chair is notified of any delinquent IBC registrations when the Principle Investigator of the registration fails to respond to notifications by the Department of Biological Safety.

3.2 Principal Investigator

In regard to safety and regulatory compliance, the key person in the laboratory is the Principal Investigator (PI). This is the individual who has been assigned the responsibility and discretionary authority to set work practices. The attitude of this person will be reflected by others working in the facility. It is the policy of the University that the Principal Investigator is responsible for complying with this Biosafety Manual, the procedures recommended in the CDC/NIH publication Biosafety in Microbiological and Biomedical Labs, and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). The Principal Investigator is also responsible for compliance with CDC Select Agent, Occupational Safety and Health Administration (OSHA) regulations, and IBC or other regulatory compliance committee approved protocols.

Specifically, the Principal Investigator has the primary responsibility for:

a. Determining the real and potential biohazards of the proposed research
b. Performing risk assessments for procedures to be performed in the lab
c. In consultation with the Biological Safety Department and considering recommendations from the IBC, determining the appropriate equipment, practices and procedures to ensure containment of biohazards
d. Selecting the microbiological practices and laboratory techniques for handling potentially infectious agents and recombinant or synthetic nucleic acid materials
e. Preparing procedures for dealing with accidental spills and personnel and environmental contamination
f. Identifying risks associated with the work performed in the lab and determining the applicability of various precautionary medical practices, serological monitoring, and immunization in conjunction with procedures established by and the assistance of the Department of Occupational Health
g. Securing approval by the appropriate University compliance committees (e.g. IBC, IRB, IACUC) of the proposed research prior to initiation of work
h. Completing annual reviews and renewals of compliance committees, as required, in a timely manner
i. Providing copies of all approved University compliance committee protocols/registrations to laboratory personnel for review
j. Providing appropriate procedural and laboratory specific training to personnel
k. Providing all appropriate personal protective equipment to laboratory personnel for work performed

3.3 Laboratory Supervisor

A laboratory supervisor should be designated by the Principal Investigator in laboratories where the PI is not actively involved in the daily operation of the research lab with many personnel. The PI may function as the laboratory supervisor in labs with limited personnel or in which the PI is actively engaged in the daily operation of the research lab. The responsibilities of the laboratory supervisor include enforcement of the safety practices and procedures that have been determined to be appropriate for the lab and training of lab personnel. The laboratory supervisor must ensure

that lab personnel receive appropriate training and updates as needed and are aware of the risks and risk mitigation strategies appropriate to the materials handled in the lab. The laboratory supervisor must ensure that personnel demonstrate proficiency in laboratory practices prior to working with agents requiring BSL2 or higher containment. Additional responsibilities of the lab supervisor may include 1) ensuring proper operation and preventive maintenance of safety equipment including biological safety cabinets, centrifuges and their rotors, cups and gaskets 2) maintaining supplies of personal protective equipment (PPE) 3) selecting and evaluating PPE and safety devices to mitigate potential exposure situations noted during the daily operation of the lab, and 4) maintaining the lab specific biosafety manual and training records. The laboratory supervisor may also be responsible for reporting accidents and exposure incidents to Occupational Health & Safety. Laboratory supervisors should communicate any concerns regarding lab safety to the PI or directly to the appropriate division of Environmental Health and Safety.

3.4 Research Laboratory Personnel

Research laboratory personnel are ultimately responsible for working safely in the laboratory. Personnel shall ensure that all work is conducted in compliance with University of Kentucky, NIH, CDC, OSHA and other applicable guidelines and regulations. Personnel should follow the University of Kentucky Biosafety Manual except where superseded by the University of Kentucky BSL3 Biosafety Manual, Bloodborne Pathogens requirements, or a more stringent guideline presented in the laboratory specific Biosafety Manual or approved IBC registration. Personnel need to know specific laboratory practices, potential hazards of infectious agents in use, emergency and spill procedures, and the signs and symptoms of lab acquired infections or exposures to the materials in use. Research laboratory personnel shall complete all required training and help to maintain laboratory safety through compliance with laboratory procedures and communication with the PI and the Department of Biological Safety.

3.5 Department of Biological Safety

The Department of Biological Safety, under the direction of the Biological Safety Officer, is responsible for programs concerning the safe use of recombinant or synthetic nucleic acid materials, infectious agents, and potentially infectious materials such as human sourced materials in the research and teaching laboratories at the University of Kentucky. This includes training, auditing, and consulting with researchers, laboratory personnel and teaching staff concerning compliance with the federal and state laws and regulations in these areas. The Biological Safety Officer is also the liaison between researchers and the Institutional Biosafety Committee, which reviews protocols dealing with infectious agents and/or recombinant DNA.

The Department of Biological Safety provides the following services to the research community include:

- Evaluation and inspection of laboratory facilities for work with infectious agents and other hazardous biological agents
- Consultation on the operation of the laboratory to ensure compliance with CDC, NIH, OSHA, federal, and state criteria
- Education and training of faculty, staff, and students who conduct research with rDNA, infectious agents and potentially infectious materials of their responsibilities to protect themselves and the environment from potential exposures
- Maintenance of training records for compliance with federal, state and University requirements
- Consultation to members of the University of Kentucky community in matters related to biological safety such as advice on safe methods for new procedures
- Provides guidance in the event of large or high hazard biohazardous material spills.

3.6 Institutional Biological Safety Committee

The Institutional Biological Safety Committee (IBC) performs duties for the University as defined in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic

Nucleic Acid Molecules (NIH Guidelines). As such, the Committee shall review applications for research involving recombinant or synthetic nucleic acids to determine whether the facilities, procedures, and practices meet the standards required by the University and the NIH. It shall, in addition, have the responsibility to certify annually to the NIH that such facilities, procedures, and practices, and the training and expertise of personnel meet NIH standards. Meetings called for the purpose of such review and certification may be open to the public. Minutes of these meetings shall be kept and made available for public inspection. The University of Kentucky also requires the IBC to review all research involving infectious agents and other potentially infectious materials to ensure compliance with the OSHA Blood Borne Pathogens Standard, 29CFR 1910.1030 and the guidelines set forth in the BMBL. Information on the committee membership and meeting schedules can be found on the biosafety website.

4.0 University of Kentucky Biological Safety Requirements

The following information describes the requirements for University of Kentucky researchers as defined by the Institutional Biosafety Committee and the Department of Biological Safety. It is the responsibility of each Principal Investigator to ensure the laboratory is in compliance.

The Department of Biological Safety shall be contacted before:

- Work with a new infectious agent and/or recombinant or synthetic nucleic acids is initiated
- Changes are made in the scope or location of existing work
- Infectious agents and/or recombinant or synthetic nucleic acids are provided to another investigator on or off campus
- Visiting researchers are invited to work in the laboratory

4.1 Registration with the Institutional Biosafety Committee

Any research involving any infectious agents, potentially infectious materials or recombinant or synthetic nucleic acids is required to be registered with the Institutional Biosafety Committee as established by University of Kentucky policy and the NIH. Because the University receives funding from NIH grants, ALL research conducted at the University must comply with the NIH Guidelines for Research Involving Recombinant DNA Molecules and University policies.

Biohazardous materials which are considered worthy of registration may include, but are not limited to:

- Infectious agents (viral, bacterial, fungal, parasitic, or prion)
- Recombinant nucleic acids (ex: plasmids with inserts, viral vectors, etc. or whole animals and plants with introduced recombinant materials)
- Synthetic nucleic acids administered to live animals
- Infected animal blood and/or tissues
- Human blood, blood products, or fluids
- Human derived cell lines or tissues
- Live vaccines

If you are uncertain as to whether material you are using in your research qualifies as biohazardous, please contact the University of Kentucky Department of Biological Safety at biosafety@uky.edu or call 859-257-8655.

Registration of protocols for Institutional Biosafety Committee review and approval requires the use of our online software. For more information about registration, the registration process or the software requirements, please visit: http://ehs.uky.edu/docs/pdf/bio_ibc_registration_0001.pdf

For information about IBC meeting dates and/or registration submission deadlines, please visit: http://ehs.uky.edu/docs/pdf/bio_ibc_meeting_schedule_0001.pdf

4.2 Working with Biohazardous Materials in the Laboratory

The control of biological hazards in the laboratory shall be maintained by:

- Obtaining Institutional Biosafety Committee approval for the work performed
- Maintaining proper air supply to the laboratory so that air in the laboratory always remains negative in relationship to the hallways and surrounding areas
- Keeping laboratory doors closed and locked when unoccupied.
- Limiting access to the laboratory in general
- Limiting access to the infectious or registered materials
- Limiting the handling of material to the minimum compatible with efficient usage
- Insuring proper usage of appropriate safety equipment
- Insuring proper disposal of infectious material after usage
- Insuring proper precautions, and procedures when handling the materials:
- Maintaining appropriate levels of identification, warning, and security in use and storage of the material.
- Conducting inventories of infectious agents and toxins in all laboratories to ensure that the institution has a record of which infectious agents and toxins are being utilized, has documentation that those materials are properly stored under the appropriate containment conditions, and has documentation that cites the party responsible for appropriate stewardship of the materials.

4.3 Laboratory Signage and Labeling of Equipment

The universal biohazard warning labels (image above) shall be posted on the outside door of each laboratory and on any equipment used for storage or manipulation of biohazardous material which is potentially infectious to humans. Materials considered infectious to humans include but are not limited to:

- Human pathogens, Risk Group 2* and above (viral, bacterial, fungal, parasitic, or prion)
- Animal blood and/or tissues infected with agents also infectious to humans
- Human blood, blood products, or fluids
- Human derived cell lines or tissues
- Viral vectors
- Live vaccines

* http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

Laboratories working with plants and insects should use appropriate signage not containing the universal biohazard warning symbol. Contact the Department of Biological Safety for appropriate signage.

Examples of proper placement of a label using the universal biohazard symbol include:

- Doors to laboratories which contain or manipulate materials
- Animal Housing or Procedure rooms where agents infectious to humans are present
- All equipment and materials used to manipulate, or store materials (ex: centrifuges, freezers)
- Containers used to transport biohazardous materials between locations
- Waste containers for untreated biohazardous waste

4.4 Recombinant or Synthetic Nucleic Acid Experiments

Institutional Biosafety Committee (IBC) approval is required prior to the initiation of most non-exempt experiments involving recombinant or synthetic nucleic acid materials. Many exempt experiments still require registration due to the expanded purview of the University of Kentucky IBC. A brief description of non-exempt and exempt recombinant or synthetic nucleic acid experiments can be found in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Principal Investigators shall contact the Department of Biological Safety to register all recombinant nucleic acid work, update current registration if the scope of the work has changed, or if there are any questions regarding recombinant nucleic acid work.

4.4.1 Viral Vectors in the Laboratory

A comprehensive outline of common viral vectors used in current research and their safety considerations can be accessed at: http://ehs.uky.edu/docs/pdf/bio_viral_vectors_0001.pdf

When work with viral vectors is being performed, a sign stating that viral vectors are present, entry is restricted to authorized personnel, and doors are to remain closed should be posted on laboratory doors. A sign for this purpose may be found at http://ehs.uky.edu/docs/pdf/bio_s_viral_vector_present_0001.pdf.

4.4.2 Animal Use with Nucleic Acids

- Concurrent approvals are needed from UK Institutional Biosafety Committee (IBC) and UK Institutional Animal Care and Use Committee (IACUC) prior to commencing animal work with viral vectors, siRNA, or other recombinant or synthetic nucleic acid molecules.
- Viral vector stocks must be tested for the presence of replication competent virus prior to introduction into research animals. Before use in animals, human/animal cells transfected with 1st and 2nd generation viral vectors must be tested for replication competency. Testing procedures and results should be recorded in laboratory notebooks or otherwise documented. This documentation may be requested during a laboratory audit or by the Institutional Biosafety Committee (IBC).
- Animals shall be handled in a BSL-2 area designated and approved for viral vector work.
- Depending on the viral vector in use, infected animals may excrete (shed) virus within the first 72 hours after infection. Only lab personnel or animal husbandry workers trained to handle animals infected with viral vector should be responsible for animal husbandry practices during the first 72 hours following infection of the animal.
- Precautions shall be taken not to create aerosols when emptying animal waste material, washing cages, or cleaning the room.
- Special training shall be given to all animal husbandry personnel on the specific viral vectors in use. This training shall address the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment.
- All necropsy shall be performed in a necropsy room using Animal BSL-2 precautionary practices and procedures.

4.4.3 Accidental Exposure to Recombinant Nucleic Acids

Accident reporting procedures are addressed later in this document. However, significant research related accidents and illnesses must be reported to the NIH Office of Biotechnology Activities within 30 days. However, spills or accidents in BSL2 laboratories which result in overt exposure to recombinant nucleic acid materials must be reported immediately. **In the event of any accidents, spills, or illnesses related to research with recombinant nucleic materials, please immediately contact the University of Kentucky Biological Safety Officer at 859-257-1049 after first aid or urgent care is administered.**

4.5 Tat-fusion Protein

Guidelines for tat-fusion protein use can be accessed at:
http://ehs.uky.edu/docs/pdf/bio_rna_tat_fusion_protein_use_0001.pdf

4.6 Human Gene Transfer (HGT)

Proposed clinical trials involving human gene transfer require registration and approval from both campus and federal agencies before initiation. The University of Kentucky Institutional Biosafety Committee shall approve all protocols involving HGT. Contact the Department of Biological Safety for more information and/or visit: http://ehs.uky.edu/docs/pdf/bio_hgt_guide_0001.pdf

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

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Blood borne Pathogens Standard, 29 CFR Part 1910.1030 (Blood borne Pathogens Standard) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or certain body fluids (blood borne pathogens). The Blood borne 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 (OPIM) include:

- Human cell or tissue cultures, including established cell lines obtained from a repository such as ATCC
- 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, HBV, or other blood borne pathogens

OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain blood borne pathogens (BBP). Examples of BBP include the following:

Human Immunodeficiency Virus (HIV)	Human T-lymphotrophic Virus Type 1	Colorado Tick Fever virus
Hepatitis B virus (HBV)	*Babesia* species	*Brucella* species
Hepatitis C virus (HCV)	*Leptospira* species	*Spirillum* minus

Hepatitis D virus	*Francisella* species	Creutzfeldt-Jakob virus
Borrelia species	*Streptobacillus moniliformis*	*Plasmodium* species
Arboviruses	Hemorrhagic Fever viruses	*Treponema* species

An individual is also considered occupationally exposed if they do not have direct contact with blood or other potentially infectious material, if the employee uses equipment that is used to process or store blood, other potentially infectious materials or blood borne pathogens. All occupationally exposed employees are required by OSHA to attend a Blood borne Pathogens training session prior to beginning work and annually thereafter. Blood borne Pathogens training is available on-line on the UK Department of Biological Safety website (http://ehs.uky.edu/classes/).
There are additional requirements for research laboratories and production facilities engaged in the culture, production, concentration, and manipulation of HIV and HBV. For more information, please visit:
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10051&p_table=STANDARDS

4.8 Select Agents and Toxins

In compliance with the "Public Health Security and Bioterrorism Preparedness Response Act of 2002" (Public Law 107-188) and the Select Agents regulations from the Centers for Disease Control (42 CFR 73) and USDA (9 CFR 121, 7 CFR 331) any research project which will utilize Select Agents or Toxins and High Consequence Livestock Pathogens and Toxins on campus requires special registration. Please contact the Department of Biological Safety if you plan to use any of the federally listed select agents or toxins in your research. The current list can be found at: http://www.selectagents.gov/SelectAgentsandToxinsList.html

For information on Select Agent and Toxin regulations, please visit:
http://ehs.uky.edu/docs/pdf/bio_select_agent_additional_info_0001.pdf

4.9 Research Animals

All research experiments involving animals shall be conducted in accordance with the associated Institutional Animal Care and Use Committee (IACUC) approved protocol. Animal research that involves a hazard (biological, radiological, or chemical) shall be reflected in the approved IACUC protocol. The IBC shall approve work with human pathogens or recombinant or synthetic nucleic acids in animals (including transgenic animals) prior to initiation.

Comprehensive reviews indicate that animals infected with a wide range of etiologic agents are capable of shedding infectious micro-organisms in the saliva, urine or feces. In the absence of specific information to the contrary, all infected animals should be regarded as potential shedders.

Procedures appropriate for the handling of infected animals are given below:

- Careful handling procedures should be employed to minimize the dissemination of dust from animal and cage refuse.
- Cages should be sterilized by autoclaving. Refuse, bowls, and watering devices should remain in the cage during sterilization.
- All water devices should be of the "non-drip" type.
- Cages should be examined each morning and at each feeding time so that dead animals can be removed. Dead animals should be placed in leak-proof containers (plastic bags, covered metal trays, canisters, or fiber cartons) that are appropriately marked with date, experiment, biohazard label, cage number, etc., and stored in designated refrigerators or cold rooms prior to necropsy or disposal.

- Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should never be placed in the cage to move any object therein.
- When animals are to be injected with infectious agents, the animal caretaker should wear protective gloves and the laboratory workers should wear surgeon's gloves. Animals should be properly restrained using physical restraints (e.g., use of squeeze cage for primate inoculation), anesthesia, or specific handling practices to avoid accidents that might result in disseminating infectious agents, as well as to prevent injury to the animal and to personnel. Infected animals to be transferred between buildings should be placed in microisolator cages or other aerosol-proof containers.
- Animals exposed to infectious agents in aerosols require special consideration based on the agent in use and experimental procedures. Contact the Department of Biological Safety for assistance.

There is concern for zoonotic disease transmission to some individuals handling research animals, including non-human primates, wild caught animals and any tissues or biological samples derived from these particular animals. For information on zoonotic diseases, and biosafety tips for personnel with potential for zoonotic disease exposure, please visit: http://ehs.uky.edu/docs/pdf/bio_ia_zoonotic_diseases_of_concern_0001.pdf

4.10 Research with Transgenic Plants and/or Plant Pests

Research involving transgenic plants and/or plant pest species requires safe handling, appropriate containment and is subject to federal regulations (USDA-APHIS, EPA, FDA), the NIH Guidelines, and the University of Kentucky IBC. Physical containment of plants, pollen, seeds, and pest species must be ensured to prevent release to the environment. The Principal Investigator (PI) is ultimately responsible for the research project and for ensuring compliance with biosafety standards.

Safe conduct of research with transgenic plants or plant pest species requires usage of appropriate PPE, adequate facility signage, as well as implementation of Standard Operating Procedures for the safe handling, transfer, sterilization and disposal of materials. Researchers should contact the Department of Biological Safety for more information or consultation.

For guidance on safely handling transgenic plant materials, please see Appendix P of the NIH *Guidelines for Research Involving Recombinant DNA Molecules* at*:* http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

For guidance on Plant and Plant pest containment, please see A Practical Guide to Containment, Plant Biosafety in Research Greenhouses at:
http://www.isb.vt.edu/documents/Plant%20Contain.text.PDFX-1a.pdf

4.11 Minors in Laboratories

Minors under the age of 14 are not permitted inside of any research laboratory, greenhouse, or animal facility at the University of Kentucky except for UK sponsored programs which are designed for youth under the age of 14 and which have documented training policies.

Visiting minors, not previously approved as part of a UK program, tour, or science fair, are not allowed in any UK research laboratory, greenhouse, or animal facility for any reason.

Specific policies and procedures for gaining approval for minors to conduct work in UK research laboratories, greenhouses, or animal facilities, please visit:
http://ehs.uky.edu/docs/pdf/ohs_minors_in_labs_0001.pdf

For more information and required registration forms for minors, please visit:
http://ehs.uky.edu/ohs/minors_0001.php

4.12 Personnel Training

Successful completion of a range of biosafety training programs may be required prior to the initiation of your work at the University of Kentucky. Please review the chart found at http://ehs.uky.edu/docs/xls/bio_pi_req_matrix.xls for information on required training. Please contact the Department of Biological Safety for further questions.

Laboratory specific training is also required on the part of the individual laboratory supervisor or PI, which should instruct employees on procedures unique to the laboratory. A checklist to document this laboratory specific training may be accessed at:
http://ehs.uky.edu/docs/pdf/bio_laboratory_specific_training_checklist_0001.pdf

4.13 Equipment

4.13.1 Biological Safety Cabinets (BSCs) and Laminar Flow Benches (LFBs)

The efficacy of Biological Safety Cabinets (BSCs) and Laminar Flow Benches (LFBs) depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory. All BSCs and LFBs at the University of Kentucky shall be placed in the University of Kentucky inventory database and BSCs shall be certified annually and after movement to a new location. Any BSCs not certified will be reported to the Department Chair, Executive Vice-President of Research, and Provost as a serious health and safety violation. It is the responsibility of the PI to ensure any BSC utilized in the laboratory is annually certified.

- Notify the Biosafety Office in advance when you plan to have BSCs or LFBs moved, placed in storage, transferred to a new owner, discarded, removed from the University of Kentucky, or obtained from another institution or manufacturer.

- BSCs shall be professionally gas or vapor decontaminated by a certified technician, before a unit is relocated, stored, serviced (interior), or discarded based upon the agents which have been manipulated in the cabinet and the future usage of the BSC. The PI is responsible for ensuring proper decontamination of the BSC or LFB. Contact the Department of Biological Safety for a risk assessment based upon the use/reuse of the cabinet to determine the appropriate decontamination method.

  Please follow the following procedures for the required clearance of the BSC after decontamination by a vendor and prior to removal from the laboratory space:

  A. Contact vendor to schedule decontamination of BSC.
     1. Decontamination should be scheduled as early as possible to ensure that vendor will be available before move date.
     2. Contact the Biological Safety Office if you are unsure of the vendor which services your location or you have questions regarding the level of decontamination required for your BSC.

  B. Have outside vendor decontaminate BSC per NSF standards.

  C. Tape a copy of the report on front of BSC and fax a copy to the Biological Safety Office at 323-3838.

  D. Have UK Biosafety inspect and post clearance signage.

- **BSCs must be recertified after movement prior to re-use**.

- Use of hazardous chemicals, radioactive isotopes or open flame is **STRICTLY prohibited** in BSC without prior approval from Biological Safety Department

For further guidance on the proper use of BSCs, please visit:
http://ehs.uky.edu/docs/pdf/bio_le_biological_safety_cabinet_operations_0001.pdf

The Department of Biological Safety actively discourages the purchase and use of LFBs since air is blown across the work surface into the face and torso of the operator. The Institutional Biosafety Committee and the Department of Biological Safety recognize that clean benches do not provide personnel or environmental protection from infectious or potentially infectious agents, allergens, chemicals or radioactive materials. If you are using a clean bench, contact the Department of Biological Safety for a review of your procedures.

4.13.2 Autoclaves

Any autoclave in use at the University of Kentucky must be inspected as to their construction, installation and condition and certified as a pressure vessel as required by KRS 236.110. Calibration services should be completed by the manufacturer on all new autoclave units. Documentation of this performance shall be maintained with the maintenance records for the unit. Consecutive monthly verification of calibration shall be performed on all units processing biohazardous waste materials.

For more information regarding autoclave certification, verification and maintenance, please visit:
http://ehs.uky.edu/docs/pdf/bio_le_autoclave_operations_and_verification_program_0001.pdf

4.13.3 Labeling Equipment Sent Out for Repair, Surplus, or Disposal

Biohazard contaminated and/or potentially contaminated equipment sent out for repair, surplus, or disposal shall be decontaminated as thoroughly as possible. In most cases, a solution of 10% bleach is sufficient to decontaminate the exterior and interiors of equipment such as freezers and centrifuges. Thorough decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. In these cases, decontaminate the equipment to the degree possible (flushing lines or wiping down the exterior) with appropriate disinfectant. Decontaminated equipment is required to have formal clearance from the Department of Biological Safety before it can be moved out of the laboratory space. Please contact our department for this clearance label.

For more detailed information regarding equipment for surplus, please visit: http://ehs.uky.edu/docs/pdf/bio_lab_surplusing_biohazardous_equipment_0001.pdf

4.14 Transport

4.14.1 Shipping Biological Specimens

Any laboratory wishing to ship biological specimens shall have an individual who has been properly trained by the University in the approved DOT/ IATA Shipping Training. This training should be completed by at least one individual in the laboratory. This individual shall be responsible for all package preparation and shipment of biological specimens. Be aware that additional restrictions apply to shipment destinations outside the United States. If no one in the lab has received training, contact the Department of Environmental Management to receive training.

In some cases an APHIS permit may be required to ship and or/receive biologicals. Any laboratory wishing to import biological samples may have to complete an additional CDC Importation Permit Application. Please contact the Department of Biological Safety for information and/or assistance in completing these importation permits.

For more information on shipping policies and procedures, please visit:
http://ehs.uky.edu/docs/pdf/bio_ps_shipping_and_permit_requirements_for_biologicals_0001.pdf

4.14.2 Transport of Biological Materials on Campus

Biological specimens transported between laboratories and animal facilities on campus should be properly contained in a sealed, leak-proof, shatter-proof secondary container. This container should be sealed in the laboratory and the outside should be disinfected. This will allow for safe transport of the specimen without gloves. An example of proper transportation would be a sealed tissue culture flask placed into a sealed plastic bag and then placed into a small cooler with a tight-fitting lid.

For examples of proper secondary containers for transport, please visit:
http://ehs.uky.edu/docs/pdf/bio_le_recommended_secondary_containment_devices_0001.pdf

5.0 Employee Health

Depending on the type of research being conducted, a consultation with Employee Health by University of Kentucky personnel may be necessary. For a particular employee, the recommendations from Employee Health might call for any of a number of precautionary measures, including immunizations or a periodic physical examination. In these instances, the principal investigator would provide Employee Health with guidelines and descriptions of conditions that might have significance for personnel assigned to the laboratory.

5.1 Immunizations

Employee Health currently administers the Hepatitis B vaccine program and many other employee health initiatives. For more information about this program please visit:
http://ehs.uky.edu/docs/pdf/bio_ia_immunizations_for_employees_0001.pdf

5.2 Medical Restrictions

Employee Health is available to discuss medical conditions that can affect employee safety in the laboratory. Services can be scheduled by contacting Employee Health. Payment for services must be coordinated through the individual employee's department.

5.2.1 Pregnancy

It is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Therefore, if pregnancy is possible while you are working in an infectious disease laboratory or laboratory engaged in work with infectious agents you should consult your Principal Investigator. The Department of Employee Health is also available for questions regarding the potential harm from the biological agents present within your laboratory.

Women that are pregnant or become pregnant are encouraged to inform their supervisors or Principal Investigators. Employees are urged to discuss exposure issues with their supervisors or principal investigators regarding associated risks of research being conducted and pregnancy.

5.2.2 Reproductive Biological Hazards

Consultations with an Employee Health Physician can be coordinated through the employee's department for any woman or man of childbearing age working with reproductive pathogens or other potentially infectious materials.

Reproductive biological hazards include, but are not limited to the following:

- Cytomegalovirus (CMV)
- Hepatitis B virus (HBV)
- Hepatitis E virus
- Human Immunodeficiency virus (HIV)
- Human parvovirus B19
- Rubella (German Measles)
- Lymphocytic Choriomeningitis virus
- *Toxoplasma gondii* (Toxoplasmosis)
- *Listeria monocytogenes*
- Varicella-zoster virus (chicken pox)

Whenever necessary, the Department of Biological Safety will offer an opportunity to review work procedures in the lab to ensure that potential exposure is minimized. Consideration for reassignment to other tasks that don't involve exposure to the reproductive hazard (generally with actual pathogens, not necessarily for only other potentially infectious materials such as blood or body fluids) should be given. Also, investigators actively working with reproductive hazards should explain the risk assessment at time of hire.

5.2.3 Other Restrictions

Restrictions or recommendations will be made on an individual basis. Examples of conditions that might warrant special precautions are HIV infection, immunosuppressive conditions, and drug therapies that suppress the immune system. Therefore, if you have any of the above conditions, you should inform your physician and the Department of Employee Health about the situation.

5.3 Exposure Incidents

An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials or recombinant nucleic acids that results from the performance of an employee's duties

5.3.1 Incident Response

Any employee who sustains a known or potential exposure incident shall secure any potentially infectious material, remove personal protective equipment and treat the affected area immediately by following the exposures incident response protocols on page 2 of the Occupational Injury or Exposure Protocol for Laboratories (http://ehs.uky.edu/docs/pdf/ohs_lab_exposure_protocol_0001.pdf). Please consult with the Department of Biological Safety for answers to any questions regarding proper exposure incident response procedures for your laboratory.

For life threatening injuries, call 911or proceed immediately to the Emergency Department of UK Chandler Hospital.

5.3.2 Incident Reporting

Exposure incidents must be reported immediately for the health and safety of both the employee and university community. Refer to page 1 of the University's Occupational Injury or Exposure Protocol for Laboratories for laboratory accident reporting procedures (http://ehs.uky.edu/docs/pdf/ohs_lab_exposure_protocol_0001.pdf). The employee shall report the incident to his/her supervisor. The supervisor shall complete an incident report with Workers Care documenting the route of exposure and the circumstances under which the incident occurred.

Any injuries or incidents resulting from exposure to infectious agents or recombinant nucleic acids must immediately be reported to the Department of Biological Safety (859-257-1049).

Any fatal accident, any accident requiring hospitalization of one or more people, any injury/illness that results in the loss of consciousness, any injury that results in 2nd degree burns to more than 30% of the body or 3rd degree burns to more than 20% of the body, any incident that results in amputation, or any incident that results in injuries and/or illnesses to more than two employees MUST BE REPORTED to Occupational Health and Safety IMMEDIATELY by calling (859) 227-7499.

5.3.3 Medical Assistance

Employees are urged to visit University Health Services (UHS) after they have received proper available first aid at site of exposure. Following the Protocol listing in 5.3.2, Worker's Care will assist in setting an appointment with UHS. For certain exposures; such as non-human primate bites or scratches, tick or insect bites, or exposure to infectious agents; the employee will be advised to visit UHS for evaluation and, if necessary, further treatment.

UHS will provide the post-exposure evaluation and follow-up at no cost to employees who experience "exposure incidents". The post-exposure monitoring periods are dependent on the type of exposure. This time period is related to the various incubation periods of the infectious agents.

6.0 Emergency and Accident Procedures

6.1 Investigation of Laboratory Accidents

EH&S, in cooperation with the principal investigator and his or her staff, will conduct the necessary investigation of any laboratory accident. The goal of the investigation is the prevention of similar accidents as well as obtaining information concerning the circumstances and number of employees who have been exposed to the agent in question. In addition, EH&S, in consultation with Employee Health might institute further steps to monitor the health of those who may have been exposed to the agent in question.

It should be emphasized that the reporting of accidents to the principal investigator or laboratory supervisor is the responsibility of the employee who has the accident. The principal investigator or the laboratory supervisor should then report the incident to UK Worker's Care at 1-800-440-6285, or in the case of a student, to UHS at 323-APPT.

Whenever an injury involves exposure to recombinant nucleic acids, timely evaluation is required by the Department of Biological Safety and may require immediate reporting to NIH. Please report these types of incidents to the Biological Safety Officer at 859-257-1049.

Whenever an injury involves sharpas and human source material (body fluid, tissue, cell line etc.), the Department of Occupational Health and Safety (OHS) may perform an investigation to determine if an alternate safe sharps device is available to prevent future occurrences of the injury.

Please also report incidents that did not result in an exposure (near miss) via http://ehs.uky.edu/apps/incident/ Evaluation of near misses can lead to alternative work practices and implementation of engineering controls to minimize future incidents.

6.2 Laboratory Emergency Contacts

Phone numbers of responsible individuals to be contacted in case of emergencies should be posted on the outside door of each laboratory. Also a list of the significant hazard classes (e.g., biohazard level, oxidizing agents, toxic chemicals, carcinogens) found within the laboratory is to be posted for use by emergency personnel. Specific infectious agents and chemicals should not be listed by name on door signage.

A template for door signage can be located at:
http://ehs.uky.edu/docs/pdf/bio_s_lab_signage_0001.pdf

6.3 Emergency Equipment

All personnel should be familiar with the location and use of all the emergency equipment in their laboratory. This includes fire extinguishers, eyewash stations, emergency deluge showers, chemical spill kits, absorbent materials, and fire alarm pull stations. For certain hazards, respiratory protection may be required for routine and emergency operations (see Biosafety in Microbiological and Biomedical Laboratories, 5th Ed). If respirators are required, the laboratory shall have a written Respiratory Protection Program and all users shall be fit-tested and trained in their use on an annual basis. If respirator use is optional, a written respiratory protection program is not required. Information about respirators and respiratory protection programs may be obtained from the Office of Occupational Health and Safety.

6.4 Evacuation Routes

All personnel should be familiar with the primary and secondary evacuation routes from their area to the nearest building exit. The University Fire Marshal can assist in developing these routes. The secondary route should be used when the primary route is blocked for some reason. All personnel should be told what method is used to signal a building evacuation, either a building alarm or manually operated air horn.

6.5 Spill and Accident Procedures

Spill clean-up is the responsibility of laboratory personnel. Principal investigators are responsible for developing spill and emergency guidelines suitable for biological operations in their specific areas. These guidelines shall contain proper procedures for containing and decontaminating any spills that may occur and any emergency actions to be taken. Personnel working in the areas of operation should be trained on these emergency guidelines.

6.5.1 Spill of Human Blood, Body Fluids, or Biological Materials

- Block off the spill area and alert personnel in the area to the presence of the spill.
- Put on appropriate personal protective equipment (PPE). This may include gloves, gown, eye protection, face mask, or respirator depending on the size of the spill and the type of material.
- Cover the spill with paper towels or other absorbent materials to contain spill.

- Carefully pour a freshly prepared 1:10 dilution of household bleach or other effective disinfectant around the edges of the spill and into the spill. Avoid splashing.
- Allow 20 minute contact period.
- Pick-up all absorbent material and place carefully in a biohazard bag for autoclaving and subsequent disposal. Do not seal the bag at this point.
- Use forceps, plastic scoop, or other mechanical means to remove any broken glass or other sharp objects from the spill area. Take care not to create aerosols. Place these items into a small cardboard box, plastic bag, or other container that will prevent them from puncturing the biohazard bag (or your hand). Place the enclosed sharp items into the biohazard bag for disposal. Do not seal the bag at this point.
- Clean spill area with fresh paper towels soaked in disinfectant. Place used paper towels in biohazard bag for disposal. Do not seal the bag at this point.
- Once spill is completely cleaned, place all used spill control equipment in biohazard bag for disposal. Do not seal the bag at this point.
- Remove PPE and decontaminate or place in biohazard bag for disposal.
- Do not remove PPE from face with soiled gloves. Remove soiled gloves first and place them in the biohazard bag for disposal.
- Once all used PPE, spill control equipment, and other potentially contaminated items are in the biohazard bag loosely seal the bag, place in a secondary container, and transport to autoclave facility.
- Wash your hands with soap and water.

6.5.2 Spill in a Biological Safety Cabinet

A spill that is confined to the interior of the biological safety cabinet should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet. Spray or wipe walls, work surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity, which will help clean the surfaces by removing both dirt and microorganisms. Use appropriate commercially-available chemical germicides known to be effective against the organism in use or 1 part household bleach (e.g. Clorox) to 9 parts of water with 0.7% nonionic detergent, prepared fresh daily. The operator should wear gloves and eye protection during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container. This disinfectant, gloves, wiping cloth, and sponges should be discarded into an autoclave pan and autoclaved. Note: This procedure will not disinfect the filters, blower, air ducts or other interior parts of the cabinet. Decontamination of these parts shall be performed by a contractor and the biosafety cabinet will have to be recertified. Contact the Department of Biological Safety to determine if decontamination by a contractor is necessary for your situation.

6.5.3 Catastrophic Spill: A large amount of biological material spread over a large area

If potentially hazardous biological material is spilled in the laboratory, the first essential practice is to avoid inhaling any airborne material by holding the breath and leaving the laboratory. Warn others in the area and go directly to a wash or change room area. If clothing is known or suspected to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially contaminated areas as well as the arms, face, and hands. Shower if facilities are available. Reentry into the laboratory should be delayed for a period of 30 minutes to allow reduction of the aerosol generated by the spill. Advance preparation for management of a spill is essential. A "spill kit"--including leak-proof containers, forceps, paper towels, sponges, disinfectant, eye protection, and rubber gloves--should be readily available. If the emergency involves personal injury or biological contamination, call 911 from any campus phone and ask for an ambulance to be sent to the area. Be sure to state the type of contaminant (biological,

chemical, or radiological; do not state the name of the agent) on the victim. The caller should remain available to brief emergency responders on the type of contamination and proper procedures for handling the material

- Block off the spill area and alert personnel in the area to presence of the spill
- Vacate area and allow 30 minutes for aerosols to settle
- Contact your supervisor and inform them of the situation
- Don appropriate protective equipment.
- Cover the spill with paper towels or other absorbent materials.
- Carefully pour a freshly prepared 1:10 dilution of household bleach around the edges of the spill and into the spill. Avoid splashing. Allow 20 minute contact period.
- Use paper towels to wipe up the spill, working from the edges into the center.
- Clean spill area with fresh paper towels soaked in disinfectant.
- Place paper towels into a biohazard bag for disposal.

6.5.4 Personal Contamination

- Remove any contaminated clothing or personal protective equipment.
- If skin has been contaminated, wash with soap and warm water.
- If eyes have been splashed, rinse under tepid running water (eyewash or faucet) for at least 15 minutes.
- Follow procedures to seek medical attention if needed.
- Contact your PI or supervisor and inform them of the situation.

6.5.5 If You Find a Needle in a Trash Can:

- Locate a sharps container.
- Don appropriate protective equipment; gloves, eye protection.
- Find a mechanical device with which to pick up the needle (tweezers, tongs, pliers).
- Using the mechanical device, lift the needle out of the trash can.
- Carefully place the needle into the sharps container.
- Wash the mechanical device with bleach or another approved disinfectant.
- Remove gloves and eye protection.

7.0 Laboratory Practices

7.1 Opening, Closing, or Moving a Laboratory

It is the responsibility of all laboratory directors, faculty members conducting laboratory research, and principal investigators conducting such work to ensure that the appropriate departments within the Division of Environmental Health and Safety receive advance notification when they:

- move into new laboratories,
- move into different laboratories,
- expand into new laboratory space,
- leave the University,
- cease laboratory research, or
- change, initiate, or cease their use of radioactive materials, hazardous materials, or infectious agents within their laboratories.

They will also be responsible for prompt notification of the appropriate director or department chair concerning any such changes and will provide the director or chair with copies of all correspondence taking place between them and the units concerned. Directors and chairs should send new faculty a copy of the University's safety requirements for accepting or transferring

hazardous, radioactive or infectious materials well in advance of their moving to UK. Directors and chairs are responsible for developing their own internal procedures to ensure that labs are not vacated and left in an unsafe condition.

7.1.1 Set Up of a New Laboratory

The following items are suggested:

- A folding stepstool is a much safer choice than a rolling stepstool
- A complete, contained first aid kit
- A spill kit including leak-proof containers, forceps, paper towels, absorbent material (Bentonite clay/cat litter), disinfectant, eye protection, and rubber gloves
- All personal protective equipment for procedures to be performed should be in stock in sufficient quantities
- Hand care items including antimicrobial soap, paper towels and non-petroleum based hand lotion should be available
- Sufficient training of laboratory personnel should be completed prior to the commencement of research
- IBC approval, if required, shall be secured prior to the initiation of any laboratory experiments

An example of the laboratory audit checklist, which outlines specific safety considerations evaluated by the Department Of Biological Safety during laboratory inspections can be found at: http://ehs.uky.edu/docs/pdf/bio_audit_checklist_biomedical_0001.pdf

7.1.2 Moving or Closing a Laboratory

The Department of Environmental Management, Occupational Health, Radiation Safety, Biological Safety or all will schedule an inspection of the old and new areas in advance of any move or exit.

Labs being vacated will be inspected for contamination and any hazardous or radioactive waste will be removed; unneeded chemicals and radioactive materials will also be removed. New lab spaces will be inspected for compatibility with the intended research operations and any safety deficiencies will be identified. Failure to give advance notification of laboratory moves could result in delays in startup of new labs, additional cleanup and disposal costs of old labs (which may be passed on to the department), and legal actions against the University and/or lab directors, faculty members or principal investigators. For more information about laboratory moves and/or laboratory exit inspections, please visit:
http://ehs.uky.edu/docs/pdf/bio_lab_laboratory_exit_and_closing_procedures_0001.pdf

7.2 Risk Assessment and Risk Management

Responsibility for biosafety exists at all levels and is shared throughout the University. The President and Provost acknowledge the institution's role in providing a safe workplace and have given the Institutional Biosafety Committee (IBC) and the Department of Biological Safety the authority to administer the campus biosafety program. The IBC establishes policies for the safe use of biohazards and for compliance with all applicable regulations. As an agent of the IBC, the Department of Biological Safety disseminates pertinent information; consults with faculty, staff, students and visitors; and monitors for non-compliance.

The researchers, clinicians, and technicians who perform work with biohazards are perhaps the most important component of the biosafety program, as they shall incorporate the biosafety requirements and safety precautions into all facets of their work.

The Principal Investigator is ultimately responsible for safety within the laboratory. An integral part of this responsibility is to conduct a review of proposed work to identify potential hazards (risk assessment) and to adopt appropriate safety procedures before initiation of the experiments (risk management). Certain experiments require advanced registration and IBC approval prior to initiation.

A risk assessment/risk management matrix has been prepared to illustrate key elements of the process (see below). Relevant sections providing additional details are indicated within the matrix. Information on the routes of exposure is included at the end of this section.

The five P's of risk assessment and risk management are:

- Pathogen – hazardous biological agent. This category includes potentially infectious material and/or recombinant nucleic acid materials
- Procedures – proposed experimental manipulations and safe work practices
- Personnel – appropriate training and skills
- Personal Protective Equipment (PPE) – protective clothing and safety equipment
- Place – laboratory design

Consider the five P's in each facet of laboratory work. Properly conducted, risk assessment can help prevent exposure to biohazards and minimize the potential for laboratory acquired infection. Remember that prior planning prevents poor performance. After reading this section and relevant sections of the Biosafety Manual contact the Department of Biological Safety for help applying the principles of risk assessment and risk management to experimental procedures.

The Five P's	Risk Assessment	Risk Management
Pathogen	*Agent classification *Routes of infection *Infectious disease process *Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors	*Registration Department of Biological Safety IBC USDA/CDC Select Agents IRB/FDA/NIH-Human Gene Therapy
Procedures	*Aerosol risk: sonication, blending centrifugation, homogenization, shaking, etc. *Percutaneous risk: needles, glass Pasteur pipettes, syringes, cryostat, blade/knife, scalpels *Splash/splatter risk: pipetting, pouring	*Written set of standard operating procedures (SOPs) with safety practices incorporated *Adherence to basic biosafety principles *Label labs, areas, equipment housing agents *Conduct lab inspections to review practices and containment equipment *Use trial experiments with non-Infectious material to test new procedures or equipment
Personnel	*Host immunity Neoplastic disease Infection Immunosuppressive therapy Age, race, sex, pregnancy Surgery (splenectomy, gastrectomy) Diabetes, Lupus *Immunization	*Safety training *Prior work experience with biohazards *Demonstrated proficiency with techniques *Prompt reporting of all exposure incidents, near misses, as well as signs and symptoms of related disease to PI and UHS *Investigation/review of incidents and

	*Post-exposure prophylaxis *Serum banking *Attitude toward safety *Comfort *Open wounds, non-intact skin, eczema, dermatitis	spills, etc. to prevent future occurrence.
Personal Protective Equipment (PPE)	*Protection against: Aerosols (respirable particles,5µm) Droplets/splatter Sharps	*Respirators-HEPA, N-99, N-95, etc. *Face (eye, nose, mouth) protection-mask and safety glasses, or chin length face shield *Solid front gown or lab coat *Gloves *Biological safety cabinets *Centrifuge safety buckets/rotors
Place (Laboratory Facilities)	*Risk group/biosafety level requirements *Aerosol risk *Restricted access	*Basic lab- door, sink, surfaces easily cleaned, eyewash. *Labels *Containment laboratory with directional airflow

For the purpose of safety, an attitude can be defined as an accumulation of information and experience that predisposes an individual to certain behavior. Human factors and attitudes result in tendencies on the part of the individual to react in a positive or negative fashion to a situation, a person or an objective. Laboratory supervisors and Principal Investigators should understand the importance of attitudes and human factors in their own efforts to control biohazards in their laboratory. Each employee working with biohazardous agents shall be consistently aware of the importance of the proper attitude in preventing accidents in the laboratory.

Some observations that may be of help to supervisors are listed below:

- The lack of accident perception ability is often a significant factor in laboratory accidents. Inflexibility of work habits, that tend to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.
- Working at an abnormal rate of speed is a significant causal factor.
- Intentional violations of regulations are a frequent cause of accidents. This is termed excessive risk taking.
- The performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
- Working when one is very tired is more likely to create a higher potential for accidents.
- Working at a well-organized and uncrowded laboratory bench will help in the prevention of lab accidents.

7.3 Routes of Exposures

In order for biological agents to cause disease, they shall first enter or invade the body in sufficient numbers. Routes of entry include oral, respiratory, parenteral, mucous membrane and animal contacts (bites, scratches). Once inside the body, biohazards must meet other requirements to cause disease; they shall colonize and establish in body cells, tissues and/or organs, overcome the body's natural defense mechanisms and mutate or adapt to body changes.
Other factors contribute to an individual's susceptibility to the disease process. These include age, immunological state, occupation, physical and geographic environment and predisposing conditions (such as alcoholism and other drug abuse, pregnancy and diseases such as diabetes).
It is difficult to determine a minimum infectious dose when discussing biohazards. The same dose of a pathogen may produce no disease symptoms in one individual but may cause serious or even fatal disease in another. There are microorganisms for which it is thought one organism entering the body is sufficient to invade and promote the disease process; the bacteria that causes tuberculosis is an example. For many pathogens, 10 to 100 or more organisms may enter the body

to cause infection leading to disease. See the table below for additional information on routes of exposure or contact the Department of Biological Safety.

Route of Exposure	Protection
Mucous Membranes:	
Eyes, nose or mouth via splash/splatter	Wear full face shield or safety glasses and surgical mask. Work in a biological safety cabinet or behind a protective shield following good microbiological practices
Ingestion:	
Mouth pipetting, eating or drinking in the laboratory	Use mechanical pipette and good microbiological practices.
Inhalation:	
Breathing respirable aerosols due to centrifuge leaks, spills, or aerosol generating procedures such as pipetting or vortexing.	Work within BSC, use sealed rotors or canisters in the centrifuge, use respirators if needed and follow good microbiological practices.
Percutaneous:	
Puncture with a contaminated sharp object such as needle stick, animal bite or scratch, through wound, cut or abrasion, or via previously broken skin from a previous injury or eczema.	Substitute plastic for glass, use caution with sharps. Use proper sharps disposal techniques and containers, animal restraints, cut resistant gloves, double gloves, sleeve covers, water proof bandages and good work practices.
Indirect Exposure:	
Touching mucous membranes with hands that have been in contact with contaminated surfaces such as benches, phones, computers, etc. or hands that were not washed after working.	Decontaminate work surfaces, wash hands when finished working or gloves have been compromised, do not touch face with gloves or non-gloved hands, and do not apply cosmetics in the laboratory.

7.4 Biosafety Levels

The CDC and NIH have established biosafety levels for work with biohazardous materials in the publication Biosafety in Microbiological and Biomedical Laboratories, 5th Ed (BMBL). The publication provides combinations of microbiological practices, laboratory facilities, and safety equipment as well as their recommended use in four biosafety levels (BSL) of laboratory operation with selected agents infectious to humans. Also included in the BMBL is a parallel set of biosafety levels for research involving small laboratory animals.

Below is a summary of practices, equipment and facility requirements for agents assigned to biosafety levels 1–4 (BSL 1–4). Additional information on biosafety levels may be found in the BMBL. Only work at biosafety levels 1-3 is permitted at the University of Kentucky. No work at biosafety level 4 is allowed at the University of Kentucky.

Biosafety levels should not be confused with Risk Group (RG) numbers, which categorize agents based upon their impact to human health and are used to evaluate relative risk. The BSL number designates the containment and relative procedures appropriate for the agent in use.

Biosafety Level	Agents typically in use	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices.	None required. PPE: laboratory coats; gloves; face protection as needed.	Open bench top, sink required.

2	Pose moderate hazard to personnel and the environment. Hazards are autoinoculation, ingestion, mucous membrane exposure.	BSL-1 practice plus: Limited access; Biohazard warning signs; "Sharps" precautions; Biosafety manual defining any needed waste decontamination or medical surveillance policies.	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats; gloves; face protection as needed.	BSL-1 plus an autoclave is available.
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	BSL-2 practice plus: Controlled access; Decontamination of all waste; Decontamination of lab clothing before laundering; Baseline serum or vaccination as needed.	Class I or II BSCs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing; gloves; respiratory protection as needed.	BSL-2 plus physical separation from access corridors, self-closing, double- door access, exhausted air not recirculated, negative airflow into laboratory.
4	Dangerous or exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission.	BSL-3 practices plus: Clothing change before entering; Shower on exit. All material decontaminated on exit from facility.	All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus separate building or isolated zone, dedicated supply/exhaust, vacuum, and decon systems, other requirements outlined in BMBL.

Adapted from the Office of Health and Safety, Centers for Disease Control and Prevention

7.4.1 Biosafety Level 1

The following practices taken from Biosafety in Microbiological and Biomedical Labs, 5th Edition should be instituted in any laboratory designated BSL-1:

- The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- 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.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- 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:

        - 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.
        - Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
        - Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
        - Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- 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:
    - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    - Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign must include the names and phone numbers of the PI and laboratory supervisor.
- An effective integrated pest management program is required.
- 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.

7.4.2 Biosafety Level 2

All practices followed in a BSL-1 laboratory should be instituted in a BSL-2 laboratory. Additionally, the following practices taken from Biosafety in Microbiological and Biomedical Labs, 5th Edition should be instituted in any laboratory designated BSL-2:

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

        - Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
        - Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- 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.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

7.4.3 Biosafety Level 2+

Biosafety Level 2+ (BSL2+, Biosafety Level 2 enhanced) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BSL2 procedures. Generally, BSL3 practices are mandated in a space designed for BSL2 work. It is preferred that the BSL2 laboratory be self-contained with all equipment required for the experiment located within the laboratory. A biohazard door sign listing emergency contacts and entry requirements is posted on the door while BSL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment has been decontaminated, the sign is removed and the laboratory is returned to standard BSL2 use. All manipulations of BSL2+ material are conducted in a class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures.

7.4.4 Biosafety Level 3

Biosafety Level 3 (BSL3) involves utilization of all BSL1 and BSL2 practices and procedures in addition to many more stringent requirements. BSL3 facilities require a great deal of additional laboratory equipment and facility planning. A laboratory considering work with an agent that requires BSL3 containment should contact the Department of Biological Safety to discuss the feasibility of the research. Approval must be granted by the BSL3 Advisory Committee and the IBC prior to commencing research.

7.5 Basic Microbiological Practices

The following are some standard microbiological practices and procedures which are comprehensively outlined in Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. These procedures may be referenced when completing IBC registration.

a. The laboratory supervisor must enforce the institutional policies that control access to the laboratory. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents. Personnel must receive annual updates or additional training when procedural or policy changes occur. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

b. 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 childbearing 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. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.

c. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

d. 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.

e. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

f. 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 include not recapping needles, using puncture-proof sharps containers for disposal of needles, and not directly handling broken glassware. Plastic ware should be substituted for glassware whenever possible.

g. Perform all procedures to minimize the creation of splashes and/or aerosols. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices

h. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory

i. 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.
    i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

j. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

k. Animal and plants not associated with the work being performed must not be permitted in the laboratory.

In the absence of definite accidents or obvious spillage, it is not certain that the opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means. Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a biological safety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking or vortexing will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is re-suspended, a few minutes should elapse prior to opening the container to reduce the potential for release of an aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture of colony. Following use of inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop or needle. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Condensation in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. Vented plastic Petri dishes, where the lid touches the rim at only three points, are less likely to offer this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent dispersal. If plates are obviously wet, they should be opened in the biological safety cabinet.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long sleeved laboratory garment.
Dried, infectious culture material may also collect at or near the rim or neck of culture tubes/flasks and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened in a biological safety cabinet.

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created. Aerosol creation should be prevented or minimized; opening of ampoules should be done in biological safety cabinets. When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a biological safety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then wrap the ampoule in disinfected wetted cotton, and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw the contents into a fresh container. Some researchers may desire to use commercially available ampoules pre-scored for easy opening.

However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, non-breakable leak-proof secondary containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel. The secondary container shall be labeled with a biohazard label.

7.6 Cell and Tissue Culture Practices

- Wear long sleeved gowns with knit cuffs and long gloves when working in the biosafety cabinet. Back-closing, disposable lab gowns are preferred. Buttoned laboratory coats are also permitted.
- Glassware and other contaminated items should be disinfected or autoclaved before washing, reuse or disposal.
- Maintain a clean lab gown reserved solely for cell culture work that is not to be worn outside the laboratory.
- Avoid unnecessary talking and traffic during cell culture manipulations as aerosols may be drawn into the work area.
- Place pipettes on a rack to avoid disrupting airflow when removed.
- Keep open tubes parallel to the airflow.
- After transferring inoculum always recap vials.
- Do not place tubes on work surface.
- Discard empty tubes immediately.
- Work with one specimen at a time; recap before going to the next.
- Cell culture wastes shall be decontaminated by treatment with chemical disinfectants or autoclave.
- Autoclave verification should be performed routinely.

7.7 Maintaining a Clean and Orderly Laboratory

Well-defined cleaning and organizational procedures are essential in reducing the risks associated with working with pathogenic agents. This is particularly true in the laboratory operating under less than total containment concepts and in all areas used for the housing of animals. A well-conceived and well-executed cleaning and organizational program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminates in the event of inadvertent release of an etiologic agent.

The objectives of cleaning and organizational procedures in the laboratory are to:

- Provide an orderly work area conducive to the accomplishment of the research program.
- Provide work areas devoid of physical hazards.
- Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems under study.
- Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.
- Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

8.0 Personal Protective Equipment (PPE)

Research conducted in the University of Kentucky laboratories requires that personal protective equipment (protective clothing and safety apparatus/equipment) be used to protect the researcher from contact with infectious, toxic and corrosive agents, excessive heat, cold, fire, and other physical hazards. Suitable Personal Protective Equipment (PPE) also protects the experiment from contamination. The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research operations and levels of risk associated with the research. While PPE is an important component of any biological safety program, PPE is used with the understanding that PPE serves as a second line of defense. Good laboratory techniques, procedures, and appropriate laboratory equipment are the primary barriers against potential exposure to hazardous agents. For assistance in performing risk assessments and determining PPE for your laboratory contact the Department of Biological Safety.

General Guidelines for the Use of PPE in the Laboratory:

- Overt exposure to agents at all level of risk should be followed by immediate decontamination of the PPE and change into clean PPE to protect the worker, the experiments and the environment.
- Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.
- PPE worn within the laboratory should not be worn outside the laboratory.
- Personnel should be encouraged to use disposable facial tissues instead of personal handkerchiefs.
- PPE should be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.
- All PPE should be decontaminated before being sent to the laundry or discarded. Treat contaminated areas of PPE with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
- Do not take PPE home to launder; select a laundry service that follows universal precautions.
- Change PPE as soon as feasible whenever it is compromised, soiled or torn.
- Wear appropriate sizes and keep an adequate supply of PPE available in the laboratory.
- Wash hands whenever PPE is removed.
- Do not touch door handles, elevator buttons, telephones, computers or other clean surfaces or items with gloved hands. Do not wear gloves outside the laboratory.
- Wear closed-toe shoes and long pants to guard against skin contamination or chemical exposure. Do not wear sandals or shorts in the laboratory.

8.1 Laboratory Clothing

A commonly used PPE item within the laboratory is special clothing. Both reusable and disposable clothing is available. Whichever is used, it shall be durable, designed to provide protection and prevent exposure of the skin to harmful agents, as well as be compatible with the methods of decontamination employed. Laboratory clothing serves to protect the wearer, the experiment, and the environment against contamination. Laboratory clothing includes lab coats, lab gowns, dedicated clothing or scrubs, shoe covers, and dedicated shoes and socks. The necessary laboratory clothing needed should be determined by a lab-specific risk assessment of procedures with the biohazardous material in use. For assistance in the selection of specialized laboratory clothing, contact the Department of Biological Safety.

8.2 Gowns, Lab Coats, Jumpsuits, Aprons and Other Protective Clothing

Gowns, lab coats, aprons, and jumpsuits protect the wearer's clothing and skin from contamination. As with all PPE, the type of clothing needed depends on the task being performed and the degree of exposure anticipated. Solid front wrap-around clothing offers better protection than pull-over

type clothing or clothing with front closures. Long sleeved garments with snug fitting cuffs are preferred over open or short sleeves. Snug fitting cuffs prevent splashes, splatters and aerosols from making contact with exposed skin on the lower arms. Longer single-use gloves can be pulled over snug fitting cuffs to seal out any infectious materials.
Aprons are necessary for protection against liquids spilling or splashing on clothing. It is recommended that appropriate aprons be worn to protect against the potential harmful effects of liquid waste. Aprons may also be used to provide protection from steam and hot water in locations such as animal handling facilities, autoclave rooms, and laboratory glass-washing rooms. Aprons may be used as a required second layer of PPE when working with animals in an ABSL3 facility. Aprons utilized for this purpose must be removed and discarded prior to exit from the ABSL3 facility.

8.3 Gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. No one glove can be expected to be satisfactory for all intended uses. Some gloves are pre-tested for viral penetration, chemical resistance, and puncture resistance. Ask glove manufacturers for documentation regarding any pre-testing of gloves.

Disposable (single use) gloves provide a barrier between infectious agents and the skin. Glove use is a basic precept of preventing infectious agent transmission. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common and the use of hand-lotions is recommended to prevent this. Latex gloves can be degraded by common hand lotions which contain petroleum based products therefore, it is important to choose a water-based hand lotion for laboratory applications. The Department of Biological Safety website provides recommendations for appropriate products.

Gloves should be removed and hands washed before exiting the laboratory. Use the one glove method or an appropriate secondary container when transporting materials through common use areas.

General Guidelines for the Use of Gloves in the Laboratory:

- Change gloves periodically, when gloves become soiled, and always wash hands after removing gloves or other PPE.
- Gloves will not prevent needle sticks or other puncture injuries.
- Check gloves for visible tears before use.
- **Do not reuse disposable latex or nitrile gloves.**
- Discard contaminated gloves in a biohazard bag immediately after use.
- Double glove when cleaning spills.

8.4 Best Procedure for Removing PPE

When removing gloves, grip the outside of one glove at wrist with the other gloved hand, pull glove off inside-out and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

When removing PPE, remove lab coat or solid front gown first, then remove gloves (aseptically), remove face protection last to avoid touching your face with contaminated hands. If wearing double gloves, remove outer gloves before removing lab coat or solid front gown.

8.5 Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid, to splash on the head, face and eyes, or contact lenses. To enter the lab, eye protection must be worn. Safety glasses shall be worn at all times. A variety of face shields, head covers/hoods, protective goggles, and lenses are available from safety supply houses. The selection is dependent upon materials of construction, fit, comfort, and compatibility with the work and the overall facial area requiring protection. Face shields and goggles can provide splash protection. Safety glasses provide impact protection from projectiles in the laboratory.

Contact lenses do not provide eye protection. It is recommended that contact lenses not be worn when working around chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses are worn, eye protection, such as tight fitting goggles, shall be worn. Contact lenses should never be handled in the laboratory.

8.6 Respiratory Protection

For certain hazards, respiratory protection may be required for routine and emergency operations (see Biosafety in Microbiological and Biomedical Laboratories, 5th Ed). If respirators are provided, the laboratory shall have a written Respiratory Protection Program and all users shall be fit-tested and trained in their use on an annual basis. Information about respirators and respiratory protection programs may be obtained from the Office of Occupational Health and Safety.

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The probability of this occurring depends on the type and infectious dose of the particular organism. Engineering controls, such as the use of biological safety cabinets, should always be considered as a first line of defense against respiratory infection when working with infectious organisms. Respirators should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.

Surgical masks do not provide respiratory protection. Surgical masks were designed to protect patients and products from the wearer and to protect the wearer from splashes to the nose and mouth. The wearer of a surgical mask is not protected from infectious aerosols therefore the use of surgical masks as respiratory protection is not allowed at the University of Kentucky.

Respirators vary in design, application, and protective capability. Respirators can be placed into two categories, air purifying and supplied air. By far, the most commonly used respirators in laboratories are air purifying respirators. These protect by purifying the existing breathing air through a filter (for particulates) or cartridge (for gases and vapors). Powered air purifying respirators (PAPR) are utilized at the University of Kentucky in specific laboratories and specific situations. These rely on the proper cartridge selection to filter out the contaminant. Dust masks that have been approved by NIOSH are also considered to be air purifying respirators. These are ranked by their filtering efficiencies and by whether they can be used in an environment containing oil aerosols. Approved dust masks will have one of the following designations – N95, N99, N100, R95, R99, R100, P95, P99, or P100. Proper selection of cartridges and respirators is very important and should not be made without input from the Office of Occupational Health and Safety.

9.0 Laboratory Equipment

Information in this section of the University of Kentucky Biosafety Manual may be incorporated into protocols submitted to the IBC. Procedures listed for specific laboratory equipment represent the standard practices accepted by the IBC in evaluating lab safety in submitted protocols.

9.1 Biological Safety Cabinets

Biological safety cabinets (BSCs), when used properly, provide a clean work environment for research activities. Biological safety cabinets offer personnel, product, and environmental protection. The BSC provides primary containment for infectious materials. The efficacy of BSCs depends upon the behavior of the operator and the orientation of the unit in the facility. Training in the effective use of BSCs is available on-line at the University Of Kentucky Department Of Biological Safety's website.

BSCs isolate biohazards from personnel by confining the biohazardous material to the unit and remove aerosolized biohazardous material by moving air through high efficiency particulate air (HEPA) filters. The intake air is filtered through a HEPA filter before entering the BSC work area, thereby protecting the materials manipulated within the cabinet.
Exhaust air also passes through a HEPA filter, thereby protecting release of the hazardous materials to the enviroment. Aerosols generated in the work area of the BSC are contained within the BSC, as long as the efficacy of the BSC has not been compromised by the actions of the personnel.

Operating Procedures for Class II Biological Safety Cabinet:
- If used, turn off UV light; turn on fluorescent light and blower.
- Disinfect all interior surfaces with suitable disinfectant.
- Place items required for procedure into cabinet; do not obstruct grills.
- Wait 2-3 minutes for contaminants to purge from work area.
- Keep materials at least 4 inches from front opening and back grill in the BSC.
- Work should proceed from clean to contaminated areas.
- After procedure, allow cabinet to run 2-3 minutes before removing materials.
- Wipe down all work surfaces with suitable disinfectant.
- Turn off fluorescent light and blower if desired.

For additional information regarding BSC operations, please visit:
http://ehs.uky.edu/docs/pdf/bio_le_biological_safety_cabinet_operations_0001.pdf

Many BSCs are equipped with "germicidal ultraviolet" (UV) lamps. Time of exposure, distance, presence of dust or debris, and UV lamp intensity all affect the germicidal effect of the UV lamp. The visible blue-violet glow of the UV lamp does not ensure that there is germicidal effect. UV lights should never be utilized as a primary means of disinfection of a biological safety cabinet. The UV lamp needs to be cleaned periodically to remove dust. UV lamps may damage eyes, skin, and laboratory equipment. UV lamps should be turned off while the room is occupied. The Department of Biological Safety discourages the use of UV lamps due to the potential damage resulting from UV lamp use. For more information regarding UV light please see the Department of Biological Safety website.

9.2 Centrifuges

All centrifugation of potentially biohazardous material shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. Each person operating a centrifuge should be trained on the proper operating procedures.

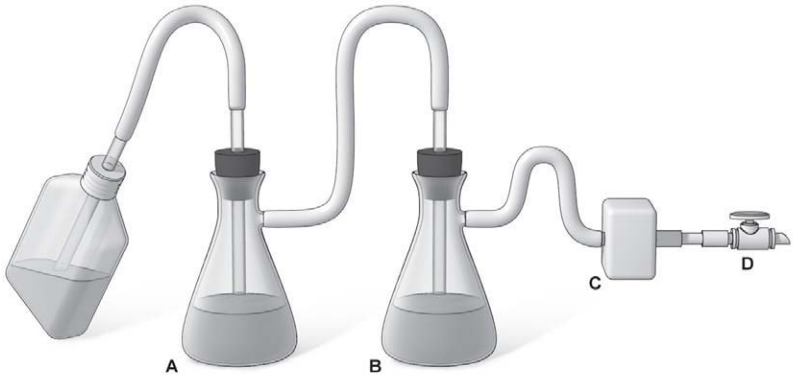
The following procedures for centrifugation are recommended:
- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet.
- Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- Always cap tubes before spinning. Use screw cap tubes.

- Place all tubes in safety buckets or sealed rotors when centrifuging infectious materials. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Open safety buckets or rotors in a biological safety cabinet.
- Never exceed safe rotor speed.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.

For additional information regarding centrifuge safety, please visit:
http://ehs.uky.edu/docs/pdf/bio_le_centrifuge_safety_0001.pdf

9.3 Vacuum Line Chemical Traps and Filters

Vacuum line chemical traps and filters prevent suction of infectious and non-infectious materials into the vacuum lines. For potentially infectious materials, add full strength chemical disinfectant to vacuum trap flasks. Allow the aspirated fluids to complete the dilution. (For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard.) Trap flasks should be emptied when 2/3 full. Trap flasks not housed in BSCs should be placed in secondary containers and placed in a safe location to prevent spillage and breakage. Recommended flasks are either heavy glass flasks with plastic coating or plastic flasks. Vacuum line chemical traps and filters should be set-up as illustrated below. Contact the Department of Biological Safety for assistance in setting up a vacuum trap system.



The catch flask (A) should contain disinfectant. An overflow flask (B) should be connected to this flask. A filter, with both hydrophobic and HEPA components (C), should be in place between the trap flasks and the vacuum source (D).

9.4 Syringes and Needles

The hypodermic needle is a dangerous instrument. To reduce the chance of accidental injection, aerosol generation, or spills, the use of syringes should be avoided when alternate methods are available. Needles used to transfer and inject biological and non-biological containers should be avoided as this is an unnecessary use of sharps in the lab. Replace sharp needles with blunt needles of the same gauge whenever possible.

The following practices are recommended for hypodermic needles and syringes:
- Use the syringe and needle in a biological safety cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.

- Examine glass syringes for chips and cracks and needles for barbs and plugs. This should be done prior to sterilization. Use needle-locking syringes only, and be sure that the needle is locked securely into the barrel. Replace glass syringes with plastic disposable syringes whenever possible.
- Expel excess air, liquid, and bubbles from a syringe vertically into a cotton square moistened with an appropriate disinfectant.
- Do not use the syringe to forcefully expel a stream of infectious fluid into an open vial for the purpose of mixing.
- If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in the transfer of infectious material to the fingers.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton square moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile cotton square may be used and immediately discarded into a biohazard bag.
- When inoculating animals, position the hand that is holding the animal "behind" the needle or use a pair of forceps to hold the animal in order to avoid puncture wounds.
- Be sure the animal is properly restrained prior to the inoculation and be on the alert for any unexpected movements of the animal.
- Before and after injection of an animal swab the injection site with an appropriate antiseptic.
- Discard syringes into an appropriate sharps container.
- Do not bend, shear, recap, or otherwise manipulate the needle.

9.5 Pipettes

The following is excerpted from Laboratory Safety, Principles and Practices 3rd Ed., ASM Press.

- Never suction or pipette by mouth; always use some type of pipetting aid when pipetting infectious materials. Preferably, all activities should be confined to a biosafety cabinet.
- Mouth pipetting should be prohibited even with mouth pipetting devices that use a hydrophobic membrane filter that does not require fingers to touch the mouthpiece. This re-useable pipetting device requires storage on the bench or other location between usages resulting in contamination of the end piece that inserts into the mouth.
- Pipetting of toxic chemicals should be performed in a chemical fume hood.
- Infectious or toxic materials should never be forcefully expelled from a pipette.
- Infectious or toxic fluids should never be mixed by bubbling air from a pipette through the fluid.
- Infectious or toxic fluids should never be mixed by alternate suction and expulsion through a pipette.
- Discharge from a pipette should be as close as possible to the fluid or agar level, and the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.
- Pipettes used for transferring infectious or toxic materials should always be plugged with cotton, even when safety pipetting aids are used.
- Avoid accidentally dropping infectious or toxic material from the pipette onto the work surface. Place absorbent material on the work surface and autoclave before discard. Plastic backed bench paper is suitable for this purpose.
- Contaminated pipettes should be placed horizontally into a pan or tray containing enough suitable disinfectant, such as hypochlorite, to allow complete immersion of the pipettes. Alternatively, a disposable container can be utilized in the BSC for pipette disposal. This can be sealed and decontaminated prior to removal from the BSC for subsequent autoclaving. Pipettes should not be placed vertically in a cylinder that, because of its height, shall be placed on the floor outside the biosafety cabinet. Removing contaminated pipettes from the biosafety cabinet and placing them vertically in a cylinder provides opportunity for dripping from the pipette onto the floor, or the rim of the cylinder, thereby creating an aerosol, and the top of the pipettes often protrude above the level of disinfectant. After suitable contact time, excess

disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together and replaced by a clean pan with fresh disinfectant.

9.6 Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding, or disrupting biohazardous materials. Even the use of a mortar and pestle can be a hazardous operation. Other devices that may produce aerosols are ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, French presses, and shakers.

The laboratory practices generally required when using equipment that may generate aerosols with biohazardous materials are as follows:

- Operate blending, cell disruption, and grinding equipment in a biological safety cabinet.
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leak-proof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline, or methylene blue solution is recommended prior to use.
- If the blender is used with infectious material place absorbent material moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use.
- Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break.
- Before opening the safety blender bowl permit the blender to rest for at least one minute to allow settling of the aerosol cloud.
- Homogenization of tissue containing infectious agents should be performed in a BSC, double bagged in a Stomacher, or in a sealed homogenizer.
- Grinding of infected tissues or materials with any open device is best done within a biological safety cabinet.

9.7 Microtome/Cryostat

Due to the very sharp blade and the nature of the materials used with the microtome/cryostat, training is essential in the use of the equipment and in the hazards of the materials used with the equipment. Users should be informed of the need to prevent cuts and scrapes as well as protect the eyes, nose, mouth and skin from exposure to the materials being used. New personnel shall be trained in the proper use and maintenance of the equipment, and demonstrate proficiency prior to use.

9.8 Miscellaneous Equipment (Waterbaths, Cold Storage, Shakers)

Water baths should be treated with chemicals to prevent growth of microorganisms and should be regularly cleaned and decontaminated. Deep freezers, liquid nitrogen containers, refrigerators, and dry ice chests should be checked and cleaned out periodically to remove any broken ampoules or tubes containing infectious material and decontaminated. All infectious or toxic material stored in refrigerators or deep freezers should be properly labeled. Security measures should be commensurate with the hazards.

The degree of hazard represented by contaminated liquid nitrogen reservoirs will be largely dependent upon the infectious potential of the stored microorganisms, their stability in liquid nitrogen, and their ability to survive in the airborne state. Investigations suggest that storing tissue culture cell lines in containers other than sealed glass ampoules might result in potential inter-contamination among cell lines stored in a common liquid nitrogen repository.

Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks/vials should be used. These should be securely fastened to the shaker platform. An additional precaution would be to line the shaker with absorbent material.

10.0 Decontamination and Disposal of Biohazardous Waste

The following guidelines describe the safe and appropriate handling and disposal of infectious waste. These wastes include human blood and body fluids, sharps, infectious microbiological materials, pathological specimens, and blood, body fluids, and tissues from infected animals.

10.1 Definitions

Autoclave - a device utilized for exposure of instruments, liquids, and potentially infectious waste to steam at a high pressure in order to decontaminate the materials or render them sterile.

Biohazardous Waste - human or animal tissue or fluids that are contaminated or may be contaminated with pathogenic organisms or recombinant DNA which may be hazardous to humans, animals, plants or the environment.

Blood and blood products - human blood, blood products such as serum, plasma and other blood components, and body fluids.

Decontamination - use of physical or chemical means to remove, inactivate, or destroy agents on a surface or item to the point where they are no longer infectious particles and the surface or item is rendered safe for handling, use, or disposal. Decontamination is generally considered to be a log 6 reduction, not sterilization.

Disinfectant - an agent that destroys harmful bacteria and/or viruses on inanimate surfaces. Common types include household bleach, quaternary ammonium compounds, phenolic compounds, and iodophors. Products making disinfectant claims must be registered with the Environmental Protection Agency (EPA), and state it on the label with a registered EPA number.

Infectious Waste - hazardous waste which is capable of causing infections in humans, including but not limited to, contaminated animal waste human blood and blood products, pathological waste, and discarded sharps.

Medical Waste - any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, including but not limited to: blood-soaked bandages, discarded surgical gloves - after surgery, used sharps, cultures, stocks, and swabs used to inoculate cultures, removed body organs.

Microbiologicals - wastes including all cultures and stocks of infectious agents. These should be collected in appropriate containers, autoclaved, and discarded with regular trash (solids) or into the sanitary sewer via sink (liquids).

Pathologicals - wastes including tissues, organs, and body parts discarded from surgical, obstetrical, autopsy, and laboratory procedures.

Sharps - includes needles, syringes, scalpels, and glass vials. These should be placed in sharps containers.

10.2 Waste Procedures

Separation and labeling of infectious waste (which may include red bagging, universal biohazard symbol, etc.) shall be done at the point of generation. During collection, storage and transportation, all waste shall be managed such that the integrity of the packaging is preserved and that rapid microbial growth and putrefaction is inhibited. Sharps containers should be rigid, impervious and puncture-resistant; plastic bags should be tear-resistant, leak-resistant, and sturdy enough to withstand handling. A waste triage flowchart to assist lab personnel in determining the appropriate disposal method for lab waste is available at: http://ehs.uky.edu/docs/pdf/bio_s_pending_waste_flowchart_0001.pdf

Whenever possible, infectious waste should be treated to render it non-infectious and non-recognizable as to its former character.

Infectious waste - Infectious waste should be properly treated to render it non-infectious. Autoclaving and chemical treatment are the most common methods. Treated waste is considered solid waste and may be safely landfilled, i.e. placed in the regular trash. This waste does not require a red bag. This material shall be placed in autoclave bags or other clearly identifiable containers and properly labeled with autoclave tape or other means that show that the waste is no longer hazardous. Blood and blood products may be disposed into the sanitary sewer.

Autoclaving of Biohazardous Waste - Orange or clear autoclave bags containing biohazardous waste should be decontaminated in an autoclave designated for this purpose which is involved in the UK Autoclave Verification Program. Autoclave bags should not be overfilled and should be placed in a secondary container prior to autoclaving. Autoclaves used for decontamination of biohazardous waste are a part of the University of Kentucky Autoclave Verification Program. If you do not have access to a designated biohazardous waste decontamination autoclave, please contact the Department of Biological Safety.

Medical waste – Medical (Pathological) waste consists of large amounts (greater than 500 ml) of human blood, unfixed human tissues and organs. At the University of Kentucky, medical waste is not autoclaved and instead is incinerated by a licensed vendor; therefore laboratory disposal of this waste requires special designation by the use of a red bag. Red bags should never be used for regular or autoclaved waste. If a research laboratory is producing legitimate red bag waste, the Department of Biological Safety must be contacted for the laboratory to be put on the authorized users list, thereby ensuring proper disposal containers and pick up service.

Animal waste - Animal waste shall be considered infectious if it is derived from animals infected with zoonotic diseases (transmissible from animals to human) or purposely infected with agents infectious to humans. Carcasses, body parts, tissue, body fluids, excreta, and bedding should be considered infectious. Animal waste can be properly disposed of in the DLAR facility. Animal carcasses that have not been intentionally infected should be returned to DLAR for disposal. Do not place animal carcasses in with other biohazardous waste to be autoclaved.
Infected animal carcasses or tissue that is also contaminated with hazardous chemicals or radioactive materials is a type of mixed waste. This type of waste poses special safety and regulatory problems and should not be generated if at all possible. The Office of Environmental Management should be consulted before generating this type of waste.

10.3 Decontamination of Laboratory Surfaces

Laboratory surfaces should be decontaminated after work with biohazardous materials and at the end of the day. Disinfectants should be examined for efficacy against the infectious agent in use. Disinfectants should be prepared according to the manufacturer directions for dilution and shelf-life. Storage of disinfectants should be in properly labeled containers. Information regarding specific disinfectants is available on the Department of Biological Safety website at http://ehs.uky.edu/docs/pdf/bio_laboratory_disinfectants_0001.pdf . For assistance in selecting an appropriate disinfectant contact the Department of Biological Safety.

11.0 References

Biosafety in Microbiological and Biomedical Laboratories, 5th Ed
http://www.cdc.gov/biosafety/publications/bmbl5/

Laboratory Safety, Principles and Practices 3rd Ed., ASM Press

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

Occupational Safety and Health Administration (OSHA) Standards for Bloodborne Pathogens (29 CFR 1910.1030)
http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10051

A Practical Guide to Containment, Plant Biosafety in Research Greenhouses
http://www.isb.vt.edu/containment-guide.aspx

University of Kentucky Environmental Health & Safety Website
http://ehs.uky.edu/biosafety/

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