KENT STATE UNIVERSITY

Biosafety Manual

September 2021

Ashtabula
College of Podiatric Medicine
East Liverpool
Geauga
Kent
Salem
Stark
Trumbull
Tuscarawas

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EMERGENCY CONTACT INFORMATION (Dial 911)

Environmental Health Safety Office	Phone number
University Biosafety Officer	330-672-4347
University Chemical Hygiene Officer	330-672-4347
Director, Environmental Health and Safety	330-672-4347

Fire Department

Campus	City Fire Department	Telephone Number
Ashtabula	Ashtabula Fire Department	440-992-7192
College of Podiatric Medicine	Independence Fire Department	216-524-4001
East Liverpool	East Liverpool Fire Department	
Geauga	Burton Fire Department	
Kent	Kent Fire Department	
Salem	Salem Fire Department	330-673-8814
Stark	North Canton Fire Department	
Trumbell	Warren Fire Department	
Tuscarawas	New Philadelphia Fire Department	
Twinsburg	Twinsburg Fire Department	

Police 911

Ashtabula	Ashtabula Police Department	440-992-7172
College of Podiatric Medicine	Independence Police Department	
East Liverpool	East Liverpool Police Department	
Geauga	Burton Police Department	
Kent	Kent Police Department	330-673-7732
Salem	Salem Police Department	
Stark	North Canton Police Department	
Trumbell	Warren Police Department	
Tuscarawas	New Philadelphia Police Department	
Twinsburg	Twinsburg Police Department	

Local Health Departments

Ashtabula		
College of Podiatric Medicine		
East Liverpool		
Geauga		
Kent		
Salem		
Stark		
Trumbell		
Tuscarawas		
Twinsburg		
Poison Control Center	Local Phone: 330-379-8562	Toll Free 1-800-872-5111

CHAPTER 1: - INTRODUCTION

1.6. Purpose

This biosafety manual has been prepared to update existing Kent State University (KSU) safety documents. This document provides guidelines for biosafety practices and procedures for the handling of known biohazardous and potentially infectious material. This manual is focused on Biosafety Level 1 and 2 as all the biological laboratories at Kent State University come under these categories. Research with Biosafety level 3 agents are permitted with special permission through Institutional Biosafety Committee (IBC). Separate safety instructions will be provided to researchers in such instances.

The Kent State institutional biosafety program is outlined in this manual and serves as guidance for researchers working with biological agents. The Environmental Health and Safety department recommends that principal investigators (PIs)use this manual as a guideline for compliance with federal, state, and local laws, as well as KSU policies. PIs should review the relevant sections of this manual, perform risk assessments for each organisms and procedure, and apply safety precautions to their research. The Institutional Biosafety Committee (IBC) and Biosafety Officer are available for consultation if a researcher has any questions pertaining to biological research. Please contact the Environmental Health and Safety office at EHS@kent.edu or 330-672-4743.

This manual has been prepared for students, staff, and faculty at KSU to provide information that is necessary to protect them, surrounding community and the environment from possible risk and hazards associated with the use of biological agents and recombinant or synthetic DNA/RNA (recDNA) molecules. For additional laboratory safety guidelines, refer to the KSU Chemical Hygiene Plan and KSU Radiation Safety Manual.

1.7. Biohazard definitions and Categories

The Kent State University's Institutional Biosafety committee defines biological agents and there by bioproducts as biohazardous material. The list of biohazardous material includes 1. Pathogenic agents (bacteria, rickettsia, fungi, viruses, protozoa, parasites, prions, and Select Agents) 2. Recombinant or synthetically derived nucleic acid, including those that are chemically or otherwise modified analogs of nucleotides (e.g., morpholinos) or both. 3. Recombinant DNA molecules, organisms, vectors (e.g., plasmids, viral vectors), and viruses containing recombinant DNA molecules 4. Human and non-human primate blood, tissue, body fluid, and cell culture (primary and established cell lines) 5. Plants, animals, or derived waste which contains or may contain pathogenic hazards (including xenotransplantation tissue). This manual provides guidelines for containment of biohazards to control the spread of contamination. The guideline described within this manual will provide additional safety during accident and fire prevention.

1.8. Regulations: Rules, regulations and guidelines

This section provides a brief summary of regular regulatory authorities that either regulate or provide guidelines for the use of biohazards.

1.8.1. Recombinant and synthetic nucleic acid molecules- NIH guidelines

NIH established a committee was established to provide an advice on recombinant DNA in early 1970. The NIH Guidelines, first published in 1976, and which continue to be updated, established carefully controlled conditions for conducting experiments involving recombinant or synthetic nucleic acids, recombinant or synthetic nucleic acid molecules. These guidelines describe the roles and responsibilities of the Institution, the IBC, and the PI planning to work with recombined nucleotide molecules.

1.8.2. NIH/ CDC biosafety in Microbiological and Biomedical laboratories (BMBL)

In 1984, CDC and NIH published first Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines. In 2020 the updated version was published as BMBL 6.0. This guideline provides specific descriptions of combinations of microbiological practices, laboratory facilities, safety equipment, and recommendations for use in the four biosafety levels of laboratory operation with selected human infectious agents.

1.8.3. The Select Agent rule

The CDC is required to regulate the possession of biological agents and toxins that have the potential to pose a severe threat to public health and safety. The CDC's Federal Select Agent Program oversees these activities. The Select Agent Program currently requires registration of facilities including government agencies, universities, research institutions, and commercial entities.

1.8.4. OSHA Bloodborne Pathogen Standard

OSHA bloodborne pathogen standard (OSHA BBP) 1030.210 applies to research staff, student with reasonably anticipated exposure to blood or other potentially infectious materials (including human cell lines) during their research laboratory work.

1.8.5. Ohio Administrative code chapter 4167-3

The Ohio Administrative code follows the standard promulgated by the Department of Labor and its Occupational Safety and Health Administration. It is the intent of the Kent State University to comply fully with the standards and regulations developed by the Department of Labor.

1.8.6. Ohio EPA regulated waste

Ohio EPA regulates the generation and treatment of infectious waste, as authorized by Chapter 3734 of the Ohio Revised Code. Businesses generating more than fifty (50) pounds of infectious waste in any calendar month are required to register with Ohio EPA and, among other requirements, ensure all infectious waste is treated prior to ultimate

disposal. Approved treatment technologies may be used onsite, or infectious wastes may be sent to a commercial treatment facility.
Ohio's infectious waste regulations contain approved treatment methods. Most commonly, autoclave and incineration technologies are used to treat infectious waste prior to disposal. Additional approved treatment methods include chemical treatment utilizing a sodium hypochlorite solution (bleach) for stocks and cultures; applied heat encapsulation for sharps; and chemical treatment utilizing peracetic acid and grinding. A business may submit a request for site-specific or statewide approval of an alternative treatment technology. Transportation of infectious waste (hazardous material) is regulated by the Public Utilities Commission of Ohio (PUCO).

Kent State University abide by the rules of Ohio EPA pertaining to regulated or infectious waste and have written biohazard waste management plan.

1.8.7. Dual Use Research of Concern (DURC): United states Government Policy for Institutional Oversight of Life Sciences DURC

United States Government Policy for Institutional Oversight of Life Sciences DURC (Dual Use Research of Concern) aims to preserve the benefits of life sciences research, while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by the research. The United States Government Policy applies to research involving high consequence pathogens and toxins. Environmental Health and Safety and the Institutional Biosafety Committee (IBC), with assistance from the Office of Research, are responsible for developing and implementing the University's DURC policy.

1.8.8. University policy

Kent State University is committed to achieving excellence in providing a safe and healthy working environment, and to supporting environmentally sound practices in the conduct of university activities. This will ensure systematic integration of safety and environmental considerations into all university activities. Safety management applies to all members of the university community and contractors whose work is directed on a day-to-day basis by university employees (KSU Policy 6-22).

1.5. Role and Responsibilities for control of biological research

The primary responsibility for the control of biohazard materials on any KSU campus, and the safety of employees and the public, rests with the PI, Department Chairs and College Deans, the Department of Environmental Health and Safety and its Biosafety Officer (BSO), and the Institutional Biosafety Committee.

1.5.1. Environmental Health and Safety Office

The Environmental Health and Safety Office is responsible for providing technical support and educational programs to keep the university in compliance with federal, state and local regulations. EHS will serve as consultants to the KSU community. Their duties will include developing policies, guidelines, recommendations and provide personnel training to ensure that the university remains in compliance.

1.5.2. Biosafety Officer (BSO)

The Institutional Biosafety Officer (BSO) is responsible for providing technical consultation to faculty and staff regarding equipment, facilities and work practices for the protection of personnel, laboratories and environment from biological hazards during use. The BSO will also review research protocols, activities, experimental design and procedures involving biological materials and recombinant or synthetic nucleic acid research to ensure safe practices are being used according to NIH Guidelines.

1.5.3. Institutional Biosafety Committee

The purpose of the Kent State University (KSU) Institutional Biosafety Committee (IBC) is to provide structured programming for teaching and/or research activities that involve recombinant or synthetic nucleic acid molecules, and biohazardous materials, agents, and toxins, conducted under the auspices of KSU or are sponsored by KSU. Institutions that receive support from the National Institutes of Health (NIH) for recombinant or synthetic nucleic research are required to establish and register an IBC with the NIH Office of Biotechnology Activities (OBA) in compliance with the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).

The IBC is responsible for the oversight, administration, and review of KSU laboratory policies, practices, and projects involving teaching and research with recombinant or synthetic nucleic acid molecules and other potentially hazardous materials that may pose health, safety, or environmental risks. The IBC assists and advises the KSU Environmental Health and Safety Office, Principal Investigators (PIs), Institutional Animal Care and Use Committee, Institutional Review Boards and other researchers to ensure that the aspects of teaching and/or research that fall within the purview of the IBC are conducted using established biosafety standards, principles, and work authorizations. Such advice includes worker safety, public health, prevention of human exposure to biological hazards, and environmental protection, ethics, and compliance with applicable biosafety standards and KSU Policies. The IBC is hereby-delegated authority to oversee teaching and/or research and approve laboratory protocols involving, but not limited to:

- Human cells, tissues, organs, blood and bodily fluids.
- Any use of recombinant or synthetic nucleic acid encoding products dangerous to humans.
- Biological agents (animal, plant, bacterial, viral, prion, and/or their products) designated as risk group 1 in which genetic alteration with external genomic or synthetic nucleic acids extend or enhance their normal biological function.

- Biological agents (animal, plant, bacterial, viral, prion, and/or their products) designated as risk group 2.
- Transfected and/or transformed cell lines that pose human risk.

The IBC will investigate any concern, including those related to accidents, injuries or illness that may have resulted from the use of recombinant or synthetic nucleic acid molecules and biohazardous materials, agents, and toxins. If any valid concern or deviation from the *NIH Guidelines* or established policy, procedure, sound practice or protocol is found, the findings will be reported to the Principal Investigator, Vice President of Research and Sponsored Programs (VPR), and relevant supporting units. Issues will be reported to the NIH according NIH Policy.

1.5.4. College Dean/ Departmental Chairs

Department Chairs/ College Deans are responsible for all employees, students, faculty, and visitors in their areas of control. These supervisors are responsible for the implementation of safe practices and procedures in their schools or departments. They must be aware of the hazards of research and approve control methods used by the PI

1.5.5. Principal Investigator

- The Principal Investigator (PI) is responsible for full compliance with approved research protocols, trainings required by the University, the University Biological Safety Manual, the NIH Guidelines (NIH Guidelines for Research Involving Recombinant DNA Molecules), the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen Standard (human-derived materials) and other local, state and federal regulations that apply to their research.
- The PI shall conduct a risk assessment to identify potentially hazardous procedures involving infectious agents, develop Standard Operating Procedures (SOPs), instruct and train all staff and students working in the lab on safe work practices, keep the lab space clean and up-to-date, and follow regulations for disposal of infectious waste. The PI must provide PPE to their staff. These actions must be documented in the Biosafety Manual.
- PIs must register research projects that require review by the IBC, such as the generation and/or use of recombinant or synthetic nucleic acids (e.g., DNA, RNA) work requiring BSL-2 or ABSL-2 containment, Select Agents, and other work with infectious agents as needed.
- The PI must be adequately trained in good microbiological techniques and is responsible for seeing that laboratory staff are adequately trained in safety practices.
- The PI is responsible for correcting work errors, identifying defective working conditions that could result in personal injury, and developing a positive attitude among laboratory staff toward accident prevention.

- The PI is responsible for hands on training for all laboratory procedures. They must ensure that all laboratory staff has fulfilled University training requirements and are current in all required training.
- The PI and/or lab personnel are responsible for initiating cleanup and disinfection in the event of a biohazard spill in a laboratory. If assistance is required contact EHS. Once the material has been contained, absorbed, and removed, housekeeping/facilities management should be contacted to finalize the cleanup and disinfection of the area.
- The PI is responsible for ensuring that all corrective actions and emergency procedures are followed in accordance with applicable University procedures and regulations.
- The PI is responsible for investigating and reporting any significant problems pertaining to containment practices and procedures to EH&S and correcting any work errors and conditions that could result in the release of biohazardous agents, including recombinant or synthetic nucleic acids.

1.5.6. Research Personnel: Scientist, Associates, Assistants, Postdocs, Students and Undergraduates

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. An incident caused by one laboratory worker can have a widespread effect on others. Specific responsibilities include:

- Follow procedures and practices established by the University and the laboratory.
- Have access to the Biosafety manual (this document) and know and understand the of requirements and procedures contained in the Manual.
- Use practices and procedures specified in this manual, presented in basic Biosafety and other research training, and other accepted good laboratory practices to minimize exposures to biological agents, and to avoid other incidents (such as fire, explosion, etc.).
- Complete CITI basic Biosafety training and other Research safety modules offered through Flash train.
- Report unsafe laboratory conditions, incidents or near incidents involving personnel exposure, releases outside of containment, or other biosafety issues to the PI, EHS, or other responsible party.
- Utilize control measures such as biological safety cabinets and personal protective equipment to prevent exposure to biological agents, and contamination of personnel and facilities.

CHAPTER 2: - REVIEW PROCESS FOR BIOLOGICAL RESRACH AT KENT STAE UNIVERSITY

2.1. Policy

The research grant and contract proposals that involve use of potential biohazard material, and/or including recombinant nucleic acids are reviewed by the Institutional Biosafety Committee. All such research proposals, regardless of funding source, are subject to this review. The IBC, not the investigator or department, is charged with the final determination of hazard classifications. Certain funding agencies also require the Kent State University to assure the biosafety compliance of the PI with the submittal of the proposal.

2.2 Procedure

2.2.1. Protocol submission

Biohazardous material use in research laboratory is reviewed by institutional Biosafety Committee when PI submits the biological material use application form to BSO. The template of biological material use application is available as Appendix A at the end of this manual

2.2.2. Protocol review

- Once the protocol is submitted to the BSO, the information is reviewed by the BSO and/ or IBC, depending the risk and complexity of the proposed work. The BSO and/or the IBC may request additional information from the PI to help in the review of research project.
- Incomplete applications may be returned to the PI. The PI will receive notification of IBC review and determination of approval or denial. If the notification letter indicates a conditional approval, it will also indicate actions or information that the IBC must receive before final approval notification can be issued. Projects may be subject to other KSU approvals such as IRB, Institutional Animal Care and Use Committee (IACUC), DURC. If animal work in involved, subsequent IACUC approval is required before initiation of the research work

2.2.2. Protocol renewal, changes to previously approved protocol

- Every protocol involving biohazardous agents, including recombinant or synthetic nucleic acids recombinant or synthetic nucleic acids is approved for two years. To renew the approval, PIs should submit to the BSO a protocol renewal form at least two months prior to the expiration date of their current approved protocol.
- For every modification or any significant changes to already approved research protocol should be approved by the IBC. Modification or significant changes involve research procedural change addition of biohazard agents, use of laboratory equipment that may generate aerosol, lab location, personnel change etc. This is done by submitting a request form (APPENDIX B) to BSO.

  At the end of two years, the PI may discontinue the research and close the protocol by submitting protocol closure form (Appendix C

3.3 Biological Risk Assessment

Risk assessment is a process used to identify the degree of risk to the laboratory worker, other personnel, and the environment. The degree of risk takes into consideration the virulence, pathogenicity, biological stability, and communicability of the organisms as well as the route of transmission. A biological risk assessment evaluates the probability of exposure to the hazard and the consequence of such an exposure. Thus, the risk assessment considers the hazard characteristics of the biological agent and the laboratory procedure hazards. If biological agents are genetically modified, the risk assessment must keep in mind how that modification may potentially change an agent's hazard characteristics such as virulence, pathogenicity or susceptibility to treatments. This information may not always be available for genetically modified organisms. (Refer to the NIH Guidelines to aid in assessing risk for work involving recombinant or synthetic nucleic acids.)

Before starting work in the laboratory, PI should perform risk assessment, which include reading SDS of materials going to be used, machine manuals, PPE requirements and SOPs of containment equipment.

3.4 Routes of Exposures

An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to public health. It is important to remember that the nature and severity of disease caused by a Laboratory-associated infection and the probable route of transmission of the infectious agent in the laboratory may differ from the route of transmission and severity associated with the naturally-acquired disease. Additionally, agents may be aerosolized during laboratory procedures although they are not naturally transmitted *via* the aerosol route. PIs should be familiar with all potential transmission routes and appropriate mitigation methods.

3.3. Risk groups

Biological agents are categorized in Risk Groups (RG) based on their relative risk. The classification into different groups take account of Pathogenicity of the organism, Mode of transmission and host range, Availability of effective preventive measures (e.g., vaccines), Availability of effective treatment (e.g., antibiotics) and other factors. A four risk group classification and assigned human etiological agents based on hazards is established into NIH guidelines.

You can search any biological agent for worldwide risk group information and locate SDS on ABSA risk group database.

3.4. Biosafety Containment levels

Biosafety levels (BSL) are used to identify the protective measures needed in a laboratory setting to protect workers, the environment, and the public. The levels are defined in Biosafety in Biomedical Laboratories (the BMBL). Biosafety Containment

Levels Biosafety is dependent on three elements: standard microbiological laboratory practices and techniques, safety equipment and facility design. There are many ways to combine equipment, practices, and laboratory design features to achieve appropriate biosafety and biocontainment. These are determined through biological risk assessments specifically conducted for each experimental protocol.

Biosafety Level 1 (BSL-1)

BSL-1 laboratories are suitable to study well-characterized infectious agents or toxins not known to cause disease consistently in immunocompetent adult humans. Biological agents handled in BSL-1 facility pose minimal potential hazards to laboratory personnel and the environment.

Biosafety Level 2 (BSL-2)

BSL-2 laboratories are built upon BSL-1 laboratories. They are suitable for work involving agents that pose moderate hazards to personnel and to the environment.

Biosafety Level 3 (BSL-3)

BSL-3 laboratories are used to study infectious agents or toxins that may be transmitted through the air and cause potentially lethal infection through inhalation exposure.

Biosafety Level 4 (BSL-4)

BSL-4 laboratories are used to study infectious agents or toxins that pose a high risk of aerosol-transmitted laboratory infections and life-threatening disease for which no vaccine or therapy is available. Biological agent with unknown risk or route of exposure are handled in BSL 4 containment level.

At present, all biological research carried out at Kent State University, require BSL-1 or BSL-2 laboratories. KSU does not currently permit use of RG3 or RG4 agents, nor does it operate BSL-3 or BSL-4 research laboratories. The College of Public Health houses a BSL-3 simulation laboratory to train individuals needing high containment laboratory skills.

CHAPTER 4: - PROCEDURE FOR BIOHAZARD CONTROL

4.1. Facility Requirements

4.1.1. BSL-1 Laboratory

The following standard practices, safety equipment, and facility specifications are recommended for BSL-1

4.1.1.1. Standard Microbiological Practices

- The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.

- The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained.
- A sign is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements.
- Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials.
    a) Glove selection is based on an appropriate risk assessment.
    b) Gloves are not worn outside the laboratory.
    c) Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    d) Do not wash or reuse disposable gloves and dispose of used gloves with other contaminated laboratory waste.
- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons 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 are not permitted in laboratory areas. Food is stored in designated food storage area outside the laboratory.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements.
- 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. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

4.1.1.2. Special practice

None required

4.1.1.3. Safety equipment: Primary Barriers and Personal Protective Equipment

- Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.

- Protective laboratory coats, gowns, or uniforms are worn to prevent contamination of personal clothing. Protective eyewear is worn by personnel when conducting procedures that have the potential to create splashes and sprays of microorganisms or other hazardous materials.
- Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
- In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

4.1.1.4. *Laboratory facilities*

- Laboratories have doors for access control.
- Laboratories have a sink for handwashing.
- An eyewash station is readily available in the laboratory.
- The laboratory is designed so that it can be easily cleaned.
    a) Carpets and rugs in laboratories are not appropriate.
    b) Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Laboratory furniture can support anticipated loads and uses.
    a) Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b) Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior are fitted with screens.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision

4.1.2. Biosafety Level 2 (BSL2)

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work with agents associated with human disease and pose moderate hazards to personnel and the environment. BSL-2 differs from BSL-1 primarily because: 1) laboratory personnel receive specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment. The following standard and special practices, safety equipment, and facility specifications are recommended for BSL-2.

4.1.2.1. *Standard Microbiological Practices*

- The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
- The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and those appropriate records are maintained.
- Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. for more information, see section VII BMBL.
- A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
    a) The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    b) The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies
- A sign is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements.
- Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
- Gloves are worn to protect hands from exposure to hazardous materials.
    a) Glove selection is based on an appropriate risk assessment.
    b) Gloves are not worn outside the laboratory.
    c) Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    d) Do not wash or reuse disposable gloves and dispose of used gloves with other contaminated laboratory waste.
- Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
- Persons 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 are not permitted in laboratory areas. Food is stored in designated food storage area outside the laboratory.
- Mouth pipetting is prohibited. Mechanical pipetting devices are used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements.
- 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. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements
- An effective integrated pest management program is implemented.
- Animals and plants not associated with the work being performed are not permitted in the laboratory

4.1.2.2. *Special practices*

- Access to the laboratory is controlled when work is being conducted.
- The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-2 containment.
- Laboratory personnel are provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
- Properly maintained BSCs or other physical containment devices are used, when possible, whenever,
    a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotors or centrifuge safety cups with loading and unloading of the rotors and centrifuge safety cups in the BSC or another containment device.
    c. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal

protective equipment and administrative controls are used, based on a risk assessment

- Laboratory equipment is decontaminated routinely; after spills, splashes, or other potential contamination; and before repair, maintenance, or removal from the laboratory.
- A method for decontaminating all laboratory waste is available (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
- Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the laboratory supervisor and any other personnel designated by the institution. Appropriate records are maintained.

4.1.2.3. *Safety Equipment (Primary Barriers and Personal Protective Equipment)*

- Protective laboratory coats, gowns, or uniforms designated for laboratory use are worn while working with hazardous materials and removed before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Protective clothing is disposed of appropriately or deposited for laundering by the institution. Laboratory clothing is not taken home.
- Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
- The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, communicate with KSU respiratory protection program
- In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

4.1.2.4. *Laboratory Facilities (Secondary Barriers)*

- Laboratory doors are self-closing and have locks in accordance with the institutional policies.
- Laboratories have a sink for handwashing. It should be located near the exit door.
- An eyewash station is readily available in the laboratory.
- The laboratory is designed so that it can be easily cleaned.
    a) Carpets and rugs in laboratories are not appropriate.
    b) Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Laboratory furniture can support anticipated loads and uses.
    a) Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b) Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

- Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
- Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
- Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent as shown in picture below. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment.
- BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
    a) BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions
    b) BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
    c) BSCs are certified at least annually to ensure correct performance.

4.2. Safe Laboratory Practices and Good Techniques

4.2.1. Technical Proficiency

Lab researcher must be aware of the potential hazards and must be trained and proficient in the necessary practices and techniques required for safe handling of biohazardous agents. Laboratory personnel must have documented training in handling biohazardous agents. The PI is responsible for providing or arranging for appropriate training for all personnel working in their laboratory. All required Research safety trainings (e.g., CITI Basic biosafety training, CITI bloodborne pathogens training, Flashtrain laboratory safety training) must be current.

4.2.2. Hazard Awareness Training

PIs, scientists, research staff, lab managers and students from the laboratories performing research with biohazard material must complete CITI Basic biosafety training module. This training is required before initiating research with biohazardous agents, including recDNA, and every year thereafter. Researchers planning to work with human blood, human source material, all human cell lines,

and other potentially infectious materials must take CITI OSHA Bloodborne Pathogens (ID 98577). The training is required initially and every year thereafter.

4.2.3. Prohibited Activities

- Eating, drinking, handling contact lenses, applying cosmetics, chewing gum, and storing food for human consumption is not allowed in the laboratory.
- Smoking is not permitted in any University building.
- Food shall not be stored in laboratory refrigerators or prepared/consumed with laboratory glassware or utensils.
- Mouth pipetting is prohibited in research laboratories; only mechanical pipetting devices can be used.
- Laboratory equipments should not be stored in in public corridors.
- Animals and plants not associated with the work being performed are not permitted in the laboratory.

4.2.4. Personal protective equipment (PPE)

- Personal protective equipment PPE is considered the last line of defense for protection and when possible should not be only form of exposure control.
- PPE should be used in combination with engineering controls, such as biological safety cabinets, and work practice controls, such as minimizing aerosol generation and using good microbiological practices. PPE prevents exposure of biohazardous material reaching to clothing, skin, eyes, mouth.
- All individuals working in the laboratory environment must wear closed toed shoes and long pants.
- All lab workers that work in laboratories that use hazardous chemicals must wear a lab coat, safety glasses / goggles and disposable gloves at a minimum.
- Personal protective requirements should be outlined in the Laboratory Chemical Hygiene Plan (LCHP) and in the Standard Operation Procedures (SOP's) and Lab Biosafety manual
- The Principal Investigator is responsible for ensuring that the appropriate PPE is used at all times. The type of PPE required must be based on a completed risk assessment of the hazards, exposure and the controls in place to protect lab workers during experiments or procedures.
- Each laboratory is responsible for providing a sufficient supply of personal protective equipment for all workers.

4.2.4.1. *Lab coat*

- Laboratory coats are recommended for general biological work in a BSL-1 laboratory and when working with BSL-1 biohazardous agents, including BSL 1 recombinant or synthetic nucleic acids.
- Wear dedicated laboratory coats, gowns, or smocks while working in the BSL-2 laboratory area. Before moving from the BSL-2 laboratory area to a non-BSL-2 laboratory area remove or change laboratory coat.
- Never take lab coats home for cleaning

4.2.4.2. *Gloves*

- Researchers should select gloves based on the results of an appropriate risk assessment
- Both latex and nitrile disposable gloves will prevent exposure to biohazardous agents but do not provide protection from punctures caused by sharp items or broken glass. However, latex is associated with allergies; provide non-latex glove options if allergies exist.
- If work involves the use of chemicals with biohazardous agents, select gloves according to recommendations in the Laboratory Chemical Hygiene plan and refer to associated chemical's Safety Data Sheet (SDS).
- Always visually check gloves for defects before using (e.g., look at gloved hands). For best protection, the cuffs of the gloves should overlap the lower sleeves of the lab coat. Change gloves when contaminated, torn, or punctured. Take care not to touch your skin with the outer surface of the gloves when removing them. Wash hands immediately after gloves are removed and before leaving the laboratory.
- Remove gloves prior to handling non-contaminated items such as doorknobs or telephones. Do not wear gloves outside the laboratory area
- Do not wash or disinfect and then reuse disposable gloves. Detergents may cause enhanced penetration of liquids through undetected holes, and disinfectants may cause deterioration.
- Used gloves must be treated as biohazardous waste and decontaminated prior to disposal. Utility gloves, such as rubber dish washing gloves, may be disinfected for re-use if they do not show signs of wear or degradation.

4.2.4.3. *Facial protection*

Facial barrier protection is required for activities in which there is a potential for splash/splatter of biohazardous agents onto the mucous membranes of the mouth, nose, and eyes.

4.2.4.3.1. *Eye and face protection*

- Use goggles, safety glasses with side shields, surgical masks, face shields, or other splatter guards for anticipated splashes or splatters of biohazards when agents must be handled outside the BSC or containment device.
- Dispose of eye and face protection with other biohazardous waste or decontaminate before reuse. Eye protection is required for persons who wear contact lenses in laboratories.

4.2.4.3.2. *Face Shields*

- Full-face shields made of lightweight transparent plastic are the preferred means of facial protection.

- They can offer excellent protection of the entire face and neck region and can easily be decontaminated. Face shields can also be used with a mask or respirator.
- If face shields are not used, use a combination of face mask and eye protection whenever splashes, spray, or splatter of biohazardous agents may be generated and where eyes, nose, or mouth contamination can be reasonably anticipated.

4.2.4.3.3. *Surgical Masks with liquid barriers*

- Surgical masks protect the mucous membranes in the mouth and nose from splashes or splatters but do not protect against aerosols. Either soft or preformed masks are more effective.

4.2.4.3.4. *Goggles/Safety Glasses with side shields*

- Ordinary prescription glasses do not provide adequate eye protection.
- Use plastic safety glasses with side shields that fit over regular glasses or goggles.
- If there is a substantial hazard for splattering, use safety goggles with a seal. Goggles that seal around the eyes are preferred over safety glasses with side shields.

4.2.4.4. *Respirators*

- A respirator is a CDC and OSHA-defined piece of equipment that protects the nose, mouth, and respiratory tract from aerosols. Respirators are used to prevent inhalation of fine particles. Respirators should not be confused with other face coverings that may act in some capacity to prevent inhalation of variously sized particles. Based on EH&S risk assessment, a respirator may be needed if aerosols are generated outside of appropriate containment.

- Laboratory procedures which generate aerosols of biological material and/ or expose researcher to aerosols may require the use of a respirator.

- If a half mask, full mask, or air supplied respirator is needed you must contact EHS at (330) 672-4347 to get enrolled into the Kent State University Respiratory Protection Program before using a respirator. For more information contact at (330) 672-4347 or visit the EHS website.

4.2.4.5. Handling Infected Animals

- Eye, face, and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.
- Gloves are worn when handling infected animals and when there is potential skin contact with biohazardous agents.

4.2.4.6. Alternatives of facial barrier protection

- Perform biohazardous work in biosafety cabinet or use a slash shield. Clean plastic shield provides an effective barrier for potential splashes caused by simple actions like opening of a tube.
- If laboratory techniques create aerosols, physical barrier will not be effective for such procedures, handle these procedures in Biosafety cabinets

4.2.4.7. Restrict Traffic in Laboratories

- BSL1 laboratories lab door can remain open during research procedure but access should limit to researchers working in those laboratories
- BSL-2 laboratories: When biohazardous agents are in use, the door to a BSL-2 laboratory should remain closed with the BSL-2 Biohazard Warning Sign displayed. The door is locked when the laboratory is unoccupied

4.2.4.8. Biohazard Warning Door Sign

- For BSL1 laboratories, universal biohazard sign should be included in laboratory hazard warning door sign. The sign includes the name and phone number of the PI or, and the name and phone number of other responsible personnel.
- For BSL2 laboratories, in addition to regular laboratory hazard warning door sign, a sperate Biohazard Warning door Sign with clear biosafety level two (BSL2) should be posted outside the laboratory. The sign should include name of the agent(s) in use, Special instructions pre and post exit and PPE requirement.

4.2.4.9. Handwashing

- Laboratory workers must wash their hands after handling biohazardous agents or animals, after removing gloves, and before leaving the laboratory area.

4.2.4.10. Good Housekeeping

- Keep work areas free of clutter and cleaned regularly. Work surfaces should decontaminate once a day and after any spill of potentially viable material. Use decontamination procedures provided in section 4.6. and spill cleanup procedures given in section 5.1 of this manual.
- Wet mopping is preferred over dry sweeping or the use of vacuums, which create aerosols.
- Laboratory should maintain inventory of infectious agents. Document and label all microorganism present in the lab. Properly decontaminate and dispose of any stocks or cultures that are not needed.
- If laboratory plan to work with select agent or select agent contact BSO. If you discover select agent or select toxin in your laboratory contact EHS immediately for assistance.
- Follow biological, chemical and radiation waste guidelines for accurately store, collect and disposal of solid and liquid waste material.

- Decontaminate all biohazardous liquid or solid wastes before disposal. This includes waste from research with all forms of recDNA.
- Use BSCs or other physical containment devices whenever aerosol-generating procedures are conducted (e.g., pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, or opening containers of biohazardous agents).

4.2.4.11. University Biosafety Manual

- **BSL-1 and BSL-2 laboratories must provide access to a current copy of the KSU Biosafety Manual AND a printed copy of the laboratory-specific biosafety manual (stored just inside the door of each laboratory).**

4.2.4.12. Laboratory-specific Biosafety Manual

- The PI of each laboratory using infectious agents, their products, or recombinant or synthetic nucleic acids must create, and annually update, a laboratory-specific biosafety manual. A template for a standard biosafety manual is found in appendix ____. Importantly, an assessment of risk for each hazard agent and each experimental procedure for which the hazardous agent is manipulated in the lab must be completed to document proper safety precautions and personal protective equipment.

4.3. Laboratory Equipment

This section describes the different types and proper use of laboratory safety equipment. All laboratory equipment used for biohazardous material research must be marked with the universal biohazard symbol to alert personnel of the potential for exposure. Equipment used in biohazardous material use labs should be decontaminated prior to servicing. A Notice of Laboratory Equipment Decontamination (Add a link or add an appendix in this manual) must be completed to certify decontamination.

Class II biological safety cabinets are typically used in laboratories intending to prevent aerosol transmission of hazardous materials. PIs should consult with the BSO to determine the appropriate BSC required for the defined laboratory research.

4.3.1.4. *Certification of BSCs*

All BSCs should be certified annually for sufficient airflow and filter integrity. KSU does not provide BSC certification services not does it pay for certifications. These are the responsibility of the PI.

- All research materials must be removed from the BSC prior to testing and certification. Plan and schedule in advance as the BSC cannot be used until certification is complete.
- The University's IBC requires that all BSCs be tested and certified prior to initial use, relocation, after HEPA filters are changed, and at least annually. The testing and certification process includes,

a) A leak test to assure that the airflow plenums are gas tight in certain installations.
b) A HEPA filter leak test to assure that the filter, the filter frame, and filter gaskets are all properly in place and free from leaks. A properly tested HEPA filter will provide a minimum efficiency of 99.99% on particles 0.3 microns in diameter and larger.
c) Measurement of airflow to assure that velocity is uniform and unidirectional.
d) Measurement and balance of intake and exhaust air.

4.3.1.6. *General guidelines for Working in a Biosafety cabinet*

- Users must receive training prior to use of BSCs. This training is the responsibility of the PI.
- Never place anything over the intake or rear exhaust grill. Keep equipment at least four inches inside the cabinet window and perform all transfer operations of viable material as deeply into the BSC as possible.
- Do not overload BSC with equipment and other items. Only bring in items needed for work.
- Plan in advance to have all required equipment inside the BSC. Good laboratory technique minimizes arm movements through the air barrier until the procedure is completed.
- During manipulations inside the BSC, segregate contaminated and clean items. Keep clean items out of the work area, and place discard containers to the rear of the BSC.
- Avoid entrance and exit from the workroom. Foot traffic can cause disruptive drafts that allow microorganisms to escape through the air barrier of the BSC.
- Equipment should be kept as parallel as possible to the downflow of the airstream.
- To purge airborne contaminants from the work area, allow the BSC to run following completion of work. The BSC can be turned off after 20 minutes but it is recommended that it be left on continuously.
- Decontaminate the BSC after use (see Section 4.6). Choose a disinfectant that does not corrode the stainless steel surface, or follow disinfection with an ethanol or water wipe to remove corrosive chemicals
- Do not use an open flame Bunsen burner inside a BSC. If required, a touch-a-matic burner or infrared loop sterilizer should be used. An open flame Bunsen burner disrupts the unidirectional air stream. The flame could damage the filter or set fire to the BSC when the BSC is turned off.
- Do not use the BSC for storage when not in use.

4.3.2. <u>Blenders, Ultrasonic Disintegrators, Grinders, and Mortar and Pestle</u>

- All of these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of biohazards, the following practices should be observed:
    - Operate blending, cell disruption, and grinding equipment in a BSC.
    - Use a heat-sealed flexible plastic film enclosure for a grinder or blender. The grinder or blender must be opened in a BSC.
- Automated Equipment Clinical or other laboratory personnel handling human blood, non-human primate blood, and other biohazards should be aware of aerosols produced by the micro-hematocrit centrifuge, the autoanalyzer, and the microtonometer.

4.3.3. Centrifuges

Centrifuges generate a lot of energy and can walk off the counter, launch projectiles, and spray aerosols if not operated correctly. Even the low speed centrifuges go fast enough to pose dangers. For centrifugation of biohazards samples, following practices should be observed,

- Read the operator manual for each centrifuge you operate – it has specific information about how to maintain that model.
- For all higher level and larger volume biohazardous material centrifuge needs, use sealed rotors or centrifuge safety cups.
- Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.
- When operating the centrifuge, use a log (especially for an ultracentrifuge) to track use and stick around to watch for signs of an improperly loaded centrifuge.
- If you detect smell, sound, small vibrations or anything concerns you, stop centrifuge immediately, turn off the equipment, notify you supervisor and seek help of an experienced researcher
- Do not open lid until fully stopped and allowed to rest for at least 30 minutes, which allows most aerosols to settle
- For spill cleanup review the procedures outlined biological spill cleanup in your laboratory.
    a) Don PPE
    b) USE tongs to handle sharps
    c) Remove sealed buckets and place them in BSC
    d) Disassemble and place other affected parts in BSD
    e) Recover samples in BSC
    f) Disinfect the centrifuge rotor

4.3.4. Water Baths and Incubators

After use, decontaminate water baths and incubators with an appropriate decontaminant (see Section 4.6). Maintenance service on water baths and incubators

that appear to be improperly used and/or contaminated may be denied. It is not the responsibility of maintenance personnel to clean up after laboratory personnel.

4.3.5. Refrigerators, Deep Freezers and Dry Ice Chests and refrigerators

- Deep freezers, liquid nitrogen, dry ice chests, should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing biohazards.
- Containers must be stored in proper order and sequence and properly labeled to preclude withdrawal of the wrong ampoules or tubes.
- Use of gloves and respiratory protection during cleaning of refrigerators, deep freeze or dry ice chests is recommended.
- All materials that are stored should be properly labeled with the scientific name, the date stored, and the name of the individual storing the material.
- Flammable solutions that require 4 degree storage conditions must be stored in a refrigerator approved for flammable storage.

4.3.6. Laboratory Vacuum Lines

- Vacuum lines used with biohazardous material should be protected with liquid disinfectant traps and in-line HEPA filters or their equivalent.
- Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment.
- Aspirator bottles or suction flasks (Figure 1) should be connected to an overflow collection flask (Figure 1, B) containing appropriate disinfectant and to an in-line HEPA or equivalent filter (Figure 1, C). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment.
- Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution (e.g., bleach) into the flask to inactivate the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of in the sink.
- If glass flasks are used, they should be placed in leak-proof secondary containment in the event of a break or spill

Figure 1. Protection of vacuum line

CDC/NIH Biosafety in Microbial and Biological Laboratories (BMBL) 6th edition

Two vacuum flasks and an in-line HEPA filter are connected in series by vacuum lines to a port for house vacuum. Material is drawn into the first flask, which contains a decontamination solution. The first flask is connected by a vacuum line to a second empty flask, which provides overflow protection for the first flask. An in-line HEPA filter is located between the overflow flask and the house vacuum port.

4.3.7. Microtomes and Cryostats

The microtome and the cryostat are used for cutting thin sections of fixed and unfixed tissue. The use of microtomes and cryostats in the laboratory presents a laceration hazard in addition to generating potentially infectious aerosols. Unfixed tissues should be considered capable of causing infection and should be treated with care. Employees who handle or could be exposed to tissue of human origin must be enrolled in the KSU BBP Program. Following procedures should be followed when using microtomes/cryostats:

- Always keep hands away from blades.
- Position the sample first and then put in the blade with the blade edge positioned away from hands.
- Use engineering controls like forceps, tweezers, dissecting probes, and small brushes to retrieve samples, change blades, dislodge blocks, or clean equipment.
- Use protectors/guards for knife-edges that may extend beyond the microtome knife holder.
- Wear appropriate personal protective equipment (PPE) such as gloves, lab coat or gown, mask, and safety glasses or goggles.
- Consider the use of cut resistant gloves when using a cryostat to provide additional protection from cuts and scrapes.
- Do not leave motorized microtomes running unattended.
- Discard and handle trimmings and sections of tissue as biohazardous waste.
- Do not move or transport a microtome with the knife in position.
- Always lock the chuck rotating mechanism (wheel) to immobilize the block when not actively cutting tissue and before insertion or removal of the blade
- Never walk away from an exposed blade.

- At the end of each session with the microtome or cryostat, either dispose of the blade immediately in a sharps container or secure reusable blades in a container.

4.4. Laboratory procedures

This section describes proper techniques used when working with biohazardous agents (e.g., pipetting; working outside a BSC; using syringes and needles; opening culture plates, test tubes, bottles, or ampoules; handling laboratory glassware; cell sorting; and centrifugation).

4.4.1. Pipetting

- Pipetting Delivery with the tip of the pipette resting against the container allows the fluid to flow down the surface and minimizes aerosols.
- Allowing a droplet to fall from the tip of a pipette, intentionally or accidentally, results in aerosol production, the extent of which depends on the height of the fall and the surface upon which the droplet lands.
- The following procedures should be followed for pipetting:
    a. Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.
    b. Infectious mixtures should not be prepared by bubbling air through the liquid with the pipette.
    c. Infectious materials should not be forcibly discharged from pipettes (e.g., the last drop forcefully removed).
    d. A towel wetted with disinfectant or a soft absorbent pad covering the immediate work surface is most useful in absorbing droplets and small spills.

4.4.2. Sharps (Needles, syringes, Razor Blades, other sharps) use

Sharps are items that are used to cut or puncture skin or body parts, including needles, scalpels and lancets. Other sharp items can still cause injuries although they do not fit the regulatory definition of sharps, such as broken glass, glass septum vials, glass pipets, razor blades, and the sharps teeth and nails of research animals. Safety precautions are necessary to prevent injury and exposure. Identify sharps devices to be used in laboratory procedures. When possible, substitute a nonsharp alternative such as a blunt needle or plastic pipette, or consider using a safe sharps device. If a sharp must be used, training and practice are essential to prevent injury. Avoid factors and conditions that can lead to a sharps injury, such as hurrying or rushing or working when you are tired or not feeling well. Keep your work area organized and uncrowded so that sharps items are always visible. For more information review sharps section in BBP policy.

- Avoid recapping needles. If a needle must be recapped, use a needle holder to do so.

- Never leave an uncapped needle exposed in the work area.
- Store reusable sharp items in a labeled storage container such as a bucket or tray. Use a magnet to contain reusable metal sharp items like razor blades.
- Promptly place all sharps waste into a red sharps container.
- When working with needles or syringes:
  - Use extreme caution to avoid accidental injection and the generation of aerosols during use and disposal.
  - Use syringes and needles only for injection and aspiration of fluid from laboratory animals and diaphragm bottles.
  - Do not use a syringe and needle as a substitute for a pipette when making dilutions of fluids. Syringe-type pipettes with blunt ended delivery are permissible.
  - Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe.
  - Prior to beginning an animal inoculation, be sure the animal is properly restrained. Swab the site of the injection with a suitable disinfectant.
  - Inoculate the animal with a hand behind the needle to avoid punctures. Swab the injection site again with a suitable disinfectant.
  - Following use, needles should not be bent, sheared, or removed from the syringe. If you need to change a needle after drawing up a dose, use a tool to remove. See Sharps and Laboratory Glass for disposal information.

4.4.3. Resuspending Sediment of Centrifuged Material

Use a swirling, rotary motion rather than shaking to resuspend the sediment of packed biohazardous materials. This motion minimizes the amount of aerosol created. Perform these operations inside a BSC. If vigorous shaking is essential to suspend the material or achieve homogeneity, allow a few minutes to elapse before opening the container to allow the aerosol to settle. Shaking always contaminates the closure and creates the added hazard of liquid escaping and running down the outside of the container or dropping from the closure when it is removed.

4.4.4. Working Outside a BSC Using a Splash Guard and/or Additional PPE

In cases where the biohazardous agent is not transmitted via a route of inhalation (e.g., opening tubes containing blood or body fluids), it is permissible to work outside a BSC using a splash guard. A splash guard is an example of a barrier type engineering control that protects by providing a shield between the user and any activity that could cause an aerosol or splatter. An example of such a splash guard is a simple clear plastic panel formed to stand on its own and provide a barrier between the user and activities such as opening tubes that contain blood or other potentially infectious materials (OPIM). Additional PPE (e.g., safety goggles, glasses, face shield) may be required for splash protection when working with biohazardous materials outside a BSC.

4.4.5. Opening Culture Plates, Tubes, Bottles, and Ampoules

Aerosol formation is the primary concern when plugs or screw caps are removed from tubes and bottles. Slow and smooth manipulations will minimize aerosols. Opening ampoules is potentially hazardous since, after the seal is broken, the air rushes in causing the dry contents to be dispersed. A BSC should be used. The bottom of the ampoule should be held in several layers of lab wipes to protect the hands. Nick the neck of the ampoule with a file. A hot glass rod should be carefully applied to the mark. The glass will crack, allowing air to enter the ampoule and equalize the pressure. After a few seconds the ampoule should be wrapped in a few layers of lab wipes and broken along the crack. An alternative method of opening an ampoule involves wearing gloves and other PPE, nicking the ampoule with a file, and wrapping the ampoule in disinfectant wetted cotton for breaking. In both methods the ampoule neck and other waste is handled as biohazardous sharps waste

4.4.5. Using Test Tubes and Other Laboratory Glassware

Tubes containing biohazards should be manipulated with extreme care. Studies have shown that simple procedures such as removing the tube cap or transferring an inoculant can create a potentially hazardous aerosol. Tubes and racks of tubes containing biohazards should be clearly marked with agent identification. Safety test tube trays should be used in place of conventional test tube racks to minimize spillage from broken tubes. A safety test tube tray is one that has a solid bottom and sides that are deep enough to hold all liquids if a tube should break. Glassware breakage is a major risk for puncture infections. Use non-breakable containers when. Avoid unnecessary use of glass Pasteur pipettes. Whenever possible, use flexible plastic pipettes or other alternatives. It is the responsibility of the PI and/or laboratory manager to assure that all glassware/plasticware is properly decontaminated prior to washing or disposal.

4.5. Control of Biohazards associated with Laboratory Animals

Before beginning research, testing, or teaching activities that involve the use of live vertebrate animals, the principal investigator must receive the approval from Kent State Institutional Animal Care and Use Committee (IACUC). Both naturally occurring diseases of laboratory animals transmissible to humans and experimentally induced disease, which may be harmful to humans, must be considered. The ultimate responsibility for reducing or eliminating such risks lies with the PI. KSU's IACUC program provides in depth guideline for the safe handling and ultimate disposition of potentially contaminated animals and animal wastes and well-being of the employee, to minimize the hazard to non-program personnel or animals in adjacent areas. The program is developed based on an understanding of the hazard potential involved in working with animals. Procedures, equipment, and facilities must be selected to minimize or eliminate such risks. For more information, follow information posted on office of research compliance

4.6. Decontamination

The primary target of decontamination is the microorganism that is under active investigation. Laboratory preparations of infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high titer materials can present certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve the viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interference with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube. Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however, common to all potentially infectious agents and must always be considered in selecting decontaminants and procedures for their use. Additional information on decontaminants can be found in "*Disinfection, Sterilization and Preservation*" by S.S. Block (4th edition).

4.6.1. Cleaning

Cleaning is the removal of gross contamination from a surface to the extent necessary for further processing for intended use. In these cases, cleaning can be used to remove microorganisms and other associated contaminants (e.g., blood, tissues, culture media) from a surface by physical means but may not provide any antimicrobial activity. Cleaning is often an essential pre-requisite to disinfection or sterilization processes to ensure the optimal activity of the antimicrobial effects of disinfectants or sterilization processes.

4.6.2. Disinfection

Disinfection is generally a less-lethal process than sterilization; it eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g., bacterial spores) present on inanimate objects. Disinfection does not ensure a kill level and lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled by several factors, each one of which may have a pronounced effect on the end results. Factors affecting disinfection include the following:

- Nature and number of contaminating microorganisms (especially the presence of bacterial spores)
- Amount of organic matter present (e.g., soil, feces, blood)
- Type and condition of surfaces, instruments, devices, and materials to be disinfected
- Temperature
- Contact (exposure) time.

4.6.3. Resistance

Microorganisms exhibit a range of resistance to chemical decontaminants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid containing viruses are relatively susceptible to chemical decontamination. The non-lipid containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Bacterial spores are the most resistant. The relative resistance to the action of chemical decontaminants can be substantially altered by factors such as concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility, within the limits of sensitivity of the assay systems employed.

4.6.4. Ineffectiveness

Ineffectiveness of a decontaminant is due primarily to the failure to contact the microorganisms rather than failure of the decontaminant to act. If an item is placed in a liquid decontaminant, the item becomes covered with tiny bubbles. The area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. If there are spots of grease, rust, or dirt on the object, microorganisms under these protective coatings will also not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful.

4.6.5. Residual Action

Many chemical decontaminants have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. However, consider residual properties carefully. Ethylene oxide can leave residues which cause skin irritation. In a concentrated form, phenol readily penetrates the skin and causes severe burns. Animal cell cultures, as well as viruses of interest, are also inhibited or inactivated by decontaminants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in liquid decontaminants prior to autoclaving and cleaning require careful selection of detergents for washing and must be thoroughly rinsed.

4.6.6. Exposure Time

Specific exposure times for the decontamination of soiled items by autoclaving, dry heat, or chemical decontaminants cannot be specifically stated. The volume of material treated, its contamination level, the soil load and type(s), moisture content, and other factors all play a role in the inactivation rate of microorganisms. Inactivation of microorganisms by chemical decontaminants may be achieved in one or more of the following ways: a. Coagulation and denaturation of protein b. Lysis c. Binding to enzymes, inactivation of an essential enzyme by binding, or destruction of enzyme substrate d. Oxidation Dozens of decontaminants are available under a wide variety of trade names.

4.6.7. Decontamination

Decontamination renders an area, device, item, or material safe to handle in the context of being reasonably free from a risk of disease transmission. The primary objective of decontamination is to reduce the level of microbial contamination so that transmission of infection is prevented. The decontamination process may involve the cleaning of an instrument, device, or area with ordinary soap and water. In laboratory settings, decontamination of items, used laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, which may be the most cost-effective way to decontaminate a device or an item.

4.6.8. Selection of Decontaminant

- Before selecting an appropriate decontaminant, consider collecting the answers to the following questions:
- What is the target microorganism(s)?
- What decontaminants, in what form, are known to, or can be expected to, inactivate the target microorganism(s)?
- What degree of inactivation is required?
- Is the situation complicated by the presence of organic matter such as blood, agar, etc.?
- What types of surfaces are being targeted: solid or porous and/or airborne?
- What is the highest anticipated concentration of cells?
- Can the decontaminant, either as an aqueous solution, a vapor, or a gas, reasonably be expected to contact the microorganisms and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility of materials?
- Do the anticipated procedures require immediate availability of an effective concentration of the decontaminant, or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?
- Will the toxicity of the decontaminant harm the researcher or other workers in the area?
- Several terms are used when discussing decontamination:
    - Sterilization refers to methods that destroy all forms of microbial life.
    - Disinfection refers to methods that remove or destroy pathogens.
    - Sanitization refers to methods that reduce the level of microorganisms.
    - The ending "cide" (as in "bactericide") refers to killing.
    - The ending "stat" (as in "bacteriostat") refers to inhibiting growth.

4.6.9. Sterilization

Any item, device, or solution is sterile when it is completely free of all forms of living microorganisms, including spores and viruses. This definition is categorical and absolute; an item is either sterile or it is not. Sterilization can be accomplished

by dry or moist heat, gases and vapors (e.g., chlorine dioxide, ethylene oxide, formaldehyde, hydrogen peroxide, methyl bromide, nitrogen dioxide, ozone, propylene oxide), plasma sterilization technology, and radiation (e.g., gamma, e-beam in industry). From an operational standpoint, a sterilization procedure cannot be categorically defined because the likelihood that an individual microorganism survives is never zero. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million. This is referred to as a sterility assurance level (SAL) of 10-5. Laboratories use sterilization techniques for producing media, sterilizing glassware, and other items, and for decontaminating waste

4.6.10. General procedures

- Biohazardous liquid and solid wastes, as well as all items such as labware, equipment, or apparatuses contaminated with biohazards, must be decontaminated before being washed, sorted, or discarded. Each individual working with biohazardous material or contaminated items is responsible for its decontamination.
- Whenever possible, contaminated items or biohazardous liquid or solid waste should be decontaminated by autoclaving.
- All floors, laboratory benches, and other surfaces or areas where biohazardous materials are handled should be chemically decontaminated as often as deemed necessary by the PI/lab manager. The choice of the chemical decontaminant used is at the discretion of the PI/lab manager.
- Upon completion of operations involving plating, pipetting, centrifugation, and similar procedures with biohazardous materials, the surrounding area should be chemically decontaminated.
- Floors should be wet mopped. Dry sweeping and dusting leads to the formation of secondary aerosols. Stock solutions of suitable chemical decontaminants should be maintained on each laboratory bench.

4.6.7. Treatment and Disposal of Biological Material

Various categories of waste are generated in research laboratories. These categories are specifically defined by the EPA, OSHA, DOT, and other agencies whose role is to regulate waste. Treatment, disposal, and hauling of waste are also regulated by various agencies. PIs are responsible for proper waste decontamination and disposal. Some waste is not collected nor processed by KSU. That category of waste is the responsibility of the PI, who should contract with appropriate waste haulers. Otherwise, the KSU EHS biological waste disposal guidelines provide proper procedures for the collection, treatment and disposal of laboratory generated biological waste. These guidelines have been created to prevent the release of potentially infectious agents and reduce the risk of exposure to faculty, staff and the KSU community during handling of biological waste generated from research

laboratories. Questions regarding these procedures should be directed to the office of Environmental Health and Safety at 330-672-4347 or mkulkar3@kent.edu

Ohio EPA [Chapter 3734 of the Ohio Revised Code] regulates the generation and treatment of infectious waste. All infectious waste or regulated waste must be treated prior to ultimate disposal. Approved treatment technologies may be used onsite, or infectious wastes may be sent to a commercial treatment facility. Any business that generates infectious waste is considered an infectious waste generator and is subject to Ohio's infectious waste regulations. An infectious waste generator is classified as a small generator when less than 50 pounds of infectious waste are generated per calendar month. Kent State University is registered as small waste generator and follows regulation provided by EPA.

4.6.7.1 *Infectious waste*

Any wastes or combination of wastes that include cultures and stocks of infectious agents and associated biological, human blood and blood products, and substances that were or are likely to have been exposed to or contaminated with or are likely to transmit an infectious agent or zoonotic agent, including the following:

- laboratory wastes
- pathological wastes
- animal blood and blood products
- animal carcasses and parts
- waste materials from the rooms of humans, or the enclosures of animals, that have been isolated because of a diagnosed communicable disease
- sharp wastes used in the treatment of human beings or animals, or sharp wastes that have or are likely to have come in contact with infectious agents
- waste materials generated in the diagnosis, treatment, or immunization of human beings or animals, research pertaining to the immunization of human beings or animals, or in the production or testing of biologicals, that the public health council identifies as infectious wastes
- "blood products" does not include patient care waste such as bandages or disposable gowns that are lightly soiled with blood; and any other waste materials the generator designates as infectious wastes.

4.6.7.2. *Biohazard waste collection methods*

4.6.7.2.1. Sharps

Sharps are generally agreed to be the most hazardous items in the potentially infectious waste stream. A high degree of precaution must be taken with any sharp item, contaminated or not.

- Contaminated sharps include,
- Needles
- Razor blades
- Scalpels

- Lancets
- Syringes with and without needles
- Slides, slide coverslips
- Specimen tubes
- Pasteur pipettes

At Kent State University, biohazardous sharps must be collected in red plastic sharps containers. PIs are responsible for the purchase, decontamination and proper disposal of biohazardous sharps. Non-contaminated sharps are directly collected in sharps container with defaced biohazard label or white sharps containers without biohazard labels.

The containers should be removed once they are 2/3 are full to avoid overfilling. Decontaminate the outer surface of sharps container if it is stored in biosafety cabinet before disposal. Infectious and no infectious sharp containers should e disposed of in accordance with the departmental guidelines Small shards of contaminated broken glass must be placed into the sharps containers, large contaminated broken glass items must be autoclaved separately in a transparent biohazard autoclave bag marked with temperature indicator autoclave tape. **After autoclaving**, the glass waste must be disposed of in the regular glass waste.

4.6.7.2.2. Biohazard solids

All non-sharp laboratory materials utilized in experiments with biological materials consist of

- culture dishes and flasks
- petri dishes
- plastic culture tubes
- gloves, gowns, masks
- other solid waste potentially contaminated with biological material (e.g., microorganisms, recombinant DNA, animal or human tissues, cell cultures, etc.).

All solid biohazard waste must be collected in biohazard container (preferably red) lined with clear polyethylene autoclavable bag (without biohazard symbol). The collection containers must clearly display the international biohazard label so that they will not be mistaken as regular trash. **Never place glass in these containers.** The biohazard waste collected in polyethylene bags must be treated prior to disposal by an approved decontamination method such as autoclaving. The validation of decontamination process should be verified e.g. use temperature indicator autoclave tape while autoclaving materials

After autoclaving, biohazard symbol from bag must be defaced with tape or black sharpie marker, then the bag must be over bagged with an opaque trash bag and sealed prior to disposal in the regular waste stream. Clear autoclave bags (without the biohazard symbol) do not require over bagging and may be sealed and placed in the regular waste stream.

Solid biohazard waste

Bags with the biohazard symbol, **regardless of use**, must not be placed, without over bagging, in the regular waste stream. Other methods for decontamination exist (e.g., decontamination by bleach, ethanol, etc.). Autoclaving may not always be a suitable method. More information can be obtained from Ohio EPA infectious waste guidelines

NON-Hazardous Glass waste

4.6.7.2.3. Biohazard Liquids

Biohazard or infectious regulated liquid consist of

- Liquid waste media from cells and tissues used for culturing risk groups Biosafety Level (BSL) 1 and 2 pathogens, toxins and recombinant DNA procedures.
- Cultural stocks of microbial waste
- Animal liquid waste intentionally infected with microbial pathogens, toxins viral vectors
- Liquid culture media used for culturing human tissues or human cell lines
- Human body fluids collected for research purposes

Liquid waste must be collected in polypropylene/ plastic container. If you are collecting liquid waste in a glass container, then the container should be secured in secondary plastic container. The collection container should be marked with universal biohazard sticker.

Biohazard liquids must be decontaminated before discharging to sanitary sewer. Autoclaved biohazard liquid waste can be discharged directly to sewer. Liquid waste

can be decontaminated using chemical disinfection method. Liquid regulated waste must be decontaminated with 10 % freshly prepared household bleach by allowing 20 minutes contact time for complete decontamination. The decontaminated liquids must be disposed in sanitary sewer. When this is done take care of splashing and drains are drained with making sure drains are flushed with generous amount of water. If you are planning to use different chemical for disinfection, please follow the manufacturer's instruction for use (concentration, contact time etc.), disposal and safety.

4.6.7.2.4. Vacuum respirator flask

- These flasks are generally used for aspiration of culture media from tissue culture plates or tissue
- culture flasks. If the collection flasks are kept inside the biosafety cabinet, make sure sash is closed to correct height before work is started in the cabinet. If the flasks are kept outside the cabinet, make sure the collection tube is sterilized while moving in and out of the cabinet.
- Vacuum flask assembly should be set up as per guideline provided in Biosafety in Microbial and Biological Laboratories (BMBL) as shown earlier in section.

4.6.7.2.5. Research Animals

- All of the tissues or organs from uninfected small research animals (e.g., cats, dogs, rabbits, rats, mice, birds, etc.) are considered as pathological waste. This waste should be collected and send out to vendor for incineration.
- All of the tissues or organs from infected small research animals (e.g., cats, dogs, rabbits, rats, mice, birds, etc.) are considered as regulated biological waste. This waste should be collected and send out to vendor for incineration.
- There are no exceptions to this policy without prior notification and approval by The Institutional Biosafety Committee (IBC).

4.6.7.2.6. Research Animal bedding

Bedding from noninfected animals is collected as pathological waste and disposed by incineration. Material should be packed and labeled properly.

4.6.7.3. Biohazard waste (Regulated waste) disposal chart

Microbiological waste including microorganism (Risk group 1[RG1] and Risk group 2[RG2])	Autoclave solid waste decontaminate liquid waste with 10 % bleach or appropriate decontaminant. Discard solid waste in regular trash after proper decontamination. Discard liquid waste in sewer.

Animal waste (animal carcasses) from animals infected with RG1 and RG2 agents	Incinerated by outside vendor
Animal bedding from the cages where animal infected with RG1 and RG2 microorganisms (pathological waste)	Incinerated by outside vendor
Human blood or Human body fluids	Treat with 10% freshly prepared bleach or autoclaved and discard decontaminated waste down the drain
Human tissues or body parts	Incinerated by outside vendor
Uninfected animal waste (pathological waste)	Incinerated by outside vendor
Nonhazardous Sharps containers	
Biohazardous Sharps containers	Incinerated by outside vendor

4.6.7.4. Researcher instructions

During loading and Unloading of biohazard material, follow the instructions,

- Never leave biohazard materials in hallway or public spaces prior to autoclaving. Biohazard waste bags should remain in lab until they are autoclaved. Never place bags on the floor next to autoclave
- Biohazard waste bags must be closed when they are ¾ full and place them in a secondary containment
- If biohazard waste bags are being transported to autoclave, Biohazard waste bags must be carried, they must be closed and contained in a closed wall secondary containment (autoclave tray)
- Minimize contact with biohazard waste as much as possible. Never crush or push biohazard waste in bags. Wear appropriate PPE when handling biohazard waste
- Apply temperature sensitive indicator autoclave tape on the biohazard waste bag.
- After the proper autoclave waste decontamination steps are followed, place the decontaminated waste in a regular trash.
- If red biohazard bag with universal biohazard symbol is used for collection of biohazard waste, place the decontaminated bags in regular trash after over bagging with black trash bag.
- Maintain the biohazard waste records which should include date of collection, weight of the waste and autoclave decontamination details (date of autoclave, conditions of autoclave, name of the PI and location of waste of collection)

4.6.7.5. Steam Sterilization

Steam sterilization or autoclaving is most reliable decontamination methods of microbial pathogens. Proper temperature and exposure time are critical for the accuracy of the decontamination method. Proper temperature is accomplished by steam penetration to every part of the autoclavable load, hence the users must be careful while using the autoclave. The hazards associated with autoclave include

extreme heat, high pressure, large heavy doors and loading cart. Appropriate safety precautions must be followed.

- Each operator should review the manufacturer's manual prior to use. Even though the principles of use are similar, manufacturer recommendations for use may be different.
- Ensure gaskets are in place before you close door firmly and start autoclave cycle. If the autoclave does not have safety interlocks, take additional precautions to securely close the doors.
- Do not autoclave toxic, volatile and radioactive material. If the biohazard waste contains any of these other contaminants, then contact EHS for disposal guidelines.
- Wear appropriate PPE while using autoclave.
- In case of spills, clean the spill immediately and inform supervisor about spills.
- Report any malfunctions and accidents to supervisor.

4.6.7.6. Autoclave Validation

- Autoclave validation should be performed on all autoclaves every calendar month in which the autoclave is used for the treatment of infectious wastes [4,5]. The validation (quality assurance) testing ensures that the autoclave is capable of achieving the performance standard of a minimum four log10 reduction of *Bacillus stearothermophilus* spores.
- There are many commercially available biological indicators with a choice of spore ampoules or spore strips with growth media. Verify that the type of spores being used in the quality assurance test are appropriate for the autoclave being evaluated to treat infectious waste. Follow instructors provided by manufacturer of biological indicator.
- Make sure the device is capable to accurately register temperatures within the appropriate incubation temperature range of the spore test being used.
- Maintain the proper temperature/temperature range that the spore test is to be incubated. This information is important for ensuring whether any spores survived the autoclaving process and thus whether the autoclave is capable of treating infectious waste. This information is to be recorded on the quality assurance log required to be produced and maintained by the infectious waste treatment facility.
- Allow the amount of time needed to make a complete evaluation of spore growth. This information is important for ensuring whether any spores survived the autoclaving process and thus whether the autoclave is capable of treating infectious waste. This information is to be recorded on the quality assurance log required to be produced and maintained by the infectious waste treatment facility.

CHAPTER5: -EMERGENCY PREPAREDNESS AND RESPONSE

5.1.Biohazard Spill Clean Up Procedure

This section provides spill clean-up procedures for BSL-1 and BSL-2 laboratories. These procedures apply to biohazardous agents and all recDNA.

5.1.6 Responsibility

Each PI is responsible for preparing a spill clean-up kit and procedures that are appropriate for the biological materials used in the laboratory. The kit should be easily accessible to laboratory personnel and all personnel must be trained prior to beginning work in the lab on the cleanup procedure., ..

5.1.7 Biohazard Spill Kit

Assemble spill kit components in a single container that can be moved easily to a spill area. A large plastic bucket is ideal for the container as it can double as the secondary container for transporting waste away from the spill. Biohazard spill kit should contain following items,

- Chemical disinfectant: A freshly prepared bleach (10%) or other appropriate disinfectants agent used in the laboratory.
- Absorbent material: Specially designed absorbent material, paper towels, absorbent laboratory-diaper pads
- PPE: Disposable lab coat, regular gloves, safety glasses, face shield, surgical mask, sturdy nitrile gloves
- Tongs, forceps, scoops to pick up broken glass
- Biohazard bag to collect clean up waste
- Broken glass container to pick up contaminated broken glass
- Printed instructions for spill cleanup

5.1.8 Biohazardous Spill inside Biological Safety Cabinet (BSC)

This section provides spill clean-up procedures for biohazardous agents and all recombinant or synthetic nucleic acids that occur inside a BSC

5.1.3.1. *Biohazardous Spill Inside the Biosafety Cabinet (BSC)*

- Do not turn off the BSC during spill clean-up. Do not place your head inside the cabinet or under the sash at any time.
- Remove any sharps, contaminated objects from the spill area using mechanical instruments (e e.g., tongs or forceps).. Never use your hands with hands. Use a biohazard sharps container to discard all contaminated sharps .
- Cover the spill with paper towels or other absorbent material. Slowly pour an appropriate decontaminant solution around the spill and allow the solution to flow into the spill.
- A freshly prepared 1:10 dilution of household bleach, (~0.5% sodium hypochlorite) is suitable for most biological spills. Allow 30 minutes of contact

time. The contact time may vary depending on the decontaminant used and the microbiological agent. Follow the manufacturer's directions.

- Place contaminated paper towels and other spill clean-up materials in a biohazard bag.
- Wipe up the spill, work surfaces, walls, and any equipment in the cabinet with paper towels dampened with decontaminant. If using bleach, follow with a water rinse to protect metal surfaces from corrosion.
- Decontaminate the spill area again. Place all used spill materials into a biohazard bag.
- Remove any contaminated PPE in a manner to avoid cross-contamination; dispose of as per standard lab practices
- Wash hands thoroughly after removing gloves.

5.1.3.2. *Spill inside a BSC that flows into the front or rear grills, should be cleaned following below directions,*

- Do not turn off the BSC during spill clean-up. Do not place your head inside the cabinet or under the sash at any time.
- Close the drain valve under the BSC if open.
- Remove any sharp, contaminated objects from the spill area using mechanical means (like tongs or forceps) and never with hands. Discard contaminated sharps in a sturdy, leak-proof, biohazard labeled sharps container.
- Flood the top work surface tray and, if a Class II BSC, the drain pans and catch basins below the work surface with a decontaminating solution that is appropriate for the agent involved
- A freshly prepared 1:10 dilution of household bleach, (approximately 0.5% sodium hypochlorite) is suitable for most biological spills
- Allow 30 minutes of contact time. The contact time may vary depending on the decontaminant used and the microbiological agent. Follow the manufacturer's directions.
- Remove excess decontaminant from the work surface tray by wiping with a sponge or cloth.
- For Class II BSCs, drain the tray into the catch basin below the work surface, lift the tray and take out the removable front intake grille. Wipe the top and bottom (underside) surfaces of the grille with a sponge or cloth soaked in the decontaminant. Then place the tray in position, drain the decontaminant from the cabinet base into an appropriate container, and dispose of the decontaminant in the sewer
- Place contaminated paper towels and other clean-up materials in a biohazard bag.
- Decontaminate the spill area again. Place all used spill materials into a biohazard bag. Remove any contaminated PPE in a manner to avoid cross-contamination and dispose of as per standard lab practices.

- Wash hands thoroughly after removing gloves.

5.1.3.3. Small Spill outside the Biological Safety Cabinet

- Small spills that can easily be cleaned with one paper towel
- If biological agent is transmitted via inhalation (e.g. adenovirus, influenza virus): Hold your breath and leave the room immediately
    - Ask other lab occupants to also leave the room and close the door.
    - Warn others not to enter the contaminated area and post a sign on the door.
    - Remove contaminated garments and put into a container for autoclaving.
    - Thoroughly wash any exposed areas of the body.
    - Wait 30 minutes for aerosols to dissipate
- Put on appropriate PPE (e.g., long-sleeve lab coat, goggles, and nitrile gloves).
- Remove any sharp, contaminated objects from the spill area using mechanical means (e.g., tongs or forceps). Discard contaminated sharps in a sturdy, leak-proof, biohazard labeled sharps container.
- Cover spill with paper towels or absorbent material. Slowly pour an appropriate decontaminant solution around the spill and allow the solution to flow into the spill. Paper towels soaked with the decontaminant may also be used to cover the area.
- A freshly prepared 1:10 dilution of household bleach, (approximately 0.5% sodium hypochlorite) is suitable for most biological spills. To avoid aerosolization, never pour decontaminant solution directly onto the spill.
- Allow 30 minutes of contact time. The contact time may vary depending on the decontaminant used and the microbiological agent. Follow the manufacturer's directions.
- Wipe up the spill, work surfaces, walls, and any equipment in the cabinet with paper towels dampened with decontaminant. If using bleach, follow with a water rinse to protect metal surfaces from corrosion.
- Place contaminated paper towels and other clean-up materials into a biohazard bag.
- Decontaminate the spill area again. Place all used spill materials into a biohazard bag.
- Remove any contaminated PPE in a manner to avoid cross contamination and dispose of per standard lab practices.
- Wash hands thoroughly after removing gloves.

5.3.1.4. *Large spill in the laboratory*

- Hold your breath and leave the room immediately. Close the door. Ask other lab occupants to also exit the room
- Warn others not to enter the contaminated area and post a sign on the door
- Remove any contaminated PPE and place in a biohazard bag for autoclaving.
- Wait 30 minutes for aerosols to dissipate. 6. Assemble spill clean-up materials.
- Put on appropriate PPE (e.g., long-sleeve gown, goggles, and nitrile or heavy duty gloves) before re-entering the room.
- Slowly pour an appropriate decontaminant solution (Section 4.6) around the spill and allow the solution to flow into the spill. Paper towels soaked with the decontaminant may also be used to cover the area.
- A freshly prepared 1:10 dilution of household bleach, (approximately 0.5% sodium hypochlorite) is suitable for most biological spills. To avoid aerosolization, never pour decontaminant solution directly onto the spill.
- Allow 30 minutes of contact time. The contact time may vary depending on the decontaminant used and the microbiological agent. Follow the manufacturer's directions.
- Remove excess decontaminant by wiping with a sponge or several paper towels. Place contaminated clean-up materials in a biohazard bag.
- Slowly pour an appropriate decontaminant solution around the spill and allow the solution to flow into the spill. Paper towels soaked with the decontaminant may also be used to cover the area
- Remove any contaminated PPE in a manner to avoid cross-contamination and dispose of per standard lab practices.
- Wash hands thoroughly after removing gloves.

5.3.1.5. *Spill Outside the Laboratory in Public Spaces*

Transport biohazardous materials in secondary, leak-proof containers to minimize the potential for spills. Use a cart if necessary. If a spill does occur in a common hallway or public space, cordon off the area, restrict access, and decontaminate the spill with appropriate disinfectant. If the spill cannot be immediately decontaminated, contact EHS at 330-672-4347.

5.1.4. Mixed Waste

Laboratories planning to work with both radioactive and hazardous material should develop a spill cleanup plan appropriate for all material before initiating the work. EHS research safety office will review and approve the spill cleanup plan.

5.2. Injury Policy and Accident reporting

5.2.1. Injury Policy

An exposure incident is defined as a specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with biohazardous agents, which includes all recDNA. Examples of exposure incidents include needlesticks, splash/splatter to the mucous membranes of the face, and any other incident that involves contact between blood or OPIM and non-intact skin (cuts, scratches, chapped skin, etc.). This injury policy applies to all Kent State university student'sdffgnhh faculty and staff.

5.2.2. Immediate response

- Call 911 for any life-threatening emergency
- If the injury/accident involves a potential exposure to a biohazardous agent, including all recombinant or synthetic nucleic acids, immediately follow the below steps. If an injury exposure involves significant personal exposure to recombinant or synthetic nucleic acids, EH&S may need to notify the NIH, communicate BSO or EHS and inform about the incident.

Eye exposure	Use emergency eye wash station to flush eyes for 15 minutes with lukewarm water holding eyes open
Skin exposure	Wash skin with mild soap and water for 15 minutes
Needle stick, puncture, sharps injury or animal bite	Wash thoroughly with warm water ad mild soap for 15 minutes
Inhalation or ingestion	Move out of the contaminated area and seek fresh air Do not induce vomiting unless instructed to do so

- At Kent campus, get medical help at DeWeese Health Services during normal hours. After hours go to nearest emergency room. Reginal campuses visit emergency room or your primary care physician depending on the severity of the exposure. Include emergency medical information including phone numbers in laboratory exposure control plan.

5.2.3. Incident Reporting

For all exposures, notify your supervisor, secure the area before leaving
Notify EHS after performing first aid and getting medical help
Complete the employee incident reporting form or student incident reporting form and sent it to EHS.

Chapter 6 B BLOODBORNE PATHOGENS PROGRAM

6.1. Purpose

The Purpose of the Kent State University (KSU) Bloodborne Pathogen (BBP) Program is to protect employee, faculty and students from exposure to human blood and other potentially infectious material. During normal work activities, the possibility of exposure to bloodborne pathogens exists when research faculty and students work with human blood, human blood products and human tissue and human cell lines.
The program is in place to ensure affected University employees and students get all necessary help to manage potential exposures to bloodborne pathogens. The University must fully comply with environmental health and safety standards and improve the overall safety of University faculty, staff and students.

6.6. Exposure control plan

6.6.1. Objectives

This plan establishes the following policies and procedures:

- An exposure determination identifies research staff, students and faculty members with potential of bloodborne pathogens (Category I)
- Category I employees are trained with bloodborne pathogen training and exposure control plan is reviewed annually with these employees.
- Only employees properly trained in the use of Universal Precautions should be allowed to clean blood spills and OPIM.
- Bloodborne pathogen spill clean-up kits are provided and stored in a predetermined location in required buildings. Contact your supervisor for its location.
- University affected personnel and other employees must receive training in Universal Precautions and the safe clean-up of spills of blood and OPIM.
- Kent State University provides access to Hepatitis B vaccines through University Health Services at no cost to employees identified having exposure to occupational bloodborne pathogens (Category I) and to those employees exposed to bloodborne pathogens.

6.6.2. Scope

- This policy applies to all Kent State University employees and students identified as having the potential to be exposed to blood or OPIM during their work activities. A copy of the ECP will be made available to the employee, within 15 days, upon request.
- For academic research laboratories, principal investigators are responsible for preparing and implementing laboratory specific exposure control plan.
- The exposure control plan prepared by PI must be submitted to Biosafety Officer at EHS office along with biological material use form to seek Institutional Biosafety Committee approval.

6.6.3. Responsibilities

Environmental Health and Safety (EHS)

Director of Environmental Health and safety

- Act as exposure control officer and is responsible for approval, coordination and implementation of Exposure control plan for the entire university.
- keep updates of current legal requirements concerning bloodborne pathogens.

Biosafety officer (BSO)

- Work with Principal Investigators to develop and implement additional bloodborne pathogen related policies and practices needed to accommodate employee and their work practices.
- Provide technical assistance for evaluations of the workplace and answer biological safety questions.
- Investigate all exposure or potential exposure incidents to infectious materials to determine the cause and recommend procedures or engineering controls such as safer sharps as necessary to prevent future incidents.
- Review regulated waste policy to ensure proper packaging, labeling and decontamination before disposal
- Review and update this program annually as required by the Standard.

Occupational Health and Safety Coordinator (EHSC)

- Provide bloodborne pathogen annual training
- Review and update training annually as required by the OSHA Standard
- Investigate all exposure or potential exposure incidents to infectious materials to determine the cause and recommend procedures or engineering controls such as safer sharps as necessary to prevent future incidents.

Research departments

Principal Investigators, Safety coordinators and Managers

- Acquire the knowledge and information needed to recognize and control bloodborne pathogen hazards, develop and implement laboratory specific Exposure Control plan
- Select laboratory practices and engineering controls to reduce the potential for exposure to bloodborne pathogens
- Supervise the performance of his/her staff to ensure the required work practices are followed and ensure appropriate controls (engineering and personal protective equipment) are used and in good working order.

- Ensure that all who are exposed or injured immediately wash the affected area for 15 minutes in an eyewash for facial mucous membrane exposures or wash skin and wounds with soap and water for 15 minutes
- Ensure that exposed or injured staff contact KSU DeWeese Employee Health for follow-up after an injury or exposure.
- Ensure the exposed employee completes the Employee Report of Injury Form.

University Health Services

- Provide pre-exposure access to the Hepatitis B vaccine at no cost to KSU employees identified as having a risk of occupational exposure to bloodborne pathogens and are subject by this program (Section 6.4, category I). Cost of the vaccines shall be the responsibility of the employee's department.
- Provide post-exposure access to the Hepatitis B vaccines at no cost to University employees who had an exposure incident to bloodborne pathogens as identified in this program. Cost of the vaccines shall be the responsibility of the employee's department.
- University Health Services must create its own site-specific Bloodborne Pathogens Exposure Control Policy for employees

Employees covered by Bloodborne pathogen exposure control plan

- Complete appropriate training and complete a Hepatitis B vaccination form
- Contact your personal care physician's office to schedule an appointment to receive vaccination (if desired)
- KSU Kent Campus employees can contact University Health services (1500 East way drive, Kent campus. Phone: 330-672-2322) to schedule an appointment to receive the vaccination (if desired).
- Acquire knowledge about human blood or OPIM material present in research laboratories and understands possibility of potential for occupational exposure to bloodborne pathogens.
- Annually complete bloodborne pathogens training sessions.
- Use all the engineering controls, work practices and appropriate personal protective equipment consistently to conduct all operations in accordance with their departmental exposure control plan
- Report to their supervisor, any bloodborne pathogens exposure incidents, near miss situations and all unsafe conditions.

6.6.4. Employee exposure determination

OSHA defines occupational exposure as any reasonably anticipated skin, eye, mucous membrane or parenteral contact with blood or other potentially infectious materials that may result from the performance of an individual's tasks. Exposure determination must be made without considering the use of personal protective equipment.

Exposure determinations are made by the Biosafety Officer in conjunction with principal investigators. Research laboratory personnel exposure is viewed closely by departmental safety coordinator and principal investigators.

All research personnel working with human blood, blood related products are considered as **Category I** employees. These positions are routinely exposed to blood or other potentially infectious materials. Use of proper personal protective equipment and engineering controls are required for every employee under this category. Appendix A lists the criteria for determining the risk of occupational exposure to bloodborne pathogens in research work area

6.6.5. Methods of Compliance and Control

This section describes methods of implementation and control to minimize the blood borne pathogen exposure

6.5.5.1. Universal Precautions

All employees must follow universal precautions during activities involving contact with human blood or other potentially infectious materials (including the handling of contaminated or potentially contaminated equipment). In circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids must be treated as potentially infectious materials. When performing activities involving potential contact the following standard or Universal practices shall be followed:

- Wear gloves when working with human blood or other potentially infectious materials.
- Wash hands with soap and water immediately after removal of personal protective equipment, after glove contamination, prior to leaving the contaminated work area and after using the restroom.
- Wear an impervious gown, apron or lab coat if contamination of clothing by splash or splatter of blood or other potentially infectious materials is possible.
- Wear full face protection if liquids or tissues are handled. Full face protection may be achieved with a fullface shield or safety glasses/goggles and a surgical mask.
- Employees with breaks in the skin should not handle blood or other potentially infectious materials.
- Employees should consult with immediate supervisors and the Biosafety Officer for an evaluation of breaks in the skin to determine if waterproof bandages and double gloving can serve as a barrier to exposure.
- Handle Sharps carefully (See sharp disposal policy below).

6.5.5.2. Engineering Controls

Engineering controls are the devices that remove or reduce the risk of exposure to employees either by removing or isolating hazard away from the employee. Engineering

controls are mostly used in clinical facilities or research laboratories. Engineering controls include biosafety cabinets, sharps disposal containers, self-sheathing needles, needless systems etc.

6.5.5.2.1. Safe Sharp

Criteria for Safer Sharps

- Allows/requires employees' hands to stay behind the needle after use
- Safety feature an integral part of the device, present before the device is contaminated
- Safety feature stays in place throughout the waste system
- Easy to use with little instruction
- Does not interfere with patient care
- Safety feature activated with a one-handed technique

Policy for Safer Sharps

The use of safer sharps or sharp devices in the workplace setting must be an integral part of your exposure control plan. Follow OSHA guidelines to:

- Evaluate safer devices currently available on the market
- Include employees who use these devices for evaluation purposes
- Document these evaluations and choices
- Choose those safer devices that meet your needs
- Reevaluate as new devices become available at least annually

Exceptions to using safer sharps:

- No safer sharps are available for the procedure (have not been developed yet)
- Temporarily unavailable on the market (must continue attempting to obtain)
- Sharp interferes with patient care
- Poses greater safety risk to patient or employee
- Sharp is produced by only one manufacturer

Use engineered sharps injury protection devices wherever feasible. If safer sharps are not available, evaluate other methods to reduce the chance of injury, such as devices used to permanently cover the contaminated point.

Recapping needles is not recommended in workplaces, although when recapping of contaminated needles is medically necessary, a one-handed technique or a mechanical device will be used. Tube/needle holders used for collecting blood specimens will be discarded into the sharps container with the needle attached.

All sharps injuries involving contaminated sharps must be documented on the Sharps Injury Log which is maintained in the Environmental Health and Safety Office (EHS) and Human Resources Department. The log is retained for thirty years past the last day of employment. The injury log will also be documented in the same manner as all exposures.

Re-evaluate the effectiveness of your plan each year. This will include a review of our Sharps Injury Log. Also evaluate new safer medical devices each year and revise your choices if better devices come on the market.

Safe sharp evaluation procedure

This practice will use applicable safer devices where possible to reduce or eliminate the potential for sharps injuries that could lead to the transmission of bloodborne pathogens.

- Each year review your reported sharps injuries and/or device failures for the past year to determine if additional personnel training is required or if certain devices should be replaced.
- If training is needed, contact the manufacturer or appropriate training resources. Provide and document the additional training.
- If certain devices seem problematic, initiate an effort to find safer replacements by contacting:
    - Our supplier
    - The device manufacturer
    - Other appropriate sources for suggestions
- Even if there are no sharps injuries or device failures, each year evaluate new safer devices that are available by:
- Requesting information from our supplier
- Contacting manufacturers
- Searching the internet
- When improved devices are available, determine if they would be useful in your setting and request samples from our supplier and/or the manufacturer.
- Evaluate these devices and document your findings. Employee-users must be included in the evaluation process.

6.5.5.2.5. <u>General work Practices in laboratories</u>

Researchers must follow good work practices when working in research laboratories

- Activities like eating, drinking, smoking, applying cosmetics or lip balm and handling contact lenses are prohibited in laboratories
- Do not store food and drink in refrigerators, freezers, shelves, cabinets or on countertops or benchtops in the laboratory or where blood or other potentially infectious materials are stored.
- Remove personal protective equipment worn in the laboratory and wash hands before entering offices, lunch area or break area. Protective clothing worn in the laboratory is not to be worn outside the laboratory or patient care area.
- All procedures involving blood or other potentially infectious materials must be performed with precautions to minimize splashing, spraying, spattering, and generation of droplets of these substances.
- All shippers of infectious material must be trained on DOT/IATA shipping

regulations. IATA training is required every two years and DOT training every three years. For information on where to receive training, contact the EHS office.

6.5.5.2.6. Hand washing

Employees must wash their hands and any other body part potentially contaminated with blood or other potentially infectious materials with soap and running water immediately. Employees must also wash their hands immediately after removing gloves. Each department must provide all covered employees and students with readily accessible hand washing facilities. If this is not possible due to the nature and location of the activity being conducted, antiseptic towelettes/cleansers must be provided. When antiseptic hand cleansers or towelettes are used, hands must be washed with soap and running water as soon as feasible and should be dried with forced air or disposable paper towel.

6.5.5.2.7. Specimen handling and processing

Departments and laboratories involved handling specimens of blood and potentially infected material must maintain standard operating procedures for handling, processing, spill control and decontamination of these materials. These specimens must be stored in a leak-proof container during collection, handling, processing and storage. When transporting specimens between labs or buildings, following requirements must be followed a) A sealed primary container b) A sealed secondary container c) secondary container lined with absorbent material d) a biohazard sticker on the outside of secondary container with agent name listed and e) name and phone number of the contact person's information.

6.5.5.2.8. Equipment decontamination

Cleaning equipment, Laboratory equipment may become contaminated with blood and other potentially infectious materials during cleaning, sample processing, must be examined for contamination after each use. Routine decontamination and cleaning protocol must be established for such equipment. A readily observable biohazard label must be attached to the laboratory equipment. Principal Investigator must ensure this information is conveyed to all students, technicians and employees, prior to handling, and processing samples with these equipments so that appropriate precautions must be taken.

6.5.5.2.9. Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) is specialized clothing worn by employees for protection against potential exposure hazard. Individual departments are responsible for ensuring that employees with potential exposure of bloodborne pathogen during their daily responsibilities are trained and understand the appropriate use of PPE needed to perform specific tasks or procedures. Departments and principal investigators must provide necessary PPE to category I employees and students and when required to category II employee. PPE

storage location and individual responsible for maintaining the stock, must be listed in individual department exposure control plan.

The following PPE must be used when appropriate:

- **Gloves:** Gloves shall be worn when it is reasonably anticipated that employees may have hand contact with blood, OPIM non-intact skin and mucous membranes, and when handling or touching contaminated items or surfaces. Disposable gloves are not to be washed or decontaminated for reuse and are to be replaced as soon as feasibly possible after contamination, or if they are torn or punctured.
  Utility gloves may be decontaminated for reuse provided their integrity is not compromised. Gloves must be discarded if they show signs of cracking, peeling, tearing, puncturing or deterioration.

- **Masks:** Masks are required to be worn to shield nose and mouth whenever splashes, spray, splatter or droplets of blood or OPIM may be generated. The disposable surgical mask must be used while working with blood or OPIM. Do not use washable cloth mask during the activities with blood work.

  Employees must participate in the University's respiratory protection program if they engage in work that requires a respirator. Personnel must have prior medical clearance to wear a respirator and must consult with EHS on the selection and use of respiratory protection equipment. Annual fit testing is also required.

- **Lab coats:** Lab coats are required to be worn in research laboratories where research activities involve work with blood or other potentially infectious material.

- **Face shields or Safety goggles:** Safety goggles are required during laboratory research activities whenever splashes, spray, splatter or droplets of blood or OPIM may be generated.

6.5.5.2.10. Housekeeping

- For research laboratories, Principal Investigators must provide methods of cleaning and decontamination of laboratory work area to students, technical or professional staff who are involved in housekeeping activities.
- Regulated infectious (biohazardous) waste must be placed in leak proof, appropriately labeled or color-coded containers, lined with plastic bags that are closable, constructed to contain all contents and prevent leakage (as specified in section 6.2.12, "Labeling and Signage"), and closed prior to removal to prevent spillage or protrusion of contents during handling. Research laboratories must include specific infectious waste handling in laboratory Exposure Control plan.
- Researchers must use mechanical means, such as tongs or a broom and dustpan, to pick up contaminated sharps, including contaminated glassware and must dispose of these items in a sharps disposal container.

- All equipment and work surfaces are cleaned and decontaminated as soon as feasible after contamination and after completion of work procedures. Principal Investigators or area supervisors must ensure that all equipment and work surfaces are cleaned and decontaminated after contact with blood or other potentially infectious materials.

6.5.5.2.11. Regulated Waste

All infectious (regulated) waste, including but not limited to blood and OPIM must be handled, packaged, transported and disposed of in accordance with Ohio Administrative Code Chapter 3745-27: Chapter 3745-27: Solid and Infectious Waste Regulations. If you have any question about regulated waste, you must reach out to Environmental Health and Safety office.

6.5.5.2.12. Laundry

- Contaminated clothing is never taken home for laundering. Contaminated laundry must be handled as little as possible, with minimal agitation.
- DeWeese Health Center, University Facility management, Recreational Services, Public safety department, University Police department must establish protocol to handle contaminated laundry. The protocol must be part of department specific exposure control plan.
- Each department should provide laundry facilities or access to a contracted commercial cleaning service for laboratory personnel to maintain clean personal protective equipment. This procedure must be included in the laboratory exposure control plan. .

6.5.5.2.13. Bloodborne Pathogen Cleanup Procedure

Blood or other potentially infectious material (OPIM) spill cleanup procedure outlined below, however individual research laboratories with category I employees must modify this procedure to fit best to their work responsibilities

- Notify your supervisor of the need for a body fluid clean up. Give the exact location and wait for the supervisor to arrive before cleaning the spill.
- Don all required Personal Protective Equipment (PPE) required for use during a body fluid spill clean-up.
- Apply coagulant or an absorbent compound to the body fluid spilled. Use enough absorbent to soak up the spill completely. The spill must be completely absorbed for decontamination.
- Pick up the absorbent material using the scraper provided in the spill kit, or a broom and dustpan.
- Place all spill material in a trash bag for disposal.

- Place the dustpan and broom in the janitor's sink for decontamination.
- Clean and disinfect the spill area using a suitable disinfectant, following the label instructions.
- After mopping the area with disinfectant, empty the mop bucket down the sink. Rinse the mop bucket and mop well. Fill the mop bucket with water and one cup of bleach. Let the mop soak overnight in the solution.
- Rinse the broom and dustpan with clear water to remove any material on them. Spray the dustpan and broom with disinfectant and allow to air dry.
- Rinse the vinyl gloves in running water to remove any residual material. Carefully remove the gloves and place them in the trash bag.
- Wash your hands completely with soap and water.
- Remove any other PPE and dispose in the trash bag.
- The supervisor is responsible for restocking the body fluid spill cleanup kit, if necessary.

Blood & Bodily Fluid Clean-up Kit

Blood and bodily fluid cleanup kit must contain absorbent material, appropriate disinfectant solution, proper personal protective equipment and instruction sheet. Individual departments and laboratories with category I employees must maintain detailed information about clean up kit in their exposure control plan.

6.5.5.2.14. Labeling and Signage

Appropriate labelling and signage must be used to advise employees about the presence of blood or/and other potentially infectious materials.

Labeling

Universal biohazard labels must be attached to containers of regulated waste, refrigerators, freezers containing blood or other potentially infectious materials. Warning labels must be attached to other containers used to store and transport blood or other potentially infectious materials. Labels must be attached to equipment used for processing of these samples. Warning labels must contain international biohazard symbol in fluorescent orange or orange red with lettering or symbols in contrasting color. Labels must be attached with adhesive or other methods that prevent their loss or unintentional removal.

Signage

All clinical laboratories and research laboratories (working with blood or other potentially infectious material) must have door sign containing universal biohazard symbol at all entrances to work areas. The sign must contain "BIOHAZARD" and must include: 1) name of infectious agent, 2) special requirements for entering the area and 3) name and telephone number of the laboratory director or another responsible person(s). (Appendix B)

6.6.6. HIV and HBV Research Laboratories

In addition to the other protective measures outlined in this exposure control plan, HIV and HBV research laboratories must follow stringent protective measures. Work practice regulations are listed as follows. Currently there are no HIV and HBV laboratories on KSU Kent Campus and other regional campuses.

- Laboratory access must be restricted to authorized personnel who have been trained for potential hazard, specific entry and exit requirements, PPE requirements, decontamination procedure and incident reporting protocols. (Specify all protective measures in laboratory specific ECP.)
- All laboratory doors must be kept closed when work involving HIV or HBV is in progress.
- All contaminated materials must be decontaminated at a site away from the work area and must be packaged in durable, leak proof, labeled or color-coded containers that are closed prior to removal from the work area.
- When OPIM or infected animals are present in the work area or containment module, door signage must be posted at all entry ways of the work area. Door signs must include universal biohazard symbol (must be fluorescent orange-red or predominantly so, with lettering and symbols in a contrasting color); the name of the infectious agent; special requirements for entering the area; and the name and telephone number of the laboratory supervisor/investigator or other responsible person.
- All activities involving HIV, HBV and OPIM must be conducted in biological safety cabinets or other physical-containment devices within the containment module.
- Protective clothing must be decontaminated prior to laundering.
- All vacuum lines must be protected with liquid disinfectant traps and high- efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency. These must be checked routinely and maintained or replaced as often as necessary. Glass disinfectant traps must be placed in appropriate secondary containment.
- Hypodermic needles and syringes must be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e. the needle is integral to the syringe) must be used for the injection or aspiration of OPIM.
- All spills must be immediately contained and cleaned by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials. A spill or accident that results in an exposure incident must be immediately reported to the laboratory director or other responsible person.
- A biosafety manual has been adopted, is reviewed and updated at least annually and is incorporated into lab practices and procedures.
- All activities with OPIM that pose a threat of exposure to droplets, splashes, spills, or aerosols must be conducted in a certified biological safety cabinet or other appropriate combinations of personal protection of physical containment devices. Biological safety cabinets must be certified when installed, whenever they are moved, and at least annually.

- An eye wash and hand washing facility, autoclave for decontamination process must be readily available within the work area.
- Work areas must be separated from areas of unrestricted traffic flow within the building. Passage through two sets of doors must be the basic requirement for entry into the work area from access corridors or other contiguous areas. Access doors to the work area or containment module shall be self-closing.

6.7. Medical Surveillance

6.7.1. Hepatitis B Vaccination Policy

Kent State University complies with OSHA's guidelines for Hepatitis B immunization, medical evaluations and procedures and post-exposure follow-up to covered employees as well as employees experiencing an exposure incident. University Health Services (UHS) at Kent campus offer the Hepatitis B vaccine to each occupationally exposed employee from category I, before his/her initial assignment, however employees can contact their primary care physician to receive Hepatitis B Vaccine. Employees whose duties are entirely administrative and are not at risk for exposure to bloodborne pathogens are not subject to the Bloodborne Pathogen Standard. However, UHS must offer Hepatitis B vaccine to these employees using the same guidelines as is used for the occupationally exposed employee.

Employees from category I must complete Hepatitis B Vaccination consent/ declination form (Appendix C) before their first assignment. For employees who accept the offer of immunization, can receive the first injection within the first 10 days of assignment. This must be done at no cost to the employee. It must be done during the normally scheduled work time.

Students exposed to blood or other potentially infected material because of their coursework or research activities, must be provided with bloodborne pathogen trainings and Hepatitis B vaccination information. Students involved in this kind of work must sign student consent/declination form for Hep B vaccination. (Appendix D)

The immunization must be three shots of the standard dose administered in the deltoid by a licensed healthcare worker who is authorized to administer such injections within his/her scope of practice. A pre-vaccination titer for Hepatitis B antibody is not required unless 1) employee has been previously immunized 2) vaccination is medically contraindicated.

The employee may decline the immunization, in which case he/she must be required to sign the approved declination form. If the employee initially declines the Hepatitis B vaccination but later decides to accept the vaccination while still employed with the same employer, it must be provided under the same conditions.

Documentation of the Hepatitis B immunization series and titer results will be documented at respective health clinics.

In accordance with the *Health Insurance Portability and Accountability Act* or HIPPA, effective April 2003, all patient-related medical information must be kept confidential.

6.7.2. Post Exposure Follow Up

Under the Bloodborne Pathogen Standard, an occupational exposure incident is defined as "a specific eye, mouth, or other mucous membrane, non-intact skin, contaminated needle stick or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties." When such an incident occurs, certain follow-up activities must be performed. These follow-up activities must be provided by the employer at no cost to the employee and must be conducted in a confidential manner.
Employees can contact their primary care physician, other clinics or emergency room for post incidental exposure follow up. KSU health services at the Kent campus offers post incidental exposure follow up during the working hours to employees. It is a responsibility of health care provider to offer post exposure follow up as per blood borne pathogen exposure standard.

As part of the post exposure follow up, the Individual department of the incident employee must complete the following

- Document all details of the incident in "Exposure Incident Report Form". https://www.kent.edu/sites/default/files/file/employee-report-of-injury_2.pdf
- Have employee sign declination form if no follow-up is desired (Appendix E). If this is the case, you may stop here. Otherwise, you should recommend employee to seek medical help for blood borne pathogen exposure.

6.7.3. Post Exposure Follow Up Documentation

- The Environmental Health and Safety office or designee Department will maintain exposure incident report forms and follow up declination form for 30 years beyond the end of the employee's employment.
- Document summarized information on the Sharps Injury Log and PERRP300 (Bureau of Workers Compensation's (BWC) Public Employer Risk Reduction Program (PERRP) has adopted OSHA's Private industry recordkeeping standards.) log form if applicable.

Healthcare professional who offered post exposure follow up care should maintain a copy of medical record and make it available to employee on request.

6.7.4. Evaluating Circumstances Surrounding an Exposure Incident

Departmental supervisors are responsible for collecting and reviewing circumstances surrounding of exposure incidents with the help of the office of Environmental Health and Safety. The following items must be considered while evaluating exposure incident.

- Work practices in use at time of the exposure incident
- Personal protective equipment or clothing used at the time of the exposure incident (gloves, eye shields, etc.)
- Location of the incident
- Procedure being performed when the exposure incident occurred
- Employee's training

- Engineering controls in use at time of the incident (if available at the location of incident)
- A description of the device being used, if applicable (name and model)

If the review of the circumstantial surrounding of an exposure incident results in a need for changes in procedures or protocols to reduce the occupational exposure, then Exposure Control Plan (ECP) must be revised to incorporate new changes. Revisions must be reviewed by departmental directors, biosafety officer and principal investigators to ensure appropriate changes are made to ECP.

6.8. Recordkeeping

6.8.1 Training Records

EHS must maintain all training records. Individual departments must maintain training certificates and Hepatitis B vaccination consent/declination for employees from category I, and post exposure follow up declination form. EHS must ensure all required training records are maintained for 5 years from the date on which the training occurred. Departments performing their own training are responsible for maintaining records at their departments.

6.8.2. Availability of Training and Medical Records

EHS must ensure all required training records are made available upon request for examination and copying to employees, to employee representatives, to the Assistant Secretary of Labor for Occupational Safety and Health (or designated representative) and the Director of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services (or designated representative) in accordance with 29 CFR 1910.20. Medical records can be obtained from respective clinics, for examination and copying to the subject employee, anyone having written consent of the subject employee, the Director of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services (or designated representative) and the Assistant Secretary of Labor for Occupational Safety and Health (or designated representative) in accordance with 29 CFR 1910.20.

6.9. Sharps Injury Log

Kent State University has established and maintains a sharps injury log for the recording of percutaneous injuries from contaminated sharps maintained as outlined in 29 CFR 1904.6. Compliance and Risk Management is responsible for determining whether an exposure or Sharps injury meets the recordkeeping requirements of the State of Ohio Public Employment Risk Reduction Program (PERRP). Environmental Health and Safety (EHS) Maintains and records for all injuries provides this information to the applicable college/department OSHA Log Coordinator. Environmental Health and Safety (EHS) is responsible for recording

applicable cases on PERRP 300P Logs as required by PERRP; records must be kept for 5 years. This information is compiled in a University wide PERRP 300P summary by EHS and is submitted annually to PERRP.
In addition to the PERRP 300P recordkeeping requirements, all percutaneous injuries from contaminated sharps are recorded on a PERRP Sharps injury and Needlestick Reporting. All incidences must include at least the following:

- date of the injury
- type and brand of the device involved (syringe, suture needle)
- department or work area where the incident occurred
- explanation of how the incident occurred

All PERRP sharps injury and needlestick reporting forms are reviewed as part of the annual program evaluation and maintained for at least five years following the end of the calendar year covered. All needlesticks are reported to PERRP by Environmental Health and Safety.

Chapter 7 VIRAL VECTOR GUIDELINES

7.1. Introduction

Researchers commonly use viral vectors in their research. Viral vectors are recombinant molecules and regulated by the NIH guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) It is particularly important to know how these tools came in use and their implications in biological research and safety. The Guidelines provided here contain information on commonly used viral vectors, laboratory hazards, personal protective equipment (PPE) and disinfection protocols. If a researcher is planning to work with viral vectors that are not covered under these guidelines, then he/she/they should communicate with the Institutional Biosafety Committee (IBC) or the Biosafety officer (BSO) for consultation on risk assessment.

7.2. Viral Vectors

Viral vectors pose occupational and safety challenges to researchers with development of *in vivo* and *in vitro* gene editing technology. Research institutions are responsible for evaluating the risk presented by the use of viral vectors in research based on available safety information and NIH guidelines. Viral vectors are a biosafety concern because they have the potential to accidentally infect research personnel. Most viral vectors, such as those commercially purchased and/or 3rd generation or higher are considered as low risk viral vectors by the Kent State University Institutional Biosafety Committee (KSUIBC). Viral vectors which are not commercially purchased and/or 1st or 2nd generation are considered higher risk because recombination events or contamination from wild type virus can result in replication competent viruses. These guidelines provide information about biosafety levels, containment, and PPE requirements for a selected common viral vector (Appendix A). Please communicate with the Biosafety officer if your proposed work falls outside the scope of this document.

7.3. Research Oversight

All recombinant DNA work and viral vector work at Kent State University must be registered and approved by the KSUIBC as the University receives funding from the NIH for research involving recombinant or synthetic nucleic acids. All research conducted at the University must comply with NIH guidelines regardless of whether or not the research being conducted is supported by NIH funds.
In many cases, the NIH Guidelines and the Recombinant DNA Advisory Committee (RAC) guidance document on viral vectors explicitly state the containment level.

- NIH Guidelines, Section III-D-3: Recombinant viruses in tissue culture
- NIH Guidelines, Section III-D-4-a: Recombinant viruses in animals
- NIH Guidelines, Appendices B-II-D through B-IV-D: Risk Group Classification of Various Viruses

The default biological safety containment level for recombinant viruses is generally at the same level as the risk group classification (e.g. use of Risk Group 2 viruses requires BSL2, use of Risk Group 3 viruses require BSL3); however, there are exceptions.

- A lower biological safety containment level may suffice for incomplete viruses cultured *in vitro*.
- Experiments that might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen should be considered for increased containment conditions. Transgenes that result in the production of harmful products or undesirable traits in the host animal may require increased containment conditions. Animals with recombinant viruses which ordinarily require BSL 2 containment may be downgraded to ABSL 1 if and when animals are considered to be no longer shedding virus.

The KSUIBC must review research protocols involving recombinant DNA or viral vectors to determine appropriate biosafety levels, PPE and disposal methods. The biosafety levels listed in Appendix A apply to replication incompetent vector systems only for *in vitro* and *in vivo* experiments. Researchers planning to prepare viral vectors in their lab for research must use Appendix B to determine biosafety levels and complete the questionnaire

7.4. ADENOVIRUS VECTORS

Background:
Adenoviruses are non-enveloped, midsized (90-100nm) icosahedral viruses containing double-stranded DNA that cause infections in human. Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors is capable of infecting human cells. The probability of producing replication competent adenovirus (RCA) increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1- containing genomic DNA in HEK 293 cells.

Human hazard:
Adenoviruses cause mild respiratory illness, pink eye or gastroenteritis. Rare cases of severe disease can occur hence its use as a genetic vector requires the use of adequate containment equipment and practices.

Laboratory Hazard:
Possibility of inhalation of aerosolized droplets, ingestion or parenteral inoculation, mucous membrane contact.

Biosafety requirement:

- BSL-2 facility
- All work must be performed in a biosafety cabinet
- PPE requirement - Lab coat, gloves, safety glasses or full-face shield
- Minimize aerosolization while centrifuging adenoviral vectors. Use airtight centrifuge rotor/bucket lids while spinning adenoviruses. Open and Load the centrifuge rotors/buckets in Biosafety cabinet. Disinfect centrifuge rotors/buckets after each use.

Animal Biosafety requirement:

- Injections of animals must be performed in Biosafety cabinet located at ABSL-2 facility
- Animals must be kept at ABSL2 facility for 72 hours as they may shed/excrete adenovirus post administration. Animals can be moved to ABSL-1 facilities after 72 hours.

Disinfection/Deactivation:

- Susceptible to 0.5% Sodium hypochlorite (Bleach), 0.25% sodium dodecyl sulfate, 2% Glutaraldehyde, 5% Phenol, or Autoclave for 30 minutes at 121˚C under 15 psi of steam pressure.
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) is recommended.
- Alcohol is NOT an effective disinfectant against adenovirus.

Reference: Adenovirus MSDS

7.5. ADENO-ASSOCIATED VIRAL (AAV) VECTORS

Background:
Adeno-associated virus gets its name from adenovirus as it is often found in cells that simultaneously infected with adenovirus. These are non-enveloped icosahedral viruses with a

single stranded DNA genome. Wild type adenovirus or herpesvirus must be present for AAV to replicate. If these helper viruses are not present, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic cycle.

Human hazard:
AAVs are not associated with direct human disease. However, AAV may be associated with insertional mutagenesis and cancer, thereby making AAV possibly not as safe as previously thought.

Laboratory Hazard:
Mucous membrane contact, parenteral injections, ingestion or inhalation of aerosolized droplet. There is no specific treatment for infection with AAV.

Biosafety requirement

- BSL1 facility for All AAV-serotypes
- PPE requirement - Lab coat, gloves, safety glasses or full-face shield
- BSL-2 facility for AAV production
- Minimize aerosolization during AAV production and Purification. Use airtight centrifuge rotor/bucket lids while spinning AAVs. Open and Load the centrifuge rotors/buckets in Biosafety cabinet. Disinfect centrifuge rotors/buckets after each use.
- BSL-2 facility if AAV produced using helper virus

Animal biosafety requirement

- Animals are maintained in ABSL-1 facility
- ABSL2 facility must be used in case of helper virus use. Animals can be moved to ABSL-1 facilities after 72 hours.

Disinfection/Deactivation:

- Adeno-Associated viruses (AAV) are susceptible to 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 0.25% sodium dodecyl sulfate, or Autoclave for 30 minutes at 121˚C under 15 psi of steam pressure.
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) is recommended.
- Alcohol is NOT an effective disinfectant against AAV.

Reference: Adenovirus vectors MSDS

7.6. LENTIVIRAL VECTORS/ RETROVIRUSES

Background:
These are simple, enveloped single stranded viruses with the ability to integrate into host cells, infect non-dividing cells and have high mutation rate. There are 5 serotypes of lentiviruses based on the mammalian host association

- Bovine lentiviruses: Bovine immunodeficiency virus
- Feline lentiviruses: Feline immunodeficiency virus
- Equine lentiviruses: Equine infectious anemia virus
- Ovine/caprine lentiviruses: Crapine arthritis-encephalitis virus, Ovine lentivirus
- Primate lentivirus group: Human immunodeficiency virus (HIV) types 1-3, Simian AIDS retrovirus
- (SRV-1), Human T-cell lymphotrophic virus type I and II, Simian immunodeficiency virus (SIV)

Most of the lentiviral vectors currently used in research are HIV-derived vectors. A broad range of vectors are used by replacing HIV envelope with vesicular stomatitis virus G glycoprotein (VSV-G).

Human hazard:

Lentiviruses can be transmitted through direct exposure with bodily fluid, percutaneous exposure, sharing contaminated needles or sexual conduct. Lentiviruses can integrate into human DNA and replicate, mutate. Lentiviruses stay in human body lifelong and show non-specific symptoms such as weight loss, fever, fatigue, chronic diarrhea etc.

Laboratory Hazard:

Human disease can cause by direct contact with skin and mucous membranes, parenteral inoculation, ingestion.

Biosafety requirement

- A Biosafety Level 2 (BSL-2) designation requires implementation of standard practices, safety equipment, and facility specifications to laboratories in which work is performed using biological agents and toxins that are associated with causing disease in humans of varying severity.
- All work must be carried out in Biosafety cabinet
- Personal Protective Equipment (PPE) required: Disposable gown, gloves safety glasses or full-face shield
- Minimize aerosolization while concentrating viruses. Use airtight centrifuge rotor lids while spinning Lentiviruses. Load and open the centrifuge rotor/bucket in Biosafety cabinet. Disinfect centrifuge rotors/buckets after use.

Animal biosafety requirement

- Animals must be injected in ABSL2+ facility and housed in BSL2+ facility for first 72 hours
- After 72 hours, animals can be moved to ABSL-1 facility

Disinfection/Deactivation:

- Susceptible to 0.5% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) recommended

Reference: Biosafety Considerations for Research with Lentiviral Vectors

7.7. HERPES VIRUS

Background:
Herpes viruses are double stranded linear icosahedral, lipid enveloped, approximately 110-200nm in diameter. HSV types I and II can be differentiated into immunologically derived vectors as they have wide variety of host and cell tropism (dividing and nondividing cells).

Human hazard:
HSV-1 *Herpes gingivostomatitis* (Oral herpes): primary infection occurs in early childhood and present mild symptoms. Reactivation of latent infection can present fever blisters or cold sores, usually on the face and lips, which crust and heal within a few days.
HSV-2 *Herpes genitalis* (genital Herpes): primary infection is sexually transmitted, associated with aseptic meningitis; vaginal delivery may pose risk to newborn (encephalitis and death).
Both HSV-I and HSV-II are capable of infecting the genital tract or oral mucosa.

Laboratory Hazard:
Human disease can cause by inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.

Biosafety requirement
- BSL2 facility
- All work must be carried out in Biosafety cabinet
- PPE requirement: Disposable gown, gloves safety glasses or full-face shield
- Minimize aerosolization while concentrating viruses. Use airtight centrifuge rotor lids while spinning Herpes virus. Load and open the centrifuge rotor/bucket in Biosafety cabinet. Disinfect centrifuge rotors/buckets after each use.

Animal biosafety requirement
- Animal injections are carried out in ABSL2 facility
- Animals are housed in ABSL2 facility

Disinfection/Deactivation:
- Herpes virus susceptible to 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) is recommended.

Reference: Herpes Virus (HSV)MSDS

7.8. EPSTEIN BARR VIRUS (EBV)

Background:

Epstein-Barr virus is frequently referred as EBV and belongs to B-lymphotrophic herpesvirus family. It is icosahedral, lipid enveloped, double-stranded DNA virus sized 120-150 nm in diameter. EBV has been found in the tumor cells of a heterogeneous group of malignancies such as Burkitt's lymphoma, lymphomas associated with immunosuppression, other non-Hodgkin's lymphomas, Hodgkin's disease, nasopharyngeal carcinoma, gastric adenocarcinoma, lymphoepithelioma-like carcinomas, and immunodeficiency-related leiomyosarcoma.

Human Hazard:

Most EBV infections are acquired during childhood and are asymptomatic.
Infectious Mononucleosis (IM): IM is an acute, self-limiting febrile illness in young adults, characterized by fever, sore throat, abdominal discomfort, pharyngitis, tonsillitis and tender generalized lymphadenopathy.
Burkitt's lymphoma: Burkitt's lymphoma arises due to an early infection with EBV virus resulting in infected B cells. It generally affects the facial bones, particularly the jaw, maxilla, and orbit, in young children.
Other malignant diseases in immunocompetent hosts include various B-cell or T-cell lymphomas, and epithelial or mesenchymal carcinomas such as classical Hodgkin's lymphoma and nasopharyngeal carcinoma.

Laboratory Hazard:

Human disease can cause by inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.

Biosafety requirements

- BSL2 facility
- All work must be carried out in Biosafety cabinet
- PPE required: Disposable gown, gloves safety glasses or full-face shield
- Minimize aerosolization while concentrating virus. Use airtight centrifuge rotor lids while spinning EB virus. Load and open the centrifuge rotor/bucket in Biosafety cabinet. Disinfect centrifuge rotors/buckets after use.

Animal Safety requirement

- EBV vector must be administered under BSL-2 containment.
- Animals must be housed as ABSL-2 containment for first 72 hours

Decontamination/ Deactivation

- EBV is susceptible 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) recommended.

References: Epstein Barr virus MSDS

7.9. MURINE LEUKEMIA VIRUS (MLV)/ RETROVIRUSES

Background:

MLV is an enveloped, icosahedral, single stranded RNA virus with linear genome. It is 100 nm in diameter and integrates into host genome and present in infected cells as provirus. Cell division is required for the infection and host range depends on the specificity of viral envelop. The eco-tropic enveloped viral particles infect rodent cells and ampho-tropic enveloped particles can infect murine and non-murine cells.

Human Hazard:

MLV can cause chronic productive retroviral infection which may allow insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk with pseudotyped viruses.

Laboratory Hazard:

In vivo transduction in humans may occur through direct injection with ampho-tropic or pseudotyped virus. Contact with feces or urine from infected animals for 72 hours post infection pose a risk of infection. Contact with tissues and body fluids of infected animals can cause insertional mutagenesis, integration and expression of oncogenes.

Biosafety requirements:

- BSL-1 containment for eco-tropic replication incompetent MLV
- BSL-2 containment for ampho-tropic or pseudotyped MLV
- All work must be carried out in Biological Safety Cabinet (BSC)
- PPE requirement: Lab coat, disposable gloves eyeglasses or face shield
- Minimize aerosolization while concentrating virus. Use airtight centrifuge rotor lids while spinning MLV virus. Load and open the centrifuge rotor/bucket in Biosafety cabinet. Disinfect centrifuge rotors/buckets after each use.

Animal Safety requirements:

- MLV vector must be administered under BSL-2 containment
- Animals administered eco-tropic MLV can be housed under ABSL-1 conditions
- Animals administered ampho-tropic/pseudo-typed MLV must be housed under ABSL-2 conditions for 72-hours post administration, after which animals can be moved to ABSL-1 housing

Decontamination/ Deactivation

- MLV is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, 70% ethanol.
- Freshly prepared 10% household bleach is recommended

References: NIH Guidelines Appendix B-V: Animal Viral Etiologic Agents in Common Use
MLV MSDS

7.10. RABIES VIRAL VECTOR (SAD-B19)

Background:
Rabies virus is a member of the Rhabdoviridae family and can cause a common zoonotic infection from bats and other wild mammals. Rabies is an enveloped, single-stranded, negative sense RNA virus.

Human Hazard:
Rabies virus can cause an acute infection, marked by progressive encephalomyelitis, and is usually fatal. The initial symptoms of rabies resemble those of other systemic viral infections, including fever, headache, malaise, and upper respiratory and gastrointestinal tract disorders.

Laboratory hazard:
Human infection can cause by inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion. Animal bites can cause rabies infection from infected animals.

Biosafety requirement:

- BSL2 facility
- All work must be carried out in Biosafety cabinet
- PPE requirement: Disposable gown, gloves safety glasses or full-face shield
- Minimize aerosolization while concentrating viruses. Use airtight centrifuge rotor lids while spinning Rabies virus. Load and open the centrifuge rotor/bucket in Biosafety cabinet. Disinfect centrifuge rotors/buckets after use.

Animal safety requirement:

- Injections of animals must be performed in Biosafety cabinet located at ABSL-2 facility
- Animals must be kept at ABSL2 facility for 72 hours as they may shed/excrete virus post administration. Animals can be moved to ABSL-1 facilities after 72 hours.
- If B19 glycoprotein is not expressed in recombinant vector, injections of animals can be performed at ABSL-1 facility. These animals can be housed in ABSL-1 facility.

Decontamination and Deactivation:

- Rabies virus is Susceptible to 70% Ethanol, Phenol, formalin, trypsin and some detergents
- Freshly prepared 10% household bleach is recommended

Reference: Rabies virus MSDS

7.11. EXPOSURE /INCIDENT: RESPONSE and REPORTING

Personal Exposure

Personal exposure may occur through a) Splashes to mucous membrane b) Needlestick or other percutaneous injury from a contaminated sharp object c) Inhalation of aerosolized droplets d) scratches or bites from animals that have been exposed to any recombinant or synthetic nucleic acid materials (Whether or not the exposure leads to illness).

Emergency response to exposure

Skin Exposure: Immediately remove contaminated personal protective equipment or clothing and wash the contaminated area with an antibacterial soap and copious water for 15 minutes.
Eye exposure: Flush the eye with water for at least 15 minutes at an eyewash station.
Needlestick or percutaneous injury: Wash the area with soap and water for 15 minutes

Medical Attention

- Notify PI /Supervisor about exposure and seek advice for medical attention. If PI/ Supervisor is not available, proceed to next step.
- During working hours, seek medical attention at DeWeese Health Services or at a local hospital.
- After hours, seek medical help at emergency room

Reporting

- Notify Lab Manager/PI about exposure. PI will complete an incident report form and submit it to EHS within 48 hours.
- Notify Biosafety Safety Officer or EHS within 48 hours. BSO will investigate the incident, complete incident report and notify IBC chair and EHS director.
- If IBC chair and BSO determine that the incident involves **non-exempt** recombinant or Synthetic nucleic acid material, BSO will submit an NIH incident report to NIH within 30 days.

7.12. SPILL: RESPONSE and REPORTING

Spill incident

Spill incidents include a) spill or release of recombinant or synthetic nucleic acid material inside or outside containment equipment b) loss, or release of any recombinant or synthetic nucleic acid molecules to the environment, including escape or improper disposal of a transgenic animal.

Spill response

Follow the spill cleanup procedure. Most research labs at Kent State University are equipped with spill cleanup kits to handle spills involving BSL2 organisms, viruses and recombinant or synthetic nucleic acid molecules.

Spill Reporting

- Notify PI or Supervisor immediately
- Notify the BSO immediately, if the IBC Chair and BSO determine that the incident involves **non-exempt** molecules, the BSO will submit an NIH incident report to the NIH Office of Biotechnology Activities within 30 days

CHAPTER 8: LABORATORY SPECIFIC EXPOSURE CONTROL PLAN

All laboratories at Kent State University that use biohazardous material including recombinant DNA, bloodborne pathogen and other potentially infectious material as defined by OSHA must complete exposure control plan for the laboratory. This plan must be updated by principal investigator or lab manager or senior person of the laboratory on an annual basis. The plan should be updated as there are changes biohazardous material, changes to procedures or changes to laboratory personnel.

Exposure control plan provides a tool to communicate laboratory hazards to laboratory workers and to non-laboratory works including security, EHS, fire safety personnel and first responders This plan must be in the lab and should be available during laboratory audits and in emergency situations.

The Exposure control plan include the following information

- PI and laboratory staff information
- Biohazard information
- Possible exposure risk (risk analysis)
- Risk mitigation (Engineering, administrative controls, PPE requirements)
- Decontamination procedures

APPENDIX A: Biosafety Resources

Kent State University EHS department

https://www.kent.edu/compliance/environmental-health-and-safety

Kent State University Research and Compliance

https://www.kent.edu/compliance/research-safety-and-compliance

Kent State University Biological Safety

https://www.kent.edu/compliance/biological-safety

Kent State University Bloodborne Pathogen Program

https://www-s3-live.kent.edu/s3fs-root/s3fs-public/bloodborne-pathogens-brochure.pdf

https://www-s3-live.kent.edu/s3fs-root/s3fs-public/file/BBP%202017%20Revision.pdf

Kent State University Respiratory Protection Program

https://www-s3-live.kent.edu/s3fs-root/s3fs-public/respiratory-protection-brochure.pdf

https://www-s3-live.kent.edu/s3fs-root/s3fs-public/respiratory-protection-program.pdf

Biosafety in Microbiological and Biomedical Laboratories (BMBL 6th edition)

https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

WHO Laboratory Biosafety Manual, 4th edition

https://www.who.int/publications/i/item/9789240011311

NIH Biosafety and recombinant DNA policy

https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/

CDC Select Agent List

http://www.selectagents.gov/SelectAgentsandToxinsList.html

Dual Use Research of Concern

https://www.phe.gov/s3/dualuse/Pages/InstitutionalOversight.aspx

CDC Information on Hepatitis B Vaccine

https://www.cdc.gov/vaccines/hcp/vis/vis-statements/hep-b.pdf

Ohio EPA Infectious Waste

https://epa.ohio.gov/dmwm/Home/Infectious-Waste

Laboratory Acquired Infections (LAI) database

https://my.absa.org/LAI

Biological agent Risk Group Database

https://my.absa.org/tiki-index.php?page=Riskgroups