Biosafety Manual

Revised & Approved August 6, 2025.

Research Safety

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1.0 Preface

The policy of the University of Kentucky (UK) is to provide a safe and healthy working environment for all employees, students, and visitors. It is UK's intent to minimize, to the extent practical, all recognizable hazards and to comply with all Federal, State, and Local laws and regulations. The implementation of this policy is the responsibility of all employees of UK. Supervisors at all levels shall be accountable for the health and safety of employees engaged in activities under their supervision. Supervisors shall insist employees comply with all health and safety rules and work in a safe manner.

2.0 Introduction

Microbiological, molecular biology, biomedical, agricultural, and clinical research pose a number of special risks that set them apart from other types of laboratories. Most of these circumstances arise because this work often involves organisms that are potentially infectious to humans, animals, or plants. Other unique risks are posed by working with transgenic animals or plants. The purpose of this manual is to define the appropriate facilities and work practices for work with potentially biohazardous materials to reduce the probability of exposure and infection to laboratory personnel and mitigate the potential for environmental release of potentially biohazardous materials and genetically modified plants, animals, or organisms that may be infectious to humans, animals, or plants.

2.1 Office of Biological Safety

The Office of Biological Safety is responsible for programs concerning the safe use of recombinant and/or synthetic nucleic acid materials, infectious agents, and potentially infectious materials such as human-sourced materials within the research and teaching laboratories at UK. This includes training, auditing, and consulting with researchers, laboratory personnel, and teaching staff concerning compliance with all Federal, State, and Local laws and regulations. The Biological Safety Officer is the liaison between researchers and the Institutional Biosafety Committee, which reviews research utilizing infectious agents and/or recombinant and/or synthetic nucleic acid materials.

The mission of the Office of Biological Safety at UK is to ensure the safe use of recombinant and/or synthetic nucleic acids, infectious agents, and potentially infectious materials in research and teaching activities to eliminate or reduce the potential exposure to personnel or the environment. Rather than ensuring mere compliance with federal regulations, guidelines, and University policies, UK's Biological Safety Program strives to adhere to the highest ethical standards in the protection of personnel and the environment from potential exposure to potentially biohazardous materials. In service of this mission, the Biological Safety Program endeavors to:

- Continue to inform researchers about the application of federal regulations to keep researchers current with evolving standards.
- Educate faculty, staff, and students who conduct research with recombinant and/or synthetic nucleic acids, infectious agents, and potentially infectious materials on their responsibilities to protect themselves and the environment from potential exposures.
- Develop new approaches that better serve the overarching mission of the University and assess the overall effectiveness of the program.

2.2 Department of Research Safety

In response to significant growth in research funding and facilities, the Division of Environmental Health & Safety created the Department of Research Safety. Research Safety will provide tailored client-centered services to the UK research community, expanding and enhancing safety and compliance activities for labs utilizing biological, radiological, or chemical hazards. The former Departments of Radiation Safety, led by Radiation Safety Officer Jason Johnson, and Biological Safety, led by Biosafety Officer Dee Mazzetti, have been combined under the oversight of Brandy Nelson, Director of Research Safety. Additionally, chemical safety services provided to the research community previously aligned with the Occupational Safety & Health Department (OHS) will now be associated with Research Safety. Holley Hannabach will lead chemical safety and general lab safety services in the newly created role of Chemical Safety Officer within the Office of Chemical & General Laboratory Safety. In this role, Holley will develop, implement, and direct a comprehensive chemical safety program, serve as the institutional Chemical Hygiene Officer, and ensure the implementation of appropriate chemical hygiene policies and practices. The initial goals of Research Safety will include

improving the efficiency of operations and customer service, as well as expanding and enhancing training and resources for the UK research community.

Department of Research Safety Mission

- Provide tailored client-centered services to UK research and teaching labs.
- Reduce redundancy in safety and compliance activities for labs utilizing biological, radiological, or chemical hazards.
- Improve efficiency of operations and customer service.
- Increase efficiency of departmental operations with technology solutions.
- Support current research initiatives.
- Develop training and resources to simplify researcher compliance and enhance safety.
- Expand and enhance in-person training for research faculty, staff, and students.

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2.3 Division of Environmental Health & Safety

The Division of Environmental Health & Safety (EH&S) supports the University's teaching, research, and public service mission by promoting a safe, healthful, clean, and accessible campus environment.

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

Environmental Health & Safety
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Lee Poore, Director of Occupational Health & Safety	lee.poore@uky.edu	(859) 257-2924
Greg Williamson, UK Fire Marshal	ggwill2@uky.edu	(859) 257-8590

2.4 Emergency Contact Information

FOR MEDICAL EMERGENCIES, CALL 911 OR GO TO THE UK CHANDLER HOSPITAL (OR CLOSEST) EMERGENCY DEPARTMENT

All workplace incidents and workplace-acquired injuries or illnesses sustained by UK personnel shall be reported by the Principal Investigator/Laboratory Supervisor to **UK Workers Care** by calling **(800) 440-6285**. UK students should contact University Health Services (859) 323-APPT during regular business hours, or (859) 323-5321 after business hours, on weekends or holidays.

Other Useful University Contacts

E-trax & Waste Pickup	**(859) 323-5005**
Respiratory and/or Hearing Protection Programs, Ergonomic Concerns	(859) 257-2924
Fire Extinguisher Concerns	(859) 257-6326

3.0 Responsibilities

3.1 Department Chairperson

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

3.2 Principal Investigator

The Principal Investigator (PI) is ultimately responsible for all aspects of safety and regulatory compliance in a laboratory. This is the individual who has been assigned the responsibility and discretionary authority to set work practices. The attitude of this person will be reflected by others working in the facility. It is the policy of the University that the Principal Investigator is responsible for complying with this Biosafety Manual and all relevant federal, state, and local regulations regarding biological safety. This includes complying with UK IBC policies, procedures, protocols, and other regulatory compliance committees.

Specifically, the Principal Investigator has the primary responsibility for:

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

3.3 Laboratory Supervisor

A laboratory supervisor should be designated by the Principal Investigator in laboratories where the PI is not actively involved in the daily operation of the research lab with many personnel. The PI may function as the laboratory supervisor in labs with limited personnel or in which the PI is actively engaged in the daily operation of the research lab. The responsibilities of the laboratory supervisor include enforcement of the safety practices and procedures that have been determined to be appropriate for the lab and training of lab personnel. The laboratory supervisor must ensure that lab personnel receive appropriate training and updates as needed and are aware of the risks and risk mitigation strategies appropriate to the materials handled in the lab. The laboratory supervisor must ensure that personnel demonstrate proficiency in laboratory practices prior to working with agents requiring BSL2 or higher containment.

Additional responsibilities of the laboratory supervisor may include, but are not limited to:

- Ensuring proper operation and preventive maintenance of safety equipment including biological safety cabinets, centrifuges and their rotors, and rotor cups and gaskets
- Maintaining supplies of personal protective equipment (PPE)
- Selecting and evaluating PPE and safety devices to mitigate potential exposure situations noted during the daily operation of the lab
- Maintaining Laboratory Safety Binder records, including the Chemical Hygiene Plan and training records
- Reporting accidents and exposure incidents to UK Worker's Care
- Communicate any concerns regarding lab safety to the PI or directly to the appropriate department/office within the Division of Environmental Health and Safety

3.4 Research Laboratory Staff, Students & Visitors

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

3.5 Office of Biological Safety

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

The Office of Biological Safety provides the following services to the research community, including but not limited to:

- Evaluation and inspection of laboratory facilities for work with infectious agents and other hazardous biological agents
- Consultation on the operation of the laboratory to ensure compliance with CDC, NIH, OSHA, federal, and state criteria

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

3.6 Institutional Biosafety Committee (IBC)

The Institutional Biological Safety Committee (IBC) performs duties for the University as defined in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (*NIH Guidelines*) and UK Administrative Regulations 6.9. As such, the Committee shall review applications for research involving recombinant or synthetic nucleic acids and other biohazardous materials to determine whether the facilities, procedures, and practices meet the standards required by the University and the NIH. It shall, in addition, have the responsibility to annually certify to the NIH that such facilities, procedures, and practices, and the training and expertise of personnel meet NIH standards. Meetings called for the purpose of such review and certification may be open to the public. Minutes of these meetings shall be kept and made available for public inspection. The University of Kentucky also requires the IBC to review all research involving infectious agents and other potentially infectious materials to ensure compliance with the OSHA Blood Borne Pathogens Standard, 29 CFR 1910.1030, and the guidelines set forth in the CDC BMBL. Information on the committee membership and meeting schedules can be found on the biosafety website here.

4.0 Regulations & Guidelines

A number of federal, state, and local regulations and guidelines form the foundation upon which the practices and procedures of the UK Biological Safety Program are built. The content of this Biosafety Manual is based upon the policies, procedures, and regulations listed here.

4.1 CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 6th Edition

Otherwise known as the BMBL, this document is considered to be the minimum standard for biosafety practices in U.S. laboratories handling infectious microorganisms and hazardous biological materials.

4.2 NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (*NIH Guidelines*)

As a recipient of NIH support for research involving recombinant or synthetic nucleic acid research, the University of Kentucky is required to assume all responsibilities as assigned in the NIH Guidelines.

4.3 OSHA 1910.1030 Bloodborne Pathogens

The OSHA Bloodborne Pathogens standard is applicable to all occupational exposure to blood or other potentially infectious materials (including immortalized human cells).

4.4 Guidelines for Biosafety Laboratory Competency

Published in the CDC's Morbidity and Mortality Weekly Report (MMWR) in 2011, this document outlines the essential skills, knowledge, and abilities required for working with biological agents at the three highest biosafety levels (BSLs).

4.5 Dual Use Research of Concern (DURC)

Dual Use Research of Concern is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants,

animals, the environment, materiel, or national security. The United States Government's oversight of DURC is aimed at preserving the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research.

4.6 Research Involving Enhanced Potential Pandemic Pathogens (ePPP)

The U.S. Government and the Department of Health and Human Services define enhanced potential pandemic pathogen (ePPP) research as research that may be reasonably anticipated to create, transfer or use potential pandemic pathogens resulting from the enhancement of a pathogen's transmissibility and/or virulence in humans.

4.7 Practical Guide to Plant Containment

Originally published in 2001 and updated in 2008, the Practical Guide to Plant Containment addresses containment of transgenic plants or plant-associated organisms.

4.8 Arthropod Containment Guidelines, Version 3.2

A product of the American Committee of Medical Entomology, a subcommittee of the American Society of Tropical Medicine and Hygiene, the Arthropod Containment Guidelines provide guidance to research laboratories for assessing risk and establishing protocols for the safe handling of arthropod vectors of human and animal disease agents.

4.9 Federal Select Agent Program

The Federal Select Agent Program oversees the possession, use and transfer of select agents and toxins, which pose a threat to public, animal, or plant health.

4.10 OSHA Act of 1970

Otherwise known as the General Duty Clause, employers are obligated to provide a safe workplace that is free from recognized hazards.

4.11 UK Administrative Regulations

UK Administrative Regulation 6.3

The University of Kentucky (the University) endeavors to maintain a safe and healthy environment for its students, employees, and visitors through effective environmental health and safety programs. The University positions itself as a leader within the Commonwealth in environmental stewardship, health protection, and safety standards and expects all students, employees, and members of the community to comply with applicable environmental, health, and safety laws and regulations. This regulation mandates compliance and assigns specific responsibilities associated with the implementation and maintenance of the University's environmental health and safety programs.

UK Administrative Regulation 6.9

This regulation establishes the University's Environmental Health and Safety Committee, Chemical Safety Committee, Institutional Biosafety Committee, and Radiation Safety Committee. These committees exercise advisory and other stated responsibilities for the Biological, Chemical, Environmental, Fire, Accident, Industrial Hygiene, Occupational Health, and Radiation Safety programs of the University. The Committees functions within the context of established external regulations, University policies, and recognized standards for the safe conduct of operations.

5.0 Biohazardous Materials

Biohazardous materials that require registration with the UK Institutional Biosafety Committee (IBC) include, but are not limited to:

- Recombinant nucleic acid molecules (ex. plasmids with inserts, viral vectors, etc.)

- Synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (ex. antisense oligonucleotides)
- Whole animals and plants with introduced recombinant or synthetic nucleic acid molecules
- Cells, organisms, and viruses containing recombinant or synthetic nucleic acid molecules
- Infectious agents (bacterial, viral, fungal, parasitic, or prion) affecting humans, animals or plants
- Infected animal blood and/or tissues
- Human blood, blood products, or fluids
- Human-derived cell lines or tissues (including commercially acquired immortal cell lines)
- Live vaccines

5.1 Infectious Agents

Registration with the UK IBC is required for work with any infectious agents, whether they are infectious to humans, animals, or plants. The following resources are utilized by the Office of Biological Safety and are useful tools for researchers in identifying best biosafety practices when working with a variety of infectious agents.

Pathogen Safety Data Sheets (PSDSs) from Public Health Agency of Canada

Section VIII - Agent Summary Statements from the CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition

Appendix B of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

American Biological Safety Association (ABSA International) Risk Group Database

5.1.1 Generally Recognized as Safe (GRAS)

The use of probiotics as feed supplements, particularly in agricultural sciences, is increasingly popular as more probiotic products are made available to the public. Biological organisms that have been designated as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA) are not considered biohazardous materials and are exempt from registering with the UK IBC.

5.2 Human Source Material & Blood Borne Pathogens

There are inherent risks to working with human source materials, including human blood, blood products, or bodily fluids, human-derived cell lines or tissues, or other potentially infectious material (OPIM including semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva from dental procedures, or any bodily fluid that is visibly contaminated with blood), in a research laboratory environment.

The use of these materials is subject to the OSHA 29 CFR 1910.1030 Bloodborne Pathogens Standard.

Bloodborne Pathogens – What are they?

Pathogenic microorganisms present in human blood or other potentially infectious material (OPIM), which can infect and cause disease in persons exposed to materials containing these pathogens.

Common Examples –

- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Human immunodeficiency virus (HIV)

Work with human blood and OPIM involves risk of exposure to bloodborne pathogens and other opportunistic pathogens that may be present.

Non-Human Primate (NHP) Materials

Non-Human Primate (NHP) materials present many of the same risks as working with human source materials and offer some additional risks as well. Old World NHP specimens (i.e. macaques) may contain Herpes B virus (Macacine herpesvirus) and Simian Immunodeficiency Virus (SIV). Macaques with Herpes B infection may present only mild symptoms (mild oral lesions), if any symptoms at all, but Herpes B infection in humans can be fatal. Other pathogens may cross between species (e.g. influenzas, SARS Co-V, West Nile virus, etc.).

Exposure Control Plan (ECP)

In accordance with OSHA 29 CFR 1910.1030, each PI whose work involves human source materials or OPIM must establish a written Exposure Control Plan (ECP) designed to minimize employee exposure. When you complete the UK Institutional Biosafety Committee (IBC) protocol registration, you will find the ECP in the "Infectious Agents" tab.

Hepatitis B Vaccination

Per OSHA 29 CFR 1910.1030, the Hepatitis B vaccination series must be made available at no cost to all employees with occupational exposure to human source materials or OPIM. Employees are not required to take the Hepatitis B vaccine. Employees may initially decline the Hepatitis B vaccination and choose to later accept the Hepatitis B vaccination.
All lab employees with exposure to human source materials or OPIM must sign the ECP Personnel Statement form to indicate their acceptance or declination of the Hepatitis B vaccination. This form is available online HERE.

Obtaining the Hepatitis B Vaccination

Hepatitis B vaccination is obtained through UK Employee Health. Follow the procedure below to obtain the Hepatitis B vaccination.

- Call ahead and make an appointment with Employee Health, 323-APPT (2778), for Hepatitis B vaccination.
    - Employees must bring their UK ID badge to be seen by Employee Health.
    - Employees will receive documentation for services received through Employee Health, which should be returned to their supervisor.
- Speak with your Principal Investigator or departmental business manager regarding payment.
- Complete the Guarantee of Payment form PRIOR to your visit and bring it with you to all appointments.

5.3 Recombinant and/or Synthetic Nucleic Acid Materials

According to the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:

(i) Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e. recombinant nucleic acids;

(ii) Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e. synthetic nucleic acids, or

(iii) Molecules that result from the replication of those described in (i) or (ii) above.

The UK IBC requires all work with recombinant and/or synthetic nucleic acid materials to be registered with the UK IBC, including that work which is exempt from IBC registration according to the *NIH Guidelines*. Work with recombinant and/or synthetic nucleic acid materials that is exempt from IBC registration according to the *NIH Guidelines* does not require full review by the UK IBC.

5.4 Transgenic Animals

When an animal's genome has been deliberately altered by recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line, it becomes a transgenic animal. This can involve a range of genome manipulations including the expression of a gene from another species, the knockout of a gene, and the increase or decrease in expression of targeted genes.

Work with animals (transgenic or otherwise) is subject to review and approval by the UK IACUC (Institutional Animal Care and Use Committee). In addition, the *NIH Guidelines* also apply to work with animals when,

> "… *experiments involving deliberate transfer of recombinant or synthetic nucleic acid molecules, DNA or RNA derived from recombinant or synthetic nucleic acid molecules, or recombinant or synthetic nucleic acid molecule-modified microorganisms into whole animals and experiments involving whole animals in which the animal's genome has been altered by recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals). Experiments involving gene drive modified animals or experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms, except for viruses that are only vertically transmitted, may not be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required*.." (Section III-D-4)

In accordance with the *NIH Guidelines* and UK Administrative Regulations, the UK IBC shall review and approve work with biohazardous materials used in conjunction with animals (including transgenic animals) prior to initiation. All research experiments involving animals shall be conducted in accordance with the associated Institutional Animal Care and Use Committee (IACUC) approved protocol. Animal research that involves a hazard (biological, radiological, or chemical) shall be reflected in the approved IACUC protocol.

5.5 Transgenic Plants

When a plant's genome has been deliberately altered by recombinant or synthetic nucleic acid molecules, it becomes a transgenic plant. Transgenic plants can also contain expressed genes from other species, gene knockouts, and gene expression modulation.

The *NIH Guidelines* apply to work with plants when,

> "Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules, may be conducted under the containment conditions described in Sections III-D-5-a through (Section III-D-5)

Research involving transgenic plants and/or plant pest species requires safe handling and appropriate containment. In accordance with the *NIH Guidelines*, the UK IBC shall review and approve work with plant pathogens and/or recombinant or synthetic nucleic acids in plants (including transgenic plants) prior to initiation. Physical containment of plants, pollen, seeds, and pest species must be ensured to prevent release to the environment. The Principal Investigator (PI) is ultimately responsible for the research project and for ensuring compliance with biosafety standards. Details on the handling of such plants must be included in the approved IBC registration, specifically including how the hazards associated with the work will be mitigated.

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

The importation, movement, possession, and/or release of transgenic plants, modified microorganisms, and/or plant pests may be regulated by Federal agencies including the USDA Animal Plant Health Inspection Service (APHIS), the US Environmental Protection Agency (EPA), and the US Food and Drug Administration (FDA).

For guidance on safely handling transgenic plant materials, please see Appendix L of the *NIH Guidelines* at*:*
https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.htm#_Toc3457202

For guidance on Plant and Plant pest containment, please see A Practical Guide to Containment, Plant Biosafety in Research Greenhouses at: **https://vtechworks.lib.vt.edu/items/586974ff-6e77-443a-add5-c881e693b569**

5.6 Select Agents & Toxins

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Subtitle A of Public Law 107–188 requires the Department of Health and Human Services (HHS) to establish and regulate a list of biological agents and toxins that have the potential to pose a severe threat to public health and safety. The Agricultural Bioterrorism Protection Act of 2002 requires the United States Department of Agriculture (USDA) to establish and regulate a list of biological agents that have the potential to pose a severe threat to animal health and safety, plant health and safety, or to the safety of animal or plant products (Select Agents). CDC and APHIS share responsibility for some agents because they potentially threaten both humans and animals (overlap agents).

Per the Code of Federal Regulations (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73), possession, use or transfer of agents deemed Select Agents or Toxins requires registration with the Centers for Disease Control and Prevention and/or the United States Department of Agriculture. Non-compliance with these regulations may result in criminal penalties, including fines and incarceration, affecting the University of Kentucky and/or the individual in possession of the material. Additionally, per University of Kentucky policy, research involving these materials must be registered with the Institutional Biosafety Committee.

A full list of biological agents and toxins that have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products, is available here.

5.6.1 Exempt Quantities of Select Toxins

The UK Department of Research Safety Office of Biological Safety administers the UK Exempt Quantities Program to ensure compliance with Federal Select Agent Regulations. The program is designed to ensure that laboratories with exempt quantities of listed toxins maintain:

- Exempt Quantities
- Secure Storage
- Safe Handling Practices

If at any time the exempt amount is exceeded, the laboratory is in violation of Federal Select Agent Regulations. Violations of Federal Select Agent Regulations may result in serious monetary and/or criminal penalties.

As part of this program, an annual survey is sent out across the UK community from the UK Responsible Official, Delena Mazzetti, requesting that labs identify what, if any, Select Agents, Toxins, or other High Consequence Biohazardous Materials (HCBM) possessed by the lab.

The Responsible Official and/or Alternate Responsible Office will conduct an annual Federal Select Agent Program (FSAP) Select Toxin Inspection to verify the quantity of toxin, method of storage, and ensure safe handling practices.

The following toxins are not regulated by the Federal Select Agent Program (FSAP) if the amount under the control of the principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the aggregate toxin limit specified in the HHS select agent and toxin regulations [42 CFR 73.3(d)(7), or the amounts indicated in the table below. Please note, these amount limits are superseded at any given time by the information published on the associated federal *Permissible Toxin Amounts* webpage, here.

HHS Toxins [§73.3(d)(7)]	**Amount**
Abrin	1000mg
Botulinum neurotoxins (BoNT)	1mg
Short, paralytic alpha conotoxins	100mg
Diacetoxyscirpenol (DAS)	10,000mg
Ricin	1000mg
Saxitoxin	500mg
Staphylococcal Entertoxins (Subtypes A, B, C, D, and E)	100mg
T-2 toxin	10,000mg
Tetrodotoxin	500mg

Secure Storage is required to prevent unauthorized usage or theft, access to toxins should be always restricted. Recommendations for secure storage include:

- 2 levels of security
    - In a standard lab setting, this would mean locking the main lab door when the lab is unoccupied AND locking the freezer/refrigerator where toxin is stored. Alternatively, a locked box stored within the freezer/refrigerator can be utilized if the freezer/refrigerator is not equipped with a lock.

    - In an open lab setting, the main entrance to the lab must be restricted by card access (or other means) AND the freezer/refrigerator must be locked. If the freezer/refrigerator is not equipped with a lock, a chain or cable lock can be retrofitted for the freezer/refrigerator.

- Primary container of toxin and all dilutions must be labeled with the toxin symbol. Additionally, if material is stored in a box or other secondary container, this container must also be labeled. The outside of the freezer/refrigerator need not be labeled. Contact biosafety@uky.edu for labels.

- It is crucial to maintain inventory records for toxins to prevent theft and/or loss. Inventory records must include the amount of toxin currently on hand, the date the toxin is used, amount used, and the name of the user. FSAP Select Toxin Inventory Record form is available online here.

Manipulation of Select Toxins requires Safe Handling Practices. Such practices include:

- Toxins should be utilized inside a containment device such as a fume hood or biological safety cabinet (BSC).

- Many toxins are utilized in minute quantities and can be difficult to weigh. It is recommended that stock solutions of toxins be made by adding diluent directly to the original containers. Dilutions for experimental use can then be prepared from this stock solution.

- Appropriate personal protective equipment should always be utilized when working with toxins. This includes lab coats and gloves.
    - Double gloving is recommended, and glove selection may vary based upon the solvent that will be used to dilute the toxin.

    - For aqueous diluents, a nitrile inner glove and latex outer glove would be ideal. This allows for easy visualization of holes or tears in the outer glove.

    - Be careful to remove gloves inside out and always wash hands after removing gloves.

- Proper disposal is crucial for all materials that come in to contact with toxins.
    - Autoclaving is not an effective means of destruction for many toxins. Chemical inactivation is preferred.

    - Contact the Office of Biological Safety for recommendations for inactivation of the specific toxin utilized in your laboratory.

    - Undiluted toxins or high concentration stock solutions MUST be inactivated prior to being ticketed as hazardous waste and picked up by Environmental Quality Management. Inactivation must be witnessed & documented by UK Office of Biological Safety representatives. Email biosafety@uky.edu for more information.

5.7 Poliovirus Infectious Materials (IM) and Poliovirus Potentially Infectious Materials (PIM)

Poliovirus containment is a key objective of the World Health Organization's (WHO) Global Polio Eradication Initiative. The U.S. National Authority for Containment of Poliovirus (NAC) helps reduce the risk of polioviruses being released from the places where they are worked with or stored. The NAC is responsible for implementing the containment plan in the U.S.

U.S. National Authority for Containment of Poliovirus (NAC) policies and guidance outline containment requirements for poliovirus-essential facility (PEF) programs to possess wild type poliovirus (WPV), vaccine-derived poliovirus (VDPV), and oral polio vaccine (OPV) infectious (IM) and potentially infectious (PIM) materials.

The U.S. NAC partners with biosafety, emergency response, occupational health, security, and poliovirus subject matter experts to create policies that balance PEF work practices with containment requirements. U.S. NAC policies are based on WHO's Global Action Plan, 3rd Edition (GAPIII) as well as all applicable U.S. regulations and guidance.

Any UK researchers who believe they may possess any of the materials listed below should contact UK Biosafety for guidance by emailing biosafety@uky.edu.

Poliovirus Infectious Materials (IM)

Oral Polio Vaccine IM

- Cell culture isolates and reference OPV/Sabin strains;
- Seed stocks and live virus materials from OPV production;
- Respiratory secretion or fecal samples from recent OPV recipients;
- Environmental sewage or water samples that have tested positive for the presence of OPV/Sabin strains;
- Infected animals or samples from such animals, including poliovirus receptor transgenic mice;
- Derivatives produced in the laboratory that have capsid sequences from OPV/Sabin strains;
- Full-length RNA or cDNA that includes capsid sequences derived from OPV/Sabin strains; *(Note: These nucleic acids will be captured in the national inventory/reported on the survey. Nucleic acid that has been extracted/purified using methods demonstrated to inactivate PV can be handled outside of PV containment under the condition that these materials will not be introduced into PV-permissive cells or animals with or without a transfection reagent.)*;
- OPV/Sabin strains of viruses proven to be safer than Sabin strains, but that include OPV/Sabin poliovirus capsid sequences;
- Cells persistently infected with poliovirus strains whose capsid sequences are derived from OPV/Sabin strains.

Wild Poliovirus IM (includes VDPV)

- Clinical materials from confirmed wild poliovirus (including VDPV) infections;
- Environmental sewage or water samples that have tested positive for the presence of wild polioviruses;
- Cell culture isolates and reference strains of wild poliovirus;
- Seed stocks and infectious materials from IPV production;
- Infected animals or samples from such animals, including human poliovirus receptor transgenic mice;
- Viruses or derivatives produced in the laboratory that have capsid sequences from wild polioviruses, unless demonstrably proven to be safer than Sabin strains.
- Viruses that include capsid sequences derived from wild poliovirus, unless viruses derived from them are demonstrably of viruses proven to be safer than Sabin strains as assessed by WHO, but that includes wild poliovirus capsid sequences;
- Full-length RNA or cDNA that includes capsid sequences derived from wild poliovirus, unless viruses derived from them are demonstrably proven to be safer than Sabin strains. *(Note: These nucleic acids will be captured in the national inventory/reported on survey. Nucleic acid that has been extracted/purified using methods demonstrated to inactivate PV can be handled outside of PV containment under the condition that these materials will note be introduced into PV-permissive cells or animals with or without a transfection reagent.)*
- Cells persistently infected with poliovirus strains whose capsid sequences are derived from wild poliovirus.

Examples of IM

The examples listed below include materials that meet the definitions of infectious and materials.

Oral Polio Vaccine IM Examples

- Respiratory secretion or fecal clinical samples, or environmental samples collected from sewage or water sources, in which an accepted diagnostic test (e.g., virus isolation or polymerase chain reaction [PCR]) has identified the presence of an OPV/Sabin strain.
- Respiratory secretion or fecal clinical samples collected from recent OPV recipients.
- Virus stocks of, or permissive cells (e.g., A549, L20B, or Vero) infected with OPV/Sabin.
- OPV/Sabin infected animals including, but not limited to, non-human primates and transgenic mice expressing the human PV receptor (CD155).

Wild Poliovirus IM Examples

- Respiratory secretion or fecal clinical samples, or environmental sewage or water samples, in which an accepted diagnostic test (e.g., virus isolation or polymerase chain reaction [PCR]) has identified the presence of WPV or VDPV.
- Virus stocks of, or permissive cells (e.g., A549, L20B, or Vero) infected with, WPV strains.
- Live WPV batch materials that will be inactivated to produce IPV
- WPV infected animals including, but not limited to, non-human primates and transgenic mice expressing the human PV receptor (CD155).

Poliovirus Potentially Infectious Materials (PIM)

Definitions of PIM

If you have materials that meet these definitions contact the U.S. National Authority for Containment.

Oral Polio Vaccine PIM

- Respiratory secretion, fecal, or untreated environmental surface water samples collected for any purpose in a time and geographic area of OPV use;
- Products of such materials from poliovirus permissive cells or animals;
- Respiratory and enteric virus stocks handled under conditions where OPV/Sabin strain contamination or replication is possible.

Wild Poliovirus PIM (includes VDPV)

- Respiratory secretion, fecal, or untreated environmental surface water samples collected for any purpose in a time and geographic area of wild poliovirus (including VDPV) circulation;
- Products of such materials from poliovirus permissive cells or animals;
- Uncharacterized enterovirus-like cell culture isolates from countries known or suspected to have circulating wild poliovirus or VDPV at the time of collection;
- Respiratory and enteric virus stocks handled under conditions where poliovirus contamination or replication is possible.

Examples of PIM

The examples listed below include materials that meet the definitions of potentially infectious materials.

Oral Polio Vaccine PIM Examples

- Upper respiratory secretions, fecal, or sewage samples collected from country at a time when OPV was in use and stored at -20°C or colder.
- Products derived from poliovirus permissive cells or poliovirus susceptible animals inoculated with upper respiratory secretions, fecal, or sewage samples collected from country at a time when OPV was in use.
- Stocks of respiratory (e.g., influenza) and enteric (e.g., coxsackie B) viruses created in a laboratory that also used OPV IM or PIM and where cross-contamination was possible.

Wild Poliovirus PIM Examples

- Upper respiratory secretions, fecal, or sewage samples collected from country at a time when WPV or VDPV was circulating and stored at -20°C or colder.
- Products derived from poliovirus permissive cells or poliovirus susceptible animals inoculated with upper respiratory secretions, fecal, or sewage samples collected from country at a time when WPV or VDPV was circulating.
- Enterovirus isolates cultured from a stool or sewage sample, collected from a country at a time when WPV or VDPV were circulating, that has not been characterized.
- Stocks of respiratory (e.g., influenza) and enteric (e.g., coxsackie B) viruses created in a laboratory that also used WPV or VDPV IM or PIM and where cross-contamination was possible.

6.0 Institutional Biosafety Committee (IBC)

The University of Kentucky Institutional Biosafety Committee (IBC) is responsible for carrying out the functions required under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and UK Administrative Regulation 6.9.

The IBC is responsible for reviewing recombinant and/or synthetic nucleic acid molecules and biohazardous research conducted at or sponsored by the institution, regardless of funding source or location of research, for compliance with the NIH Guidelines as specified in Section III, Experiments Covered by the NIH and other appropriate guidelines and University policies, and approving those research projects that are found to conform to the NIH Guidelines, UK policies, and other federal, state, and local regulations regarding biological safety.

6.1 IBC Membership

A current list of IBC Members can be found on the Biosafety webpage, here. UK IBC Members consist of experts in multiple fields including but not limited to: Microbiology, Molecular Genetics, Biochemistry, Cancer Biology, Gene Transfer/Gene Therapy, Animal Containment Principles, Plant Pathogens, Plant Pest Containment Principles, Agriculture/Botany, Neuroscience, Toxicology & Pharmacology, and many other related fields of study.

In addition to these subject matter experts, the UK IBC also includes the UK Biological Safety Officer (BSO), at least two Community Members representing the interests of the local community at large, and one Laboratory Technical Staff Member who is also currently working in one of UK's research laboratories.

6.2 IBC By-Laws

The UK IBC By-Laws are available via the Biosafety webpage, here.

6.3 IBC Meeting Schedule

The current UK IBC meeting schedule is located on the Biosafety webpage, here.

6.4 IBC Coordination with Other Regulatory Committees

The UK IBC plays a central role in ensuring the safe and compliant conduct of research involving biohazardous materials. To promote a comprehensive and integrated oversight framework, the IBC and UK Biosafety actively coordinate with other UK institutional regulatory bodies, including the Institutional Animal Care and Use Committee (IACUC), the Institutional Review Board (IRB), and Radiation Safety Committee, and others where necessary.

This coordination is achieved through the following mechanisms:

- Cross-committee representation: Select members and UK Research Safety representatives serve on multiple committees to facilitate communication and alignment.
- Shared protocol review: The IBC collaborates with the IACUC, IRB, or other committees on research protocols that involve overlapping regulatory requirements.

The UK IBC seeks to help maintain a culture of safety and compliance across the university's research enterprise.

6.5 IBC Registration

An overview of the IBC registration review process, along with other registration details, can be found on the Biosafety webpage here.

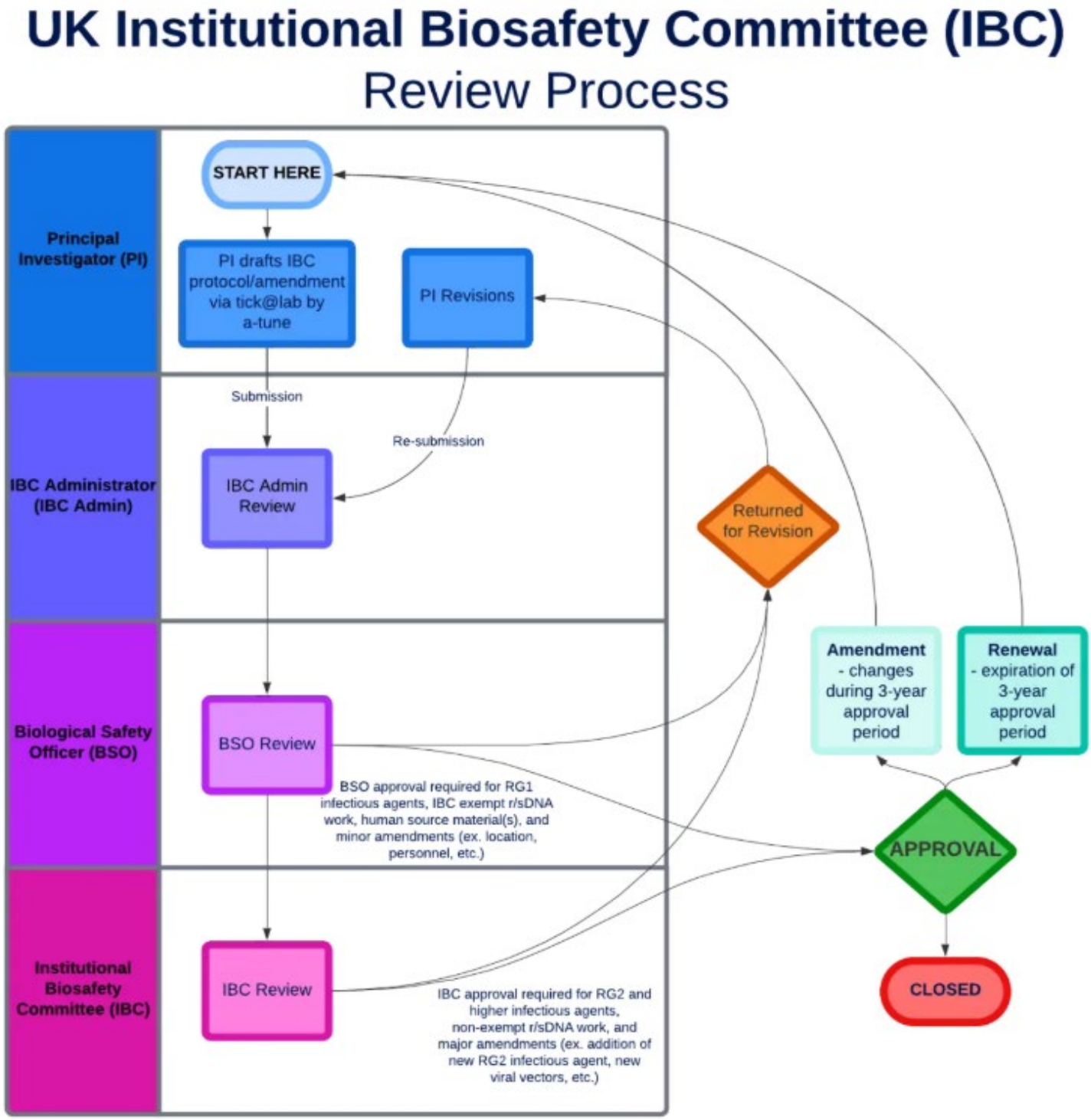
Registration of protocols for UK Institutional Biosafety Committee (IBC) review and approval requires the use of our web-based software - tick@lab by a-tune.

If you are new to UK and IBC registration, you must contact the Office of Biological Safety at biosafety@uky.edu and provide a list of all personnel associated with your IBC protocol registration. Please include first & last name and UK email address in your list.

Training for the use of the software is offered regularly via Zoom or in-person. Please contact the Office of Biological Safety at biosafety@uky.edu if you are interested in software training.

You can find how-to instructions for creating a new IBC protocol, amending an existing IBC protocol, and more in the tick@lab for IBC Protocol Management library, located here.

A diagram depicting the UK IBC review process and flow of IBC protocols can be seen below –



7.0 Personnel Training

Training of personnel is an integral part of ensuring the safe use of biohazardous materials on UK campus. All personnel working with biohazardous materials must receive training commensurate to the hazards in use prior to beginning work in the laboratory. These requirements apply to all individuals, including faculty, staff, students, post-docs, visiting researchers, and volunteers.

7.1 Biological Safety, General

The Institutional Biosafety Committee and Biological Safety Officer require all personnel working with biohazardous materials in research laboratories at the University of Kentucky to complete this training prior to initiation of work with biohazardous materials at UK. The training module will emphasize the basic principles and practices of biological safety. This training course must be completed prior to initiation of work with biohazardous materials and every three (3) years thereafter.

7.2 Biological Safety, Plant-Specific

In lieu of the General Biosafety course, personnel working with biohazardous materials associated with plants and/or plant pathogens may take the Plant Biosafety course prior to initiation of work with biohazardous materials at UK. This training course must be completed prior to initiation of work with biohazardous materials associated with plants and/or plant pathogens and every three (3) years thereafter.

7.3 Blood Borne Pathogens (BBP)

This training course is intended to promote general awareness of the potential hazards associated with blood and other potentially infectious materials (OPIM), such as human cell lines, in the occupational setting of the research laboratory. Additionally, the responsibilities and obligations of employers, supervisors, and employees under the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, and the University, will be discussed. This training course must be complemented by workplace-specific training conducted by the Principal Investigator or designee. It is required upon initial hire and annually thereafter for those employees who have the potential for exposure to blood or OPIM.

7.4 Biological Safety Cabinets (BSCs)

This training course is designed for individuals who utilize biological safety cabinets (BSC) for their work and will address general design, operation, best practices, and techniques. BSCs are essential engineering controls for certain laboratory procedures and must be operated properly for effective protection. This training course must be taken before working with a BSC and should be retaken as needed.

7.5 Autoclaves

This training course is intended to provide basic autoclave operation and precautions. This training is intended for any individual who will operate an autoclave on UK properties and is required before operating an autoclave. This training course should be retaken as needed.

7.6 Viral Vectors

This training course is intended to provide individuals with more detailed information on the most popular viral vector systems in use and UK-specific policies regarding the use of viral vectors. This training course is required prior to initiating work with viral vectors and should be retaken as needed (ex. prior to initiating work with a new viral vector system).

7.7 Dual Use Research of Concern (DURC)

This training course is designed to provide awareness of United States Government policies related to the concept of Dual Use Research and Dual Use Research of Concern (DURC). All Principal Investigators are asked to complete this training as they are ultimately responsible for ensuring that their research is properly reviewed for Dual Use Research to ensure continued funding from United States Government Funding Agencies and/or the National Institutes of Health.

7.8 Chemical Hygiene/Laboratory Safety

Chemical safety for laboratory workers. This training course is required for anyone who works with chemicals in a laboratory. The initial training course, Chemical Hygiene Plan/Laboratory Safety – General Awareness, must be completed prior to the initiation of work in any wet laboratory on UK campus. The annual refresher course must be taken annually thereafter.

7.9 Hazardous Waste

Initial Training - Training must be completed within six months after the date of assignment to the position requiring management of hazardous waste. Work in unsupervised positions is not allowed until the successful completion of the training and subsequent verification as outlined below.

Annual Training - Annual hazardous waste management refresher training is required on successive years after the initial training using either of the three options described below. Annual training is required if assigned job responsibilities include the management of hazardous waste.

Hazardous waste handling and disposal training is required for anyone who works with chemicals. If you have any questions about this class, please contact Robert Kjelland at (859) 257-3285. All personnel, except those noted below, can take the Hazardous Waste General course.

For personnel within any of the following, the Hazardous Waste Specific course must be completed.

- Veterinary Diagnostic Laboratory
- Center For Applied Energy Research
- Chemistry-Physics Building
- Jacob Science Building

7.10 Laboratory-Specific

This training must be provided by the Principal Investigator (PI) or their designee (i.e. Lab Manager) to all lab members who will work in the laboratory space prior to initiating work, annually thereafter, and whenever policies or procedures change. This training should cover the hazards, policies, and procedures specific to YOUR laboratory and must address the following:

- Safe performance of lab work
- Instructions for emergency response, including UK Fire and Emergency policies and evacuation procedures
- How/when to activate fire alarms, evacuation routes, and detection of natural gas or relevant hazardous chemical odors
- Awareness of all hazardous materials stored or used in the laboratory
- Engineering controls required for the use of hazardous materials
- Appropriate disposal and spill response
- Instructions for incident reporting
- Instructions for use of safety showers/emergency eyewashes

Completion of this training must be documented, and documentation must be maintained in your Laboratory Safety Manual. A form checklist is available online here.

7.11 IBC Member

This training is offered in-person to new IBC members on an annual basis and in the SciShield course directory. All IBC members must complete IBC member training prior to beginning their term with the UK IBC and annually thereafter. This training is designed to ensure IBC members are aware of their role and responsibilities as specified in Section IV-B-2 of the NIH Guidelines.

8.0 Standard Microbiological Practices

Standard microbiological practices are the first steps to protect against exposure to biohazardous materials utilized in a research laboratory. These are considered good laboratory practices, regardless of the materials in use.

Laboratory Access

The laboratory supervisor (Principal Investigator, Laboratory Manager, or their designee) ensures that laboratory access is limited to trained laboratory members and that laboratory door(s) are closed and locked when space is unoccupied. This ensures proper building HVAC function and security of laboratory materials, equipment, etc.

Handwashing

All laboratories should have a sink available for handwashing stocked with liquid hand soap and paper towels. Laboratory personnel should wash their hands often, particularly:

- After finishing laboratory work with potentially hazardous materials,
- Any time gloves are compromised (torn, ripped, etc.) or removed,
- Prior to leaving the laboratory to go eat, use the restroom, go home, etc.

Emergency Equipment & Evacuation

Laboratory personnel should be familiar with the location and use of all emergency equipment in their laboratory, including eyewash station, emergency shower, fire extinguisher(s), first aid kit(s), spill kit(s), and fire alarm pull stations.

Laboratory personnel should familiarize themselves with primary and secondary evacuation routes from their work area to the nearest building exit. Plan for where to meet in a safe space after a building evacuation has been ordered.

Laboratory Behaviors & Practices

There should be no eating, drinking, chewing gum, smoking, handling contact lenses, applying cosmetics (including chapstick or lip balm), taking medicines, or storing food/drink for human consumption in laboratory areas where biohazardous materials are stored and/or used. Human food or drink utilized for research purposed must be labeled “Not for human consumption”.

Mouth pipetting is strictly prohibited. Mechanical pipetting devices must be utilized.

Animals and/or plants not associated with the work being performed are not permitted in laboratory areas.

Long hair should be restrained such that it cannot contact hands, specimens, containers, or equipment.

Basic laboratory attire includes long-pants and closed-toe shoes. Shorts, sandals, etc. are not acceptable laboratory attire. During summer months, lab members are encouraged to maintain a spare pair of closed-toe shoes and long-pants (scrubs, sweat pants, etc.) on campus so that they can change into appropriate attire prior to working in the laboratory.

Personal Protective Equipment (PPE)

Gloves

Gloves are worn to protect hands from exposure to hazardous materials. The selection of suitable gloves is not arbitrary but rather based on an appropriate risk assessment that considers the materials utilized and procedures.

Disposable, single-use, nitrile gloves are the most common type of gloves in laboratories utilizing biohazardous materials and provide a barrier between biohazardous materials and the skin. Gloves should fit comfortably and be designed for flexibility, strength, dexterity, impermeability, and resistance.

Gloves should…

- NOT be worn outside of the laboratory.
- Be changed frequently, including when contaminated, whenever glove integrity is compromised, or when otherwise necessary.
- NEVER be washed or reused. Gloves should be found in only 3 places – 1) in the box, 2) on your hands, or 3) in the trash.
- Be removed in a manner to minimize contamination. How-To: Safely Remove Gloves is available online here.

Laboratory Coats

Laboratory coats may be disposable or reusable and must be worn appropriately to provide protection. Laboratory coats protect a user's body and prevent the spread of contaminants outside of the laboratory space. Lab coats must be buttoned/closed when worn. Laboratory coats with wrist cuffs should be worn with the wrist cuff UNDER the glove. Reusable laboratory coats must NEVER be taken home for laundry.

Face Protection

To enter an active research laboratory, eye protection must be worn. Contact lenses or personal prescription eyeglasses do not provide eye protection. A variety of options are available for eye protection, including face shields (disposable and reusable) and protective googles. The selection should be based upon the materials used for construction, fit, comfort, and compatibility with the work and required level of protection.

Face shields may also provide protection against splashes to other mucous membranes, including nose and mouth. Occasionally, fluid-resistant surgical masks may be utilized to provide splash protection for nose and mouth. Surgical masks do not provide protection against exposure to aerosols and are not substitutes for respiratory protection.

Respiratory Protection

In some cases, respiratory protection will be required for routine or emergency operations. If work with biohazardous materials requires the use of respiratory protection, this will be specified in the corresponding IBC protocol, and the laboratory must have a written Respiratory Protection Plan. More information on the UK Respiratory Protection Program is available from the UK Occupational Health and Safety Department online here. Users will be trained on the appropriate use, donning, and doffing of respiratory protection.

Safe Sharps

Laboratory work often requires the use of sharps, and special care must be taken to ensure the proper handling and disposal of sharps. Sharps may include needles, scalpels, broken glassware, slides, serological pipettes, micropipette tips, etc. Use of sharps should be limited as much as possible to situations where there is no alternative to minimize the risk of injury.

- Sharps utilized in conjunction with biohazardous materials should be immediately disposed in a designated, hard-sided sharps container placed as close as possible to the point of waste generation.
- Whenever possible, substitute plasticware for glassware (example – use "Plasteur" pipettes as opposed to glass Pasteur pipettes).
- Broken glassware and other sharps are never handled directly. Instead, use a brush and dustpan, tongs, or forceps to handle broken glassware.
- Needles must never be bent, sheared, broken, recapped, or otherwise manipulated before disposal.
    - If experimental procedures require the recapping of needles, utilize a hands-free device or one-hand scoop technique to safely recap needles.

One-Hand Scoop Technique

- When recapping a needle, use one hand ONLY.
- Place the cap on a level surface.

- Slowly slide the needle into the cap.
- Gently scoop up the cap. Allow the cap to cover the needle.
- Carefully press the cap against a firm inanimate surface to fix the cap in place.
- Alternatively, you may use a needle recapping device.
- NEVER place your fingers near the needle tip.

Laboratory Signage

Signage must be posted at the laboratory entrance to communicate the hazards present and the steps anyone entering the laboratory must take to mitigate exposure risk. Laboratory signage must include:

- Biological Safety Level (ex. BSL1, BSL2, BSL2+, ABSL1, ABSL2, BSL1P, BSL2P, etc)
- Universal biohazard symbol (for BSL2 laboratories and higher)
- PI name and phone number
- Emergency contact name and phone number
- PPE requirements
- Occupational Health Requirements
- Other required procedures for entry/exit

Laboratory signage is generated using SciShield, the web-based research management platform. Instructions on how to generate a door sign in SciShield are located in the SciShield Document Library online here.

8.1 Biological Safety Levels

Biological Safety Levels (BSLs) are a combination of standard microbiological practices, special practices, safety equipment, and laboratory facilities. Each biosafety level builds upon the level below it. For example, laboratories operating at BSL2 will observe practices of BSL1 + additional BSL2 practices.

Biosafety Level should not be confused with Risk Group (RG). An agent's Risk Group is based on its impact to human health and is used to evaluate relative risk. An agent's Risk Group is the starting point of a risk assessment, whereas the Biosafety Level is the result of the risk assessment process and provides instructions on how work with biohazardous materials can be conducted safely.

Below is a summary of practices, equipment, and facility requirements for Biosafety Levels utilized at UK. The IBC protocol will dictate the Biosafety level and additional practices or procedures required.

Biosafety Level	Typical Biological Agents in Use	Practices	Safety Equipment	Facilities
1	Not known to cause disease in healthy adults. Example – *Saccharomyces cerevisiae*	Standard Microbiological Practices	None PPE – laboratory coat, gloves, eye protection	Open lab bench; handwashing sink required
2	Associated with human disease that is rarely serious. Example – *Staphylococcus aureus*	BSL1 + limited access, biohazard warning sign, biosafety manual	Biological Safety Cabinet (BSC) for all manipulations that may cause splash or aerosols PPE – laboratory coat, gloves, eye protection	BSL1 + autoclave
3	Associated with serious or lethal human disease. Preventive or therapeutic interventions may be available. Example – *Mycobacterium tuberculosis*	BSL2+ controlled access, all waste and lab clothing decontaminated; vaccinations (if available)	Biological Safety Cabinet (BSC) used for ALL manipulations PPE – protective lab clothing, gloves, face protection, respiratory protection (as needed)	BSL2 + physical separation from access corridors, self-closing doors, double-door access, exhausted air not recirculated, negative airflow

8.1.1 BSL2 Enhanced (BSL2+)

Some work at BSL2 may require enhanced practices and procedures, depending on the biohazardous materials in use. Oftentimes, BSL2+ (or BSL2 Enhanced) containment will be required for work with certain viral vectors (examples – lentivirus, adenovirus, etc.).

At UK, BSL2+ indicates work utilizing BSL2 facilities in combination with BSL3 practices. Specifically –

- NO open benchwork. All procedures with biohazardous materials will be conducted inside a Biological Safety Cabinet (BSC) until inactivation.
- Centrifugation of biohazardous materials requires the use of safety buckets/cups/rotors with aerosol-tight lids. Centrifuge buckets/cups/rotors must be loaded and unloaded in the BSC and wiped with an appropriate disinfectant prior to removal from the BSC.
- Centrifuge tubes must be sealed (i.e. plates sealed with parafilm) or capped
- The use of sharps (needles, syringes, other sharps) is limited.
- PPE required includes eye protection, disposable gloves, and dedicated laboratory coat or disposable lab gown with wrist-cuffs.
- Facility must meet the following requirements for Tissue Culture facilities as approved by the UK IBC on May 12, 2010.
    - Air shall flow from the hallway to the inner lab (negative to the hallway). All room air shall be exhausted through ducts to the outside of the building and not recirculated within the building. Room air which is exhausted to a common plenum is **NOT** acceptable.
    - The number of BSCs, the amount of space within the BSC and amount of room space provided is required to accommodate all the tissue culture and viral vector work. This is for the protection of research materials and for the protection of the researchers and facilities.
    - The BSCs may be recirculating models (Class II, A2) or thimble ducted or hard ducted.
        - BSCs shall be installed such that:
            - Fluctuations of the room air supply and exhaust do not interfere with proper operations.
            - Manufacturers' guidelines are followed.
            - They can be certified according to the National Sanitary Foundation (NSF) criteria.
        - BSCs shall be certified on an annual basis by a vendor who meets the requirements of the Biological Safety Department, follows NSF criteria, and is on contract with UK.
            - Coordination of this certification is through the Biological Safety Department.
            - Payment for this service is the responsibility of the Principal Investigator or Department
    - Rooms housing any BSC shall be configured to allow storage of supplies and equipment used with the biohazardous materials. Typical equipment in these rooms includes incubators, centrifuges, microscopes, CO2 tanks, vacuum source, refrigerators.
    - A hand washing sink with eyewash shall be present in the tissue culture room facility. The eyewash may be "drench hose" if approved by UK Occupational Health and Safety Department. An exemption from the eyewash requirement may be granted by the Biological Safety Officer if the risk assessment of the proposed research warrants it.
    - There must be restricted entry to the outer laboratory, which is locked when no one is present.
    - All surfaces must be easily cleaned and decontaminated. Room casework shall be easily cleanable, and finishes should be compatible with materials used for cleaning and disinfection. Chairs must be covered with non-porous material. Rugs or carpets are not permitted.
    - Vacuum lines shall be protected with High Efficiency Particulate Air (HEPA) filters or their equivalent. This applies to central vacuum systems and to individual vacuum pumps.
    - Open flames **SHALL NOT** be used in BSCs. Therefore, gas lines **SHALL NOT** be connected to BSCs.
    - A functioning and validated autoclave enrolled in the UK Autoclave Verification program shall be available within a reasonable distance of the facilities creating the biohazardous waste.
        - IBC approved procedures for transport shall be followed when unprocessed waste is carried through public hallways and elevators.
    - Tissue culture rooms which will contain research deemed by the IBC to be BSL2+ (enhanced) shall be in an inner lab, with two doors between the BSC and the hallway.
    - Appropriate signage shall be displayed on the door of the main laboratory and of the tissue culture room.

9.0 Special Biosafety Practices

9.1 Animal Biosafety

Work with research animals can present additional challenges, especially when biohazardous materials are also utilized. All research experiments involving animals shall be conducted in accordance with the associated Institutional Animal Care and Use Committee (IACUC) approved protocol. The IBC shall approve work with recombinant or synthetic nucleic acids, infectious agents, or other potentially infectious materials used in conjunction with animals (including transgenic animals) prior to initiation of the proposed work.

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

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

- Careful handling procedures should be employed to minimize the dissemination of dust from animal and cage refuse.
- Cages should be sterilized by autoclave. Refuse, bowls, and watering devices should remain in the cage during sterilization.
- All water devices should be of the "non-drip" type.
- Cages should be examined each morning and at each feeding time so that dead animals can be removed. Dead animals should be placed in leak-proof containers (plastic bags, covered metal trays, canisters, or fiber cartons) that are appropriately marked with date, experiment, biohazard label, cage number, etc., and stored in designated refrigerators or cold rooms prior to necropsy or disposal.
- Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should never be placed in the cage to move any object therein.
- When animals are to be injected with infectious agents, the animal caretaker should wear protective gloves, and the laboratory workers should wear surgeon's gloves. Animals should be properly restrained using physical restraints (e.g., use of squeeze cage for primate inoculation), anesthesia, or specific handling practices to avoid accidents that might result in disseminating infectious agents, as well as to prevent injury to the animal and to personnel. Infected animals to be transferred between buildings should be placed in microisolator cages or other aerosol-proof containers.
- Animals exposed to infectious agents in aerosols require special consideration based on the agent in use and experimental procedures. Contact the Department of Biological Safety for assistance.

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

9.2 Clinical Biosafety and Human Gene Transfer/Human Gene Therapy

Human gene therapy and clinical trials involving recombinant or synthetical nucleic acid molecules are becoming increasingly more common on medical research campuses, like that of the University of Kentucky (UK). Being among the cutting edge of medical research, these trials sometimes involve materials and techniques that fall under both federal and institutional regulations, in addition to those of the US Food and Drug Administration (FDA) and UK Institutional Review Board (IRB).

Common Clinical Studies Requiring Institutional Biosafety Committee (IBC) Registration

Clinical studies utilizing the biohazardous materials described below require registration with the UK IBC.

- Investigational products (IP/IMP/Study Agent) that contain:
    - Live virus (ex. Live/attenuated vaccine candidates)
    - Infectious Agents (ex. bacteria, viruses, fungi, prions, parasites)

- Recombinant or synthetic nucleic acid-based agents and agents that have the ability to genetically alter a patient's cells (ex. gene therapy viral vectors, siRNA, anti-sense oligonucleotides, mRNA vaccines, LNP – lipid nanoparticle delivered nucleic acids)

- Genetically modified cell products/therapies, (ex. allogeneic or autologous CAR-T cells, and other genetically engineered cellular products)

- Studies that handle patient samples or registerable investigational products in a research laboratory (ex. Handling patient blood or tissues in a research lab outside of the patient's routine clinical care, pre- or post-administration of product.)

- Specimen Biobanks/Repositories

Within a study's documents, particularly the Investigator's Brochure and Study Protocol, certain keywords of interest can alert you to the potential need for IBC registration; these keywords are listed below. Please notify the Office of Biological Safety if any of these words are noted, or if there is any uncertainty, and we will help determine whether IBC registration is necessary.

- Gene transfer/therapy
- Pathogen/pathogenic
- Recombinant or synthetic nucleic acid (mRNA, DNA, RNA, siRNA)
- Anti-sense oligonucleotide (ASO)
- Virus
- Bacteria
- Vaccine
- Vector
- Cloning
- PCR
- Infectious
- Allogeneic/Autologous
- CAR-T
- Genetically modified/engineered
- Cellular product/component

Note: If a study's investigational product is not determined to require IBC registration, but patient samples are being sent to a research laboratory on UK's campus for analysis and/or storage, please check with the Office of Biological Safety. It is possible that this work may already be on an approved registration, or the Office of Biological Safety will assist you with the registration of this work.

Regulations and Guidelines

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

The University of Kentucky (UK) receives funding support from the National Institutes of Health (NIH). As a condition of NIH funding support, all work conducted at UK must comply with the *NIH Guidelines*, irrespective of the source of funding. As such, research subject to the *NIH Guidelines* must be registered with the UK IBC.
Human Gene Transfer (HGT) as defined by the NIH Guidelines can be found in Section III-C: Experiments Involving Human Gene Transfer that Require Institutional Biosafety Committee Approval Prior to Initiation.

Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Human gene transfer is the deliberate transfer into human research participants of either:

1. *Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or*
2. *Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:*

a. *Contain more than 100 nucleotides; or*
b. *Possess biological properties that enable introduction of stable genetic modifications into the genome (e.g., cis elements involved in integration, gene editing); or*
c. *Have the potential to replicate in a cell; or*
d. *Can be translated or transcribed."*

It is the policy of the UK IBC that ALL clinical studies that involve the deliberate transfer of recombinant or synthetic nucleic acid molecules into human research participants, even that which is exempt from IBC registration according to the *NIH Guidelines*, must be registered with the UK IBC.

Exceptions *– The following are not subject to the NIH Guidelines and do not require UK IBC registration:*

1. *The deliberate transfer of recombinant or synthetic nucleic acids into one human research participant, conducted under a Food and Drug Administration (FDA) regulated individual patient expanded access Investigational New Drug (IND) or protocol, including for emergency use, is not research subject to the NIH Guidelines and does not require UK IBC review and approval.*
2. *Use of an FDA approved or authorized product for the treatment of a clinical indication.*

FDA Center for Biologics Evaluation and Research (CBER)

The FDA Center for Biologics Evaluation and Research regulates cell therapy products, human gene therapy products, and certain devices related to cell and gene therapy.

Cellular therapy products include cellular immunotherapies, cancer vaccines, and other types of both autologous and allogeneic cells for certain therapy indications. Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use.

Cellular and gene therapy guidance documents are available from the FDA CBER online HERE.

Registering with the UK IBC

Registration of a clinical study with the IBC requires the use of the tick@lab by a-tune web-based software located here. It is highly encouraged that you enlist the assistance of a member of the Biosafety Team when drafting a clinical IBC registration. This ensures that pertinent information is collected and listed on the registration appropriately, in a timely manner before going for review. Clinical/administrative staff delegated the task of completing the IBC registration form may experience some difficulties sufficiently completing areas of the form. It is important to note that per the NIH Guidelines, the PI (Principal Investigator) is ultimately responsible for all research activities, the information included in an IBC registration, and the complete and accurate disclosure of information for the purposes of the IBC registration. Registration guidance specific to clinical IBC registrations is outlined below.

When notified, a member of the Biosafety Team will guide you through and aid in the drafting of the registration. When navigating tick@lab by a-tune on your own, keep these helpful tips in mind for a successful experience. Please reference the How-To guides for navigating tick@lab by a-tune in our reference library, located here. Also, please ensure your internet connection is consistent and stable when utilizing the software, tick@lab by a-tune is a web browser-based software. Save your work often in tick@lab by a-tune, as there is not an “auto-save” feature.

An IBC registration & approval consists of a “cradle-to-grave” evaluation of the product and procedures, including but not limited to:

- Shipping/Receiving
- Storage of investigational product and specimens
- Preparation/Dispensing
- Transport
- Investigational product administration
- Waste disposal and disinfection
- Patient sample handling
- Safety training
- Locations

This information must be gathered by the PI/UK study team. Biosafety Team members are not responsible for the direct gathering of this information. The PI and study coordinators are responsible for gathering and providing required information to Biosafety Team members. It is the responsibility of the PI and study coordinators to contact the study sponsor as necessary, when additional information is required.

As the IBC registration form is completed, please take note of the help text provided on each question. Additionally, as some of the questions are completed, additional help text will appear with a response and provide additional guidance.

This information is intended to assist the PI/authors of the registration in identifying the NIH Guidelines and other regulations that may apply to the proposed work in the registration. Always read all the form text in each question carefully and answer appropriately.

Be aware that the IBC registration form was created to be able to register all types of research involving potential biohazards, including studies with plant and animal research. Not all sections/questions may apply to the work proposed, but please keep in mind that the registration form cannot be submitted unless all questions are answered.

Getting-Started: Clinical IBC Registration Checklist

Here is a short checklist of information that will be required to begin the registration process of a new clinical IBC registration.

- List of all study personnel (physicians, nurses, pharmacists, research staff, shipping staff, etc.)
- UK site-specific study locations (Clinics, Pharmacies, Units, related academic research labs, etc.)
- Study documents (Investigator's Brochure, Study Protocol, Pharmacy Manual, etc.)
- UK site-specific procedures (Description of the investigational product's journey from receipt to administration to disposal.)
- Current copy of PI's curriculum vitae (CV)

Study Personnel

Before beginning to work in the registration system, please email a list of personnel (with linkblues) involved in the study to the Biosafety Team member you have been in contact with, or the Biosafety Office (biosafety@uky.edu). This information will be used to ensure personnel are available to be added to the IBC registration and for training verification. All personnel that would handle or otherwise come into contact with the investigational product or related patient samples (ex: UK Healthcare staff, pharmacy staff, shipping staff,) and the PI/Co-PIs, should be included in this list. If you will serve in an administrative-only role on the study (coordinator, regulatory staff, etc.), please note this in the IBC protocol registration. Anyone involved in an administrative-only role is not permitted to handle, transport, or ship investigational product or associated patient samples, related to the IBC registration.

All personnel handling or administering investigational product must be listed as personnel on the registration and complete required safety training. This may include but is not limited to individuals that:

- Receive and store the investigational product
- Prepare and/or dispense the investigational product
- Transport the investigational product from one location to another (ex: from pharmacy to clinical area)
- Administer the investigational product to patients
- Administer patient care immediately after the product is administered (post-administration)
- Dispose of the investigational product (this does not include housekeeping staff who may dispose of waste bins)
- Ship patient samples or investigational product offsite for further analysis

Training

Please check with your Biosafety Team member or the Biosafety Office for specific training requirements. Training guidance and frequency are referenced here. Required training is available on-demand online. Required training includes:

- Biological Safety – General (SciShield)*

- Chemical Hygiene Plan Laboratory Safety - General Awareness (SciShield)**
- Hazardous Waste (EH&S Training site)**
- Bloodborne Pathogens for Researchers (SciShield)***
- DOT/IATA (Shipping Dangerous Goods) (EH&S Training site) (for personnel who will handle, package, and ship patient samples, investigational products, or other hazardous materials)

*There is no UK Healthcare substitution for Biological Safety Training.

**Personnel who have already completed UK Healthcare's training WBT "Workplace Safety", may provide a copy of this certificate in substitution for both the Chemical Hygiene Plan Laboratory Safety and Hazardous Waste training modules.

***Personnel who have already completed UK Healthcare's training WBT "Bodily Fluid Exposure Bloodborne Pathogens" may provide a copy of this certificate in substitution for the Blood Borne Pathogens for Researchers module.

Please note that all required training must be completed before final IBC approval is issued.

Clinical Biosafety Inspection

An inspection, by the Office of Biological Safety, of all locations listed on the registration is required before final IBC approval may be issued. Attention will be paid to signage, available PPE, disinfection, disposal, facility conditions, and patient isolation from non-listed personnel or unenrolled participants. For clinics actively involved in patient care, an annual Clinical Biosafety Inspection can be scheduled and performed, to minimize disruption to operations and ongoing patient care throughout the year. A member of the Biosafety Team will coordinate these annual inspections with the heads of each clinical unit associated with an IBC registration.

Study Locations

All locations where the investigational product is stored, prepared, administered, or otherwise handled should be listed in the "Locations" section of the registration form. This would include the pharmacy location(s), patient treatment areas or clinics, and the UK Healthcare Cell Therapy Lab, as applicable. In addition to these areas, any specimen/tissue/biobank locations and academic research laboratories involved with the study should be listed. If patient samples will be processed in a CLIA-certified and inspected clinical laboratory within UK Healthcare, that location does not need to be included in this section, but please make a statement to this effect in the "Scientific Summary" section of the IBC registration.

If the clinical study being registered will be administered at a remote University site, please consult with the Biological Safety Officer.

UK-Site Specific Procedures & Scientific Summary

The "Scientific Summary" free-text field of the IBC registration requires special attention. This section of the registration should provide IBC reviewers with all the details necessary to make an informed risk assessment. This should include pertinent study background information (including non-clinical and clinical study data), study plan, risks, mitigations, and site-specific details. Please do not simply paste the entire study document(s). While the information in the study documents is useful, some of it does not pertain to the biosafety considerations of the clinical study. The information provided should be specific to the biohazardous risk(s) of the study, the materials and procedures involved, and the mitigation steps employed to minimize risk.

Below is an outline of the sections and information of particular interest within the "Scientific Summary":

- Rationale/Summary/Mechanism of Action
- Abbreviations List
    - List and define all abbreviations in use, including common abbreviations.

- Study Design
    - Often found in the Study Protocol
- Non-Clinical Studies Summary
    - Often in the Investigator's Brochure
- Clinical Studies Summary
    - Often in the Investigator's Brochure
- Manufacturing and Safety
    - How is the investigational product manufactured?
    - What standards and/or guidelines are followed during manufacturing?
    - What procedures & biological materials are used?
    - What quality control assays are performed before product release?
    - What are the anticipated risks posed by the investigational product?
- Sample Collection, Handling, and Shipping
    - What samples will be collected?
    - Where are samples collected?
    - Where will they be handled/processed?
    - How are samples transported between campus locations?
    - Will they be shipped offsite? If so, where?
- UK Site-Specific Procedures
    - Cradle-to-grave approach
    - Where will investigational product be received, stored, prepared, dosed, transported, administered, and disposed of?
    - How many patients will be enrolled at UK?
    - How are potentially contaminated surfaces decontaminated?

The following documents should also be provided in the registration:

- Investigator's Brochure (sponsor provided)
- Study Protocol (sponsor provided)
- Pharmacy Manual (sponsor provided)
- Any sponsor provided training materials for clinical staff
- Site-specific SOPs and related documents
- Patient Consent Form
- PI's Curriculum Vitae (CV)
- Any additional information reference information

Submission of Registration

The PI is the **only** person allowed to perform the electronic signature and acknowledgment step to submit the IBC registration for review, via the "Sign & Submit" workflow within tick@lab by a-tune. These steps are detailed in all of the How-To Library documents and also outlined here.

Clinical IBC Registration Amendment, Renewal, and Closure

Clinical IBC Amendments

Changes to the clinical study must be reflected in an amendment to the clinical IBC registration. Changes that necessitate an IBC amendment include, but are not limited to:

- Addition/changes of personnel
- Addition/changes in locations
- Changes in the investigational product
- Changes in dose amount, or frequency of administration of the investigational product
- Changes in investigation product handling, or administration procedures at the UK study site
- Changes in the population enrolled in the study (ex: study drug will now be given to children or sufferers of a different disease)
- Updated product literature

Instructions for the creation and submission of an IBC amendment are available in the How-To reference library, here. It is highly encouraged that you contact the Office of Biological Safety to assist you with any clinical IBC registration amendments.

Renewal

IBC registrations are approved for a period of three (3) years. The clinical study's PI and co-investigators will be contacted by the Office of Biological Safety well in advance of the IBC registration's expiration. Please consult with a member of the Biosafety Team if you have questions or are uncertain about renewing your registration.

Closure

If a clinical study has concluded, an amendment to close the IBC registration may be submitted before the registration's expiration date. Please consult with a member of the Biosafety Team if you wish to close your IBC registration.

9.3 Plant Biosafety

The goal of biosafety regarding plant-related research is CONTAINMENT of transgenic and/or pathogenic materials. Most often, transgenic plants and plant pathogens do not pose biohazardous risk(s) to the personnel working with these materials, but there are exceptions! Containment is crucial in plant research in laboratories, greenhouses, and growth chambers. Field trials with transgenic plants require compliance with USDA regulations. Please consult USDA guidelines for containment requirements. This page provides information for transgenic and plant pathogen containment. Individual research projects will require different containment procedures based upon the experiments performed.

Goals of Plant Biosafety & Containment:

- Prevent interbreeding of transgenic plants with native species
- Transgenic plant waste must be decontaminated or inactivated prior to disposal
- Contain species that could detrimentally impact local and agriculturally important species
- Control insect vectors
- Contain seeds and pollen
- Prevent spread of plant pathogens

All researchers working with transgenic plants or plant pathogens must register with the UK IBC, determine the appropriate biosafety level for their work, and have standard operating procedures in place for:

- Safe storage, transport, and handling of transgenic seeds, plants, or plant materials, and plant pathogens and infected plant material(s).
- Labeling and segregation of transgenic and non-transgenic plant materials
- Preventing release of transgenic seed to the environment
- Preventing dissemination of genetic material to the environment
- Preventing release of plant pathogens

Handling Transgenic Seeds & Plant Materials

Transgenic seed should be stored in a locked cabinet located in or near the greenhouse or growth chamber. When stored or handled outside of a confined space, such as on a lab bench or potting bench, seed should be in a spill-proof container. White paper can be utilized on lab benches in conjunction with a tray to allow for easy identification and containment of stray seeds.

Labeling and Segregation of Transgenic and Non-Transgenic Plant Materials

All transgenic seeds and plants should be clearly identified and labeled to distinguish them from other stored seeds, plants, or materials. If transgenic and non-transgenic plants must be grown in the same location, such as an open lab or mixed-use greenhouse, all work must be completed at the biosafety level approved for the transgenic plant work.

Preventing Transgenic Seed Release

Seed is easily tracked out of facilities on shoes. This inadvertent dissemination can be easily prevented using shoe covers and/or sticky mats. Seed is also easily carried out of facilities on clothing, and this can be prevented with the use of disposable lab gowns that are dedicated for use in the plant growth chamber or greenhouse. Good housekeeping practices can help prevent release of transgenic seed by keeping loose seeds off the floor. Daily use of disposable cloth covered sweeper can be an easy way to remove loose seed from floors.

Preventing Dissemination of Genetic Material

Growing plants need to be contained to prevent the dissemination of genetic material. This can be achieved by covering or removing flower and seed heads to prevent seed dispersal, harvesting plant material prior to sexual maturity, or utilizing male sterile lines. Various commercial containment systems are available, or inexpensive systems can be constructed with disposable plastic sheeting. These systems contain seeds, soil, and plant parts resulting in less housekeeping and less contamination between shelves. These systems also provide better humidity control resulting in less watering of plants.

Devitalization of Materials

Experimental materials must be rendered biologically inactive (devitalized) prior to leaving the laboratory or greenhouse and final disposal. Devitalization methods may include:

- Heat via steam, autoclave, hot water, incineration
- Chemical treatment
- Freezing
- Composting
- Desiccation

Suggested Criteria for Assigning Biosafety Levels

CRITERIA	TRANSGENIC PLANTS	TRANSGENIC MICROBES		TRANSGENIC ARTHROPODS AND THEIR MICROBES
		Exotic	Non-Exotic	
Not a noxious weed or cannot outcross with one	BL1-P			
Not easily disseminated			BL1-P	
No detriment to environment		BL2-P or BL1-P +	BL1-P	BL2-P or BL1-P +
Noxious weed or can interbreed with weeds	BL2-P or BL1-P +			
Contains complete genome of non-EIA	BL2-P or BL1-P +			
Contains genome of EIA	BL3-P or BL2-P +			
Treated with an EIA	BL3-P or BL2-P +			
Detriment to environment			BL2-P or BL1-P+	BL3-P or BL2-P +
EIA with detriment to environment	BL3-P or BL2-P +			
May reconstitute genome of infectious agent *in planta*	BL3-P or BL2-P +			
Contains vertebrate toxin	BL3-P	BL3-P	BL3-P	
PMP & PMI	BL3-P			
Select Agent plant pathogens	BL3-P	BL3-P	BL3-P	BL3-P+ or BL4-P

*EIA – Exotic Infectious Agent

Source: Practical Guide to Containment: Plant Biosafety in Research Greenhouses

9.4 Viral Vectors

What is a Viral Vector?

Viral vectors work like a "nano-syringe" to deliver nucleic acid to a target. They are often more efficient than other transfection methods, are useful for whole organism studies, have a relatively low toxicity, and are a likely route for human gene transfer.

All viral vectors require a host for replication. The production of a viral vector is typically separated from the ability of the viral vector to infect cells. While viral vectors are not typically considered infectious agents, they do maintain their ability to "infect" cells. Viral vectors just don't replicate (although there are some replicating viral vectors in use) under experimental conditions. An HIV-based lentiviral vector no longer possesses the ability to infect an individual with HIV, but it does maintain the ability to enter a cell and express genetic information. This is why viral vectors are useful, but also require caution.

If a viral vector can transduce a human cell line on a plate, it can also transduce YOUR cells if accidentally exposed.

Safety Considerations for All Viral Vectors

- What potential does your method of viral vector production have to generate replication-competent virus?
    - The "Generation" of a viral vector refers to the number of recombination events required to form a replication-competent virus. For example, if you're producing a lentivirus that is split up between 4 plasmids (gag/pol, VSV-g, rev, transgene), a total of 3 recombination events must take place in order to create a replication-competent virus, therefore you are using a 3rd generation lentiviral vector.

- What specific hazard(s) does the transgene pose?
    - If your transgene is GFP, the hazard is relatively low. If your transgene is hazardous (i.e. an oncogene, toxin, immunogen, allergen, gene drive, etc.), the hazard is much greater. Consider what your transgene would do to YOU should you be exposed. Does your viral vector overexpress an oncogene? Knock down a tumor suppressor? What does your transgene DO?

- What is the tropism of the virus?
    - Ecotropic viral vectors have a narrow host range and can only infect one or a small group of species or cell lines. Amphotropic viral vectors have a wide host range and are capable of infecting numerous species or cell lines.

Common Viral Vector Systems

- <u>Adeno-Associated Virus (AAV)</u>
    - Adeno-Associated Virus (AAV) is coined as such because it is most often found in cells that are simultaneously infected with adenovirus. AAV are parvoviridae, icosahedral, single-stranded DNA viruses with a protein capsid. 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 infection cycle. AAV has a broad host range and produces little to no immune response. At only 22nm in diameter, it is one of the smallest viruses known. There are at least 11 natural serotypes of AAV. AAV2 is the basis for most recombinant AAV vectors, but it is usually pseudotyped.

    - POTENTIAL HEALTH HAZARDS: There are no known health hazards associated with AAV. It is not known to cause direct disease in humans; however, AAV may be associated with insertional mutagenesis and cancer, thereby making AAV possibly not as safe as previously thought. The low immunogenicity of AAV leads to long-term gene expression, the effects of which are not entirely understood.

    - LABORATORY HAZARDS: Routes of exposure include inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion. There is no specific treatment for infection with AAV.

    - BIOSAFETY CONTAINMENT*: Construction of AAV with helper virus (Adenovirus or Herpesvirus) must be performed at BSL2 within a BSC. Once constructed, AAV may be manipulated at BSL1. Eye protection, disposable gloves, laboratory coat required.

- ANIMAL BIOSAFETY CONTAINMENT*: Animal housing may be maintained at ABSL1. ABSL2 containment is required if helper virus is present.

- DISINFECTION: AAV is susceptible to: 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 5% Phenol, or Autoclave for 30 minutes at 121°C under 15lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach is recommended. Alcohol is NOT an effective disinfectant against AAV.

- REFERENCES:
Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. BioDrugs. 2017;31(4):317-334. doi:10.1007/s40259-017-0234-5

- *Note - The UK IBC may determine a higher biosafety containment level is appropriate due to the nature of the transgene insert.

- Adenovirus
  - There are more than 49 immunologically distinct types of adenovirus that can cause infection. Recombinant adenoviruses used for biomedical research are typically based on Adenovirus 5. These are linear, non-enveloped, icosahedral, double-stranded DNA viruses of approximately 36kb with a lytic infection cycle. Virus packaged via transfection of HEK293 cells are capable of transfecting human cells. Deletion of E1 renders the virus replication incompetent. Deletion of E3 allows for larger inserts. Because recovery of E1 is the only recombination event required to create a replication competent virus, all adenoviral vectors are 1st generation.

  - POTENTIAL HEALTH HAZARDS: Adenovirus is a pathogen of respiratory, gastrointestinal mucosa, and mucous membranes. Symptoms of respiratory illness resulting from adenovirus infection can range from asymptomatic disease, common cold, pneumonia, croup, and bronchitis. Additional clinical symptoms include conjunctivitis (pink eye), cystitis, gastroenteritis (stomach flu), tonsillitis, rash-associated illness, and rare cases of severe disease (especially in immune compromised individuals). Adenoviral vectors DO NOT have to be replication competent to cause corneal and conjunctival damage.

  - LABORATORY HAZARDS: Routes of exposure include inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion. Adenovirus is unusually stable in the environment. Adenovirus can still be infective after having been extracted with ether and/or chloroform. Adenovirus can persist for 7 days to 3 months on dry inanimate surfaces. Potential recovery of E1 from HEK293 cells can produce replication competent virus. There is no specific treatment for adenovirus infection.

  - BIOSAFETY CONTAINMENT: Handling at BSL2+ containment is required with NO open bench work. A Biological Safety Cabinet (BSC) is required. Eye protection, disposable gloves, and laboratory coat is required. When centrifuging adenovirus, rotors/buckets must be loaded/unloaded in the BSC and wiped down with appropriate disinfectant prior to removal from BSC. Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped. Use of needles, syringes, and other sharp objects must be limited.

  - ANIMAL BIOSAFETY CONTAINMENT: Adenoviral vector must be administered under BSL2/ABSL2 containment. Adenoviral vector stocks must be tested for Replication Competent Virus (RCV) prior to use directly in animals or in transduced cells administered to animals. Animals may shed/excrete adenovirus for some time post-administration. Animals must be housed at ABSL2 containment for a minimum of 72 hours during this period, after which animals may be moved to ABSL1 housing.

  - DISINFECTION: Adenovirus is susceptible to: 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 5% Phenol, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach is recommended. Alcohol is NOT an effective disinfectant against adenovirus.

  - REFERENCES:
Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Curr Gene Ther. 2013;13(6):421-433. doi:10.2174/15665232133666131125095046

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/adenovirus-types1-2-3-4-5-7-pathogen-safety-data-sheet.html

- Baculovirus
    - Baculoviruses are lytic DNA viruses that are primarily pathogenic for insects. The nucleocapsids of Baculoviruses are rod-shaped and enveloped, with circular genomes of double-stranded DNA, ranging in size from 80-180kbp. Baculoviruses produce two distinct types of virions: occlusion-derived virions (ODV), embedded in large protein crystals called occlusion bodies, and budded virions (BV). ODV are responsible for horizontal transmission between insects, whereas BV help spread infection from cell to cell. There have been more than 500 baculovirus isolates identified based on hosts of origin. Apart from their utility as gene expression vectors, they are also useful as biological pesticides. The two most common isolates used for gene expression are *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) and *Bombyx mori* (silkworm) nuclear polyhedrosis virus (BmNPV).

    - POTENTIAL HEALTH HAZARDS: Non-genetically modified, wild-type baculoviruses are typically not capable of replicating in vertebrate cells, and therefore do not pose much risk to laboratory personnel. Baculoviruses for gene expression which utilize polyhedrin or p10 promoters will only transfect insect cells. Baculoviruses that have been engineered with mammalian specific promoters do achieve expression of foreign genes in mammalian cell lines and primary cell cultures.

    - LABORATORY HAZARDS: The budded virions (BV) are not infectious to insect hosts, minimizing potential spread to the environment. Baculovirus is very sensitive to human complement. Should exposure occur, rapid inactivation of the virus is expected. Pseudotyping with VSV-G may confer resistance to complement inactivation.

    - BIOSAFETY CONTAINMENT: Baculoviruses with insect specific promoters (i.e. polyhedrin or p10) may be handled at BSL1. Baculoviruses with mammalian specific promoters must be handled at BSL2. Eye protection, disposable gloves, and a laboratory coat are required.

    - ANIMAL BIOSAFETY CONTAINMENT: Baculoviruses with mammalian specific promoters must be administered under BSL2 containment. Animals may be housed at ABSL1 containment.

    - DISINFECTION: Baculovirus is susceptible to: 70% Ethanol, 0.5% Sodium hypochlorite, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach is recommended.

      REFERENCES:
      Ono C, Okamoto T, Abe T, Matsuura Y. Baculovirus as a Tool for Gene Delivery and Gene Therapy. Viruses. 2018;10(9):510. Published 2018 Sep 19. doi:10.3390/v10090510

- Epstein-Barr Virus
    - Epstein-Barr virus (EBV) is a ubiquitous B-lymphotrophic herpesvirus. EBV causes the common childhood disease mononucleosis. It is an 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 (i.e. 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). 80-90% of adults worldwide are infected with EBV. Most wild-type EBV infections are asymptomatic and acquired during childhood, with symptoms indistinguishable from other childhood acute viral syndromes.

    - POTENTIAL HEALTH HAZARDS: Infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy; lasting one to several weeks; rarely fatal. Burkitt's lymphoma - monoclonal tumors of B cells; typically involving children; jaw involvement also common; hyper-demic in highly malarial areas. Nasopharyngeal carcinoma - malignant tumor of epithelial cells of the nasopharynx; usually involving adults between 20 and 40 years of age. In immunosuppressed individuals, oral hairy leukoplasia, interstitial lymphocytic pneumonia, B-cell or T-cell lymphomas, and mesenchymal lymphomas may occur.

    - LABORATORY HAZARDS: Inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion. Cell lines are often immortalized by transformation with EBV.

- BIOSAFETY CONTAINMENT: Handling at BSL2 containment required with NO open bench work. A Biological Safety Cabinet (BSC) is required. Eye protection, disposable gloves, and a laboratory coat are required. When centrifuging EBV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC. Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

- ANIMAL BIOSAFETY CONTAINMENT: EBV vectors must be administered under BSL2/ABSL2 containment. Animals must be housed under ABSL2 containment.

- DISINFECTION: Epstein-Barr Virus is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

- REFERENCES:
  https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/epstein-barr-virus.html

  Kazuyuki Kiyosue, Yoshihiro Miwa, Epstein-Barr virus-derived vector suitable for long-term expression in neurons, Heliyon, Volume 6, Issue 3, 2020, e03504, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2020.e03504.

- Herpesvirus
  - Herpes Simple Virus (Types I and II) are icosahedral, lipid enveloped, double-stranded linear DNA viruses approximately 110-200nm in diameter. HSV types I and II can be differentiated immunologically. HSV-I is herpes gingivostomatitis; whereas HSV-II is herpes genitalis, or genital herpes. HSV-derived vectors are unique in that the vectors have a wide host range and cell tropism in dividing and non-dividing cells, and are able to infect almost every cell type in most vertebrates. HSV has a dual life cycle: a lytic growth cycle in epithelial cells, and latent infection of neuronal cells. This latency in neuronal cells leads to persistent, long-term expression.

  - POTENTIAL HEALTH HAZARDS: Oral herpes - primary infection is typically mild and occurs early in childhood; reactivation of latent infection results in fever blisters or cold sores, usually on the face and lips, which crust and heal within a few days; possible CNS involvement (meningoencephalitis), 70% mortality rate if left untreated; causes approximately 2% of acute pharyngotonsilitis. Genital herpes - 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. Latency and reactivation from latency are not well understood.

  - LABORATORY HAZARDS: Inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion. Currently, the only available treatment is anti-viral drug therapy for symptoms.

  - BIOSAFETY CONTAINMENT: Handling at BSL2 containment required with NO open bench work. A Biological Safety Cabinet (BSC) is required. Eye protection, disposable gloves, and a laboratory coat are required. When centrifuging HSV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC. Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

  - ANIMAL BIOSAFETY CONTAINMENT: HSV vectors must be administered under BSL2/ABSL2 containment. Animals must be housed under ABSL2 containment.

  - DISINFECTION: Herpesvirus is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

    REFERENCES:
    https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/herpes-simplex-virus.html

Burton EA, Fink DJ, Glorioso JC. Gene delivery using herpes simplex virus vectors. DNA Cell Biol. 2002;21(12):915-936. doi:10.1089/104454902762053864

- Poxviruses
    - The Poxviridae family is divided into two subfamilies: Chordopoxviridae, with a vertebrate host range, and Entomopoxviridae, with an insect host range. Chordopoxviridae is further broken down into eight genera: Avipoxvirus, Capripoxvirus, Leporipoxvirus, Molluscipoxvirus, Orthopoxvirus, Parapoxvirus, Suipoxvirus, and Yatapoxvirus.

      Poxviruses are enveloped, with a double-stranded DNA genome with hairpin loops at each end and a lytic infection cycle. Poxviruses do not integrate into the host's genome because they remain in the cytoplasm and utilize virally encoded polymerases to carry out replication and transcription. Members of the Orthopoxvirus genus have both narrow and broad host range. Variola, the agent of smallpox, only infects humans. The absence of other host species has made the eradication of smallpox possible. On the other hand, Vaccinia virus has a very broad host range. Vaccinia is used as a live vaccine for protection against smallpox. Vaccinia's large genome (approximately 190kb) allows for the stable insertion of DNA as large as 25kb.

    - POTENTIAL HEALTH HAZARDS: Unlike many viral vectors utilized, vaccinia is a replication-competent vector. Vaccinia virus presents varying levels of health risk to laboratory personnel, depending on the strain utilized. Highly attenuated strains are typically unable to replicate or replicate poorly in human cells. Non-highly attenuated strains can replicate in human cells and pose a health risk. The classical symptom of poxvirus infection is a vesicular or pustular lesion on the skin at the inoculation site. Vaccinia can cause severe disease in people with active skin disorders (i.e. eczema, psoriasis), pregnant women, and immune compromised individuals.

    - LABORATORY HAZARDS: Ingestion, parenteral inoculation, droplet or aerosol exposure to mucous membranes or exposure to broken skin. Vaccinia and other poxviruses are stable at ambient temperatures when dried and can remain infectious for long periods of time.

    - BIOSAFETY CONTAINMENT:

        - Handling at BSL2 containment required with NO open bench work and a Biological Safety Cabinet (BSC) is required for the following strains: MVA (Modified Vaccinia Ankara), WR (Western Reserve), and NYCBOH (used in vaccinia vaccine), Copenhagen, Temple of Heaven, Lister, Cowpox, and Monkeypox. Eye protection, disposable gloves, and a laboratory coat are required.

        - The following strains may be handled at BSL1 containment: NYVAC (derived from Copenhagen), TROVAC (Fowlpox virus), and ALVAC (Canarypoxvirus). Eye protection, disposable gloves, and a laboratory coat are required.

    - ANIMAL BIOSAFETY CONTAINMENT: Animals must be manipulated and housed under BSL2/ABSL2 containment.

    - DISINFECTION: Poxviruses are susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

    - REFERENCES:
      https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/vaccinia-virus.html

      GuoZS,LuB,GuoZ, et alVaccinia virus-mediated cancer immunotherapy: cancer vaccines and oncolyticsJournal for ImmunoTherapy of Cancer2019;7:6.doi:10.1186/s40425-018-0495-7

- Retroviruses/Murine Leukemia Virus

- Murine Leukemia Virus (MLV) is an enveloped, icosahedral, single-stranded virus with a linear RNA genome, approximately 100nm in diameter. MLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

  The host range of MLV is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles that infect only rodent cells. Amphotropic env gene allows infection of both murine and non-murine cells, including human. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells.

- POTENTIAL HEALTH HAZARDS: Recent data suggests a pathogenic mechanism by which chronic productive retroviral infection allows insertional mutagenesis leading to cell transformation and tumor formation. The nature of the transgene or additional introduced genetic element(s) may pose additional risk. The provirus integrates randomly into the genome, which can lead to inactivation of genes for protein expression. The 5' and 3' LTRs have promoter functions that can deregulate the expression of genes.

- LABORATORY HAZARDS: In mice, virus is transmitted via blood from infected mother to offspring; may also occur via germline infection. In vivo infection in humans appears to require direct parenteral injection with amphotropic or pseudotyped MLV. Exposures associated with a hazardous transgene (i.e. an oncogene, toxin, etc.) should consider the use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

- BIOSAFETY CONTAINMENT: Ecotropic MLV demonstrated to be replication incompetent may be handled at BSL1 containment. Eye protection, disposable gloves, and a laboratory coat are required. Amphotropic or pseudotyped MLV requires BSL2 containment with NO open bench work and a Biological Safety Cabinet (BSC) is required. Eye protection, disposable gloves, and a laboratory coat are required. When centrifuging MLV, rotors/buckets must be loaded/unloaded within BSC and wiped down with appropriate disinfectant prior to being removed from the BSC. Centrifuge tubes must be sealed (i.e. plates sealed with parafilm) or capped.

- ANIMAL BIOSAFETY CONTAINMENT: MLV vector must be administered under BSL2/ABSL2 containment. Animals administered ecotropic MLV demonstrated to be replication incompetent by acceptable RCV testing, may be housed under ABSL1 containment. Animals administered amphotropic/pseudotyped MLV must be housed under ABSL2 conditions for 72 hours post administration, after which animals may be moved to ABSL1 housing.

- DISINFECTION: Retroviruses/Murine Leukemia Virus is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

- REFERENCES:
  Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, Methods, Volume 33, Issue 2, 2004, Pages 164-172, ISSN 1046-2023, https://doi.org/10.1016/j.ymeth.2003.11.005

- Lentivirus
  - The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. There are five (5) serotypes recognized, based upon the mammalian hosts with which they are associated: Bovine, Equine, Feline, Ovine/Caprine, and Primate.

    Most lentiviral vectors in use today are HIV-derived vectors. The cis- and trans- acting factors of the lentiviruses are often on separate plasmid vectors, with packaging being provided in trans. The vector constructs contain the viral cis elements, packaging sequences, the Rev response element (RRE), and a transgene. Lentiviral vectors can transfect dividing and non-dividing cells. Replacing the HIV envelope glycoprotein with VSV-G allows a broad host-range for the vectors, allows the viral particles to be concentrated via centrifugation, and alters the mode of transmission.

  - POTENTIAL HEALTH HAZARDS: Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, and sharing unclean needles. Lentiviruses may persist lifelong, being both a function of their ability to integrate into the host chromosome and ability to evade host immunity.

Lentiviruses replicate, mutate, and undergo selection by host immune responses. The clinical manifestations of infection include non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue. The use of lentiviruses also presents the risk of insertional mutagenesis, potentially leading to cancer. The nature of the transgene may pose additional risk.

- LABORATORY HAZARDS: Routes of exposure include direct contact with skin and mucous membranes, parenteral inoculation, or ingestion. Exposures associated with a hazardous transgene (i.e. an oncogene or toxin) should consider use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

- BIOSAFETY CONTAINMENT: Handling at BSL2+ containment required with NO open bench work. A Biological Safety Cabinet (BSC) is required. Eye protection, disposable gloves, and a laboratory coat are required. When centrifuging lentivirus, rotors/buckets must be loaded/unloaded in the BSC and wiped down with appropriate disinfectant prior to removal from the BSC. Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped. The use of needles, syringes, and other sharp objects must be limited.

- ANIMAL BIOSAFETY CONTAINMENT: Lentivirus must be administered under BSL2/ABSL2 containment. Animals must be housed under ABSL2 containment for 72 hours post administration, after which animals may be moved to ABSL1 containment. 1st or 2nd generation Lentiviral vectors stocks must be tested for RCV prior to use directly in animals or in transduced cells administered to animals.

- DISINFECTION: Lentiviruses are susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure. Use of freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

- REFERENCES:
Milone, M.C., O?Doherty, U. Clinical use of lentiviral vectors. Leukemia 32,1529?1541 (2018). https://doi.org/10.1038/s41375-018-0106-0

Replication Competent Virus (RCV) Testing

Most viral vectors used today are disabled such that replication-competent viruses are not readily formed by any biological process that might occur in normal hosts. The Office of Biological Safety encourages the use of such vectors in all relevant applications. In particularly sensitive applications, demonstrating that the viral stock used has no apparent contamination with replication-competent vectors is essential. **The issue is not whether replication-competent virus (RCV) is present, but how much replication-competent virus is present.** Of course, assays for replication competence will never be perfect or absolute, so the Institutional Biosafety Committee (IBC) asks that one use a current procedure of demonstrated sensitivity and specificity. Even more rigorous testing may be required in some instances, such as a vector bearing a pathogenic gene, in human gene transfer, or in any materials that could be released to the environment.

- When is RCV Testing Required?
  - Use of ANY viral vector (regardless of generation) in Human gene transfer/therapy where replication-defective viral vector or products containing replication-defective viral vector is administered to human study participants
  - Use of 1st or 2nd generation viral vectors in animals (in vivo)
  - Use of cells transduced with 1st or 2nd generation viral vectors in animals (in vivo)

- When is RCV Testing NOT Required?
  - Experiments conducted entirely in cell or tissue culture (in vitro)
  - Use of 3rd generation (or higher) viral vectors in animals (in vivo)
  - Use of cells transduced with 3rd generation (or higher) viral vectors in animals (in vivo)

- Considerations for an Appropriate RCV Test
  - The test is an experiment: Any experiment should have adequate controls. A positive control is necessary to demonstrate that you would have detected replication-competent virus if it were present.
  - The test must be quantitative: What is the level of detection for replication-competent virus? Typically, this requires running a dilution curve. This should also be reported with the results of your test.

- What is an appropriate cut-off for declaring it is safe to use this virus in animals?

- Setting an Appropriate Cut-Off
  - FDA requirements for human use of recombinant adenovirus is <1 replication competent virus per 10^13 viruses. This is a very strict requirement because quantity of virus used is high, containment is very difficult, and accidental release is potentially disastrous.
  - When working with small, easily sequestered animals (i.e. rats, mice), the cut-off can be considerably less.
    - One replication competent virus per 10^6 viruses is common, but is it appropriate?
      - How many virus particles are being introduced into animals?
      - Best estimate for number of replication competent virus in bolus?
      - How many animals are likely to receive replication competent virus?
      - What is an acceptable risk?

- Designing the Test
  - What does a recombinant virus need to regain replication competency?
    - Adenovirus needs: E1
    - Lentivirus needs: gag, pol, env
  - Where can recombinant virus pick up the sequences needed to regain replication competency?
    - Adenovirus has easy access to E1 in HEK293 cells
    - Retrovirus may pick up “assets” from endogenous retroviruses
  - How does replication competent virus present itself?

- Methodological Approaches
  - Plaque assays (for lytic viruses, i.e. adenovirus)
    - Must screen for more viruses than the cut-off limit (If 1 in 10^6 is the cut-off, must screen >10^6 viruses)
  - ELISA for production of viral protein essential for replication
    - p24 assay for HIV (lentivirus)
    - Sensitivity is poor
    - Attempt amplification in a competent host
  - Quantitative PCR for essential viral gene
    - Very sensitive
    - Problem with background

- Things to Remember!
  - Confirmation of absence of RCV must be documented by researcher PRIOR to use
    - Documentation of methodology and results must be made available to the Department of Research Safety staff on request
  - Procedure must be of demonstrated sensitivity and specificity
  - Must use a positive control

Virus	Method	Reference(s)
Adenovirus	Test for RCV by PCR for E1a	Dion LD, Fang J, Garver RI Jr. Supernatant rescue assay vs. polymerase chain reaction for detection of wild type adenovirus-contaminating recombinant adenovirus stocks. J Virol Methods. 1996;56(1):99-107. doi:10.1016/0166-0934(95)01973-1
Adeno-Associated Virus (w/ Adenovirus Helper)	Test for RCV by PCR for E1a	Hehir KM, Armentano D, Cardoza LM, et al. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. J Virol. 1996;70(12):8459-8467. doi:10.1128/JVI.70.12.8459-8467.1996
Retrovirus (amphotropic & ecotropic)	Test for RCV by amplification in permissive cell line followed by	Wilson CA, Ng TH, Miller AE. Evaluation of recommendations for replication-competent retrovirus testing associated with use of retroviral vectors. Hum Gene Ther. 1997;8(7):869-874. doi:10.1089/hum.1997.8.7-869

	screening by appropriate detection assay	Uchida E, Sato K, Iwata A, et al. An improved method for detection of replication-competent retrovirus in retrovirus vector products. Biologicals. 2004;32(3):139-146. doi:10.1016/j.biologicals.2004.08.002 U.S. Food and Drug Administration. Center for Biologics Evaluation and Research. Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. January 2020. Accessed February 28, 2022. https://www.fda.gov/media/113790/download
Lentivirus	Test for RCV by PCR for psi-gag and VSV-G sequences	Sastry L, Xu Y, Johnson T, et al. Certification assays for HIV-1-based vectors: frequent passage of gag sequences without evidence of replication-competent viruses. Mol Ther. 2003;8(5):830-839. doi:10.1016/j.ymthe.2003.08.003

9.5 Cell & Tissue Culture

The following requirements for tissue culture rooms must be met:

- Air shall flow from the hallway to the inner lab (negative to the hallway). All room air shall be exhausted through ducts to the outside of the building and not recirculated within the building. Room air which is exhausted to a common plenum is **NOT** acceptable.
- The number of BSCs, the amount of space within the BSC and amount of room space provided is required to accommodate all of the tissue culture and viral vector work. This is for the protection of the research materials and for the protection of the researchers and facility.
- The BSCs may be recirculating models (Class II, A2) or thimble ducted or hard ducted.
    - BSCs shall be installed such that:
        - Fluctuations of the room air supply and exhaust do not interfere with proper operations.
        - Manufacturers' guidelines are followed.
        - They can be certified follow the National Sanitary Foundation (NSF) criteria.
    - BSCs shall be certified on an annual basis by a vendor who meets the requirements of the Biological Safety Department, follows NSF criteria, and is on contract with UK.
        - Coordination of this certification is through the Biological Safety Department.
        - Payment for this service is the responsibility of the Principal Investigator or Department
- Rooms housing any BSC shall be configured to allow storage of supplies and equipment used with the biohazardous materials. Typical equipment in these rooms includes: incubators, centrifuges, microscopes, CO2 tanks, vacuum source, refrigerators.
- A hand washing sink with eyewash shall be present in the tissue culture room facility. The eyewash may be "drench hose" if approved by UK Occupational Health and Safety Department. An exemption from the eyewash requirement may be granted by the Biological Safety Officer if the risk assessment of the proposed research warrants it.
- There must be restricted entry to the outer laboratory, which is locked when no one is present.
- All surfaces must be easily cleaned and decontaminated. Room casework shall be easily cleanable, and finishes should be compatible with materials used for cleaning and disinfection. Chairs must be covered with a non-porous material. Rugs or carpets are not permitted.
- Vacuum lines shall be protected with High Efficiency Particulate Air (HEPA) filters or their equivalent. This applies to central vacuum systems and to individual vacuum pumps.
- Open flames **SHALL NOT** be used in BSCs. Therefore, gas lines **SHALL NOT** be connected to BSCs.
- A functioning and validated autoclave enrolled in the UK Autoclave Verification program shall be available within a reasonable distance of the facilities creating the biohazardous waste.
    - IBC approved procedures for transport shall be followed when unprocessed waste carried through public hallways and elevators.
- Tissue culture rooms which will contain research deemed by the IBC to be BSL2+ (enhanced) shall be in an inner lab, with two doors between the BSC and the hallway.
- Appropriate signage shall be displayed on the door of the main laboratory and of the tissue culture room.
- Users should maintain a clean laboratory gown/coat reserved solely for cell culture work that is not worn outside of the cell/tissue culture space.

10.0 Biohazardous Waste Decontamination & Disposal

10.1 Biohazardous Waste

Biohazardous waste may include human blood and bodily fluids, human tissues, pathological specimens, pathogenic or recombinant microbiological materials, infected or transgenic animal tissues and carcasses, infected or transgenic plant materials, and any disposable items contaminated with these materials.

This chapter will outline the types of biohazardous waste most often generated in UK research laboratories and detail acceptable decontamination and disposal methods for each. If you will be generating biohazardous waste not described here, please contact the UK Office of Biological Safety for guidance.

Please see the UK Research Safety Laboratory Waste Guidelines available online here for additional instructions and guidance, including hazardous chemical and radiological waste disposal.

10.1.1 Liquid Biohazardous Waste

Liquid biohazardous waste may consist of blood, blood products, and body fluids from human or animal research and culture media contaminated with pathogenic or recombinant microbiological materials which may be hazardous to humans, animals, plants, or the environment. Liquid biohazardous waste can come in many forms in the laboratory. These include, but are not limited to liquid bacterial cultures, non-adherent human tissue cultures, supernatants of tissue cultures infected with viral vectors, human blood samples and blood products (serum, plasma, and other blood components), and human tissue culture media that is aspirated into a vacuum line trap. To disinfect liquid biohazardous waste prior to disposal, add unexpired household bleach to achieve a final concentration of 10% bleach, and allow a contact time of at least 20 minutes. Once the appropriate concentration and contact time have been met, test the pH of the liquid using a pH meter or pH test strip (available upon request from Research Safety) to ensure it is within acceptable limits for drain disposal (5.5-11.5 SU). Once the mixture has met all requirements (10% bleach, 20 minutes, pH 5.5-11.5), it may be poured carefully and slowly down the laboratory sink drain with copious amounts of water. Liquid biohazardous waste must not be left to sit overnight and should be promptly decontaminated as described and disposed of at the end of each workday or when the container is 2/3 full, whichever comes first. Please note that once bleach has been added to liquid biohazardous waste, it cannot be autoclaved. If the use of bleach is not a suitable option, please contact the Office of Biological Safety for alternative options and further guidance.

The transport of liquid biohazardous waste should be avoided when possible as this introduces the heightened risk of leakage and spills. Transporting liquid biohazardous waste to an autoclave for processing, while effective for sterilization, is not advised and should only be utilized when disinfection within the lab is not possible. If transporting liquid biohazardous waste for autoclaving is unavoidable, the primary container must be securely closed and placed in an appropriate secondary container that is closed, leak-proof, and shatter-proof. The primary container must be placed in a leak-proof autoclavable container with the lid/cap loosened for autoclaving. Ensure contents are totally cooled prior to removal and dispose of autoclaved liquid down the sink with copious amounts of water.

10.1.2 Solid Biohazardous Waste (Non-Sharps)

Solid biohazardous waste consists of any non-sharp items that may be contaminated with human or animal research material (ex. bodily fluids, tissue, etc.), pathogenic organisms, or recombinant and/or synthetic nucleic acid materials. Solid biohazardous waste may consist of used gloves and other disposable PPE (Personal Protective Equipment), culture plates, specimen vials, lab bench paper, etc., and must be collected in ***orange*** or ***clear/opaque*** autoclave bags clearly marked with a biohazard symbol. Red bags are **NEVER** to be used for collection and autoclaving of solid biohazardous waste. Bags containing solid biohazardous waste must be held in a non-porous, leak-proof container, preferably with a foot operated lid.

Once a bag of solid biohazardous waste has become 2/3 full, it must be loosely closed and placed in a leak-proof autoclavable secondary container for processing. Bags should not be overfilled. This waste is then processed in a designated autoclave verified and enrolled in the Autoclave Verification Program. Biohazardous waste must always be accompanied by an individual familiar with its contents once it is transported from its collection point. Unprocessed biohazardous waste should never be left unattended waiting to be autoclaved. If an autoclave is not available, waste must be brought back to the lab space that generated it and secured until the autoclave is available. Biohazardous waste should also not be stockpiled in lab spaces and must be processed as soon as reasonably possible once collected. Once

bags of solid biohazardous waste have been successfully autoclaved, they must be placed into regular opaque trash bags before disposal with normal trash.

Most solid biohazardous waste can be autoclaved using gravity steam sterilization cycles with a target temperature of 121°C and sterilization phase of (greater than or equal to) ≥ 40 minutes. However, the length of time required for sterilization can be higher depending on the hazardous organism. Please reference Chapter 12: Autoclaves, for more details. The Office of Biological Safety can assist you in determining an appropriate sterilization time based on your lab's work and agent(s).

10.1.3 Sharps Waste

Sharps Waste can be divided into four categories: biohazardous sharps waste, biohazardous plastic sharps waste, non-biohazardous sharps waste, and non-biohazardous plastic sharps waste.

- Biohazardous sharps waste consists of objects that have the high potential to produce punctures or lacerations and have been used in conjunction with biohazards. This includes needles/syringes, lancets, unfixed glass slides, scalpel blades, glass vials, glass Pasteur pipets, razor blades, and disposable surgical instruments. Biohazardous sharps waste must be collected in a hard plastic medical sharps container that is marked with the universal biohazardous symbol or red in color. Once the container is 2/3 full, close and seal it and decontaminate its exterior with a suitable disinfectant. If in the Medical Center (Medical Sciences, HSRB, Roach, Combs, Whitney-Hendrickson, and Gill Heart buildings), housekeeping will pick up these containers for final disposal. If located outside of the Medical Center, please submit an E-trax ticket for pickup. Do NOT autoclave these types of sharps containers.

- Biohazardous plastic sharps waste typically consists of plastic serological pipettes, micropipette tips, plastic Pasteur pipets, plastic inoculating loops, plastic plate spreaders, and any other pointy plastic items that have been utilized in conjunction with biohazardous materials that may be likely to puncture an autoclave bag. While these items may not be considered as sharp as needles or glass, they do still pose a risk of puncture or laceration. Loose biohazardous plastic sharps, such as pipettes or tips, are prohibited in autoclave bags.

    - Small biohazardous plastic sharps waste (micropipette tips, etc.) may be collected in small benchtop biohazardous waste bags. Small benchtop biohazardous waste bags must be loosely closed, placed into a larger biohazardous waste bag with other solid biohazardous waste, and autoclaved before final disposal. Alternatively, small biohazardous plastic sharps waste may be collected as described directly below.

    - Large biohazardous plastic sharps waste (ex. plastic serological pipettes and plastic Pasteur pipets) must be collected in a plastic lined box (ex. a carboard box lined with a biohazardous waste bag on the interior). Once full, close the cardboard box loosely with tape, and put the closed box into a biohazardous waste bag. Boxes should not be overfilled such that they cannot be safely closed. The bagged box of biohazardous plastic sharps may then be autoclaved as solid biohazardous waste.

- Used needles, syringes, lancets, razor blades, and other sharp objects which have NOT been used in conjunction with biohazardous or hazardous chemicals shall be placed in a hard plastic or metal container with a screw-on lid. When full, reinforce the lid with heavy-duty tape, label the container "Not recyclable trash," and place with regular trash for collection by housekeeping. If unsure as to whether these items have been used in conjunction with biohazardous materials, it is advisable to dispose of them in a medical sharps container as outlined above in Biohazardous Sharps Waste.

- Non-biohazardous plastic sharps waste often consists of plastic micropipette tips, plastic transfer pipettes, plastic Pasteur pipets, and plastic serological pipettes not used with biohazardous materials. Pipettes and pipette tips which have not been utilized in conjunction with biohazardous materials or hazardous chemicals shall be disposed of in a hard plastic or metal container with a screw-on lid. Once full, the container's lid shall be taped securely shut, labeled with "Not recyclable trash," and placed with the regular trash for removal by housekeeping. Larger plastic sharps (ex. Serological pipets and plastic Pasteur pipets) not used with biohazardous materials or hazardous chemicals shall be disposed of in a plastic lined cardboard box. Once the box is filled to a capacity where it can be safely closed (not filled above the top flap seam), its opening must be secured with tape and "Trash" written on the outside. It can then be placed with regular trash for disposal. Containers and boxes should not be overfilled such that they cannot be safely closed.

10.1.4 Small Animal

Small Animal carcasses (intact or partial) must be disposed of according to how the animal/carcass was treated and where it was originally obtained. If the animal/carcass was acquired from a DLAR (Division of Laboratory Animal Resources) facility, and the animal/carcass was not exposed to chemical or biohazardous agents, it must be collected in a leak proof opaque bag and returned to the appropriate location in DLAR. If the animal/carcass was acquired from a DLAR facility, and the animal/carcass was exposed to biohazardous materials, it must be collected in a red bag and placed in designated location in DLAR. Do not place carcasses in with other biohazardous waste to be autoclaved. For small animal carcasses not originally obtained in DLAR, contact the Office of Biological Safety to determine a suitable disposal method. Infected animal carcasses or tissue that are also contaminated with hazardous chemicals or radioactive materials is a type of mixed waste, please reference the “11.1.8 Mixed Waste” section below.

10.1.5 Large Animal

Large animals that are used in conjunction with biohazardous materials pose an added challenge for final disposal and many factors must be considered. Please contact the Office of Biological Safety for guidance during planning of projects that may generate large animal carcasses and associated bedding and/or manure contaminated with biohazardous materials.

10.1.6 Transgenic Plant Material

Plants, plant parts, and other plant materials from transgenic or infected plants (ex. Plants infected with a pathogen or pest) must be devitalized/sterilized before leaving the greenhouse or laboratory housing them. Devitalization of plant material may be accomplished by steaming. Sterilization of plant material may be accomplished by autoclaving. The method utilized is dependent on the specific plant material, associated hazards, and regulatory requirements. Plant materials requiring steam devitalization, autoclaving, or other special disposal procedures can include but are not limited to transgenic/infected plant clippings, seeds, plant pests, stems, soil containing roots, flowers, plant reproductive structures, and associated plant microorganism contaminated items. For materials requiring USDA-APHIS permitting, the approved permit will often specify the required treatment. The Office of Biological Safety can assist you in determining an appropriate devitalization/sterilization procedure for your project’s specific plant material disposal requirements in accordance with all required permitting.

10.1.7 Regulated Medical Waste

Medical (Pathological) waste consists of large volumes (greater than 500 ml) of human blood, recognizable human organs, large amounts of unfixed human tissues or blood-soaked materials. This includes blood-soaked bandages, discarded surgical gloves after surgery, used sharps, cultures, stocks, and swabs used to inoculate cultures, and removed bodily organs. At the University of Kentucky, medical waste is NOT autoclaved and must instead be incinerated by a licensed vendor; therefore, laboratory disposal of this waste requires special designation using a red bag and specific containers. Red bags should never be used for regular or autoclaved waste. Please contact the Office of Biological Safety and the Department of Environmental Quality Management (EQM) if you will be generating Regulated Medical Waste outside of the UK Healthcare clinical environment.

10.1.8 Mixed Waste

Mixed Waste is waste material contaminated with a combination of chemical, biological, and/or radiological hazards. Special consideration must be given to the disposal of this type of waste. Autoclaving is often NOT appropriate for mixed waste. Please reach out to the Department of Research Safety office (Biological Safety, Chemical &Laboratory Safety, Radiation Safety) and the Department of Environmental Quality Management for guidance.

10.1.9 Prion Waste

Biohazardous waste known or suspected to contain prion materials require special consideration and disposal methods. Prions are composed of abnormal, misfolded, pathogenic isoforms of normal prion protein. Prions are highly resistant to conventional inactivation by heat and chemicals and require special biosafety precautions and disposal methods. Please contact the Office of Biological Safety for guidance when planning projects that may generate known or suspected prion contaminated waste.

11.0 Laboratory Equipment

11.1 Autoclaves

Autoclaves, also known as steam sterilizers, use a combination of wet heat, pressure, and time to sterilize materials. Autoclaving is one of the most common methods of sterilization utilized. Autoclaves are found across University of Kentucky (UK) campus and are used to sterilize lab glassware, media, and other laboratory supplies, as well as decontaminate biohazardous waste prior to disposal. The total time, temperature, and pressure necessary for sterilization depends on the materials to be sterilized and how they are packaged and loaded into the autoclave, however general cycle parameters for biohazardous waste that must be met are a temperature of 121°C at 15 psi of saturated steam for a minimum of 20 minutes. The total processing time of an autoclave cycle used to process biohazardous waste shall be between 60-120 minutes.

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

All autoclave units utilized for decontamination of biohazardous waste materials must be enrolled in the UK Autoclave Verification Program (AVP) – see below.

Definitions & Acronyms

Biohazardous Waste: Solid and/or liquid waste that contains or has been in contact with infectious agents, potentially infectious materials, or recombinant/synthetic nucleic acid materials. This may include waste from tissue culture, pipets, flasks, gloves, tips, plates, etc. Any disposable laboratory items that have been in contact with or contain materials that could be potentially infectious to humans, animals, or plants.

Biological Indicator (BI): Test system that contains viable microorganisms with a defined resistance to a specific sterilization process. Designed to verify that the necessary conditions (time, temperature, pressure) were met to kill a specific number of microorganisms for a particular sterilization process.

Chemical Integrator: Test system consisting of a paper wick with steam and temperature sensitive chemicals that undergo a color change when cycle parameters are met. Designed to provide quick visual confirmation that autoclave cycle parameters were met. NOT a substitute for Biological Indicators (BIs).

Mixed Waste: Waste material that is contaminated with a combination of chemical, biological, and/or radiological hazards. Special consideration must be given to the disposal of this type of waste. Autoclaving is often NOT appropriate for mixed waste.

Regulated Medical Waste (RMW): Pathological waste. Gross specimens of human tissues or organs and large volumes (>500mL) of human blood. Special consideration must be given to the disposal of this type of waste. Incineration is typically required.

Responsible Individual: The individual, designated by the Department or Facility owner of the autoclave facility, tasked with ensuring verification, recordkeeping, preventive maintenance, and training for autoclave facility equipment. Each autoclave on campus should have a designated Responsible Individual.

Verification: Process involving a biological challenge to ensure an autoclave is performing to an appropriate standard to effectively inactivate biohazardous waste. Autoclaves utilized for decontaminating biohazardous waste must be verified monthly.

Principles of Autoclave Operation

Autoclaving is the most common method of sterilization used in many facilities. Wet or saturated steam penetrates objects in the autoclave. Condensation creates negative pressure and draws in additional steam. As steam condenses, it transfers heat to the objects in the autoclave and causes cell destruction via coagulation of proteins. Autoclaves can be

classified into two distinct types: gravity displacement and vacuum displacement. Both types can be found on UK campuses.

Gravity Displacement: In a gravity displacement autoclave, steam enters the top or side of the chamber and, because steam is lighter than air, it forces the air out of the bottom of the chamber through a drain vent. It is especially important that all valves are clear and unobstructed, and the chamber not overfilled for this system to function efficiently. A gravity displacement autoclave can be thought of as a pressure cooker type of system. These often require longer cycle times to achieve sterilization

Vacuum Displacement: In a vacuum displacement autoclave, a vacuum removes cold air from the chamber before steam is introduced. This allows for nearly instantaneous steam penetration into even porous loads. Vacuum displacement autoclaves typically have two sterilization cycles – 1) fast exhaust or high vacuum cycles which is suitable for sterilization of dry materials, and 2) slow exhaust or pressure pulsing which is suitable for sterilization of liquids such that liquids do not bubble out and evaporate during the cycle.

New Autoclaves

Before newly installed autoclaves may be used for decontamination of biohazardous waste at UK, the following conditions must be fulfilled:

- Inspection & Certification as a Pressure Vessel
    - Performed by Boiler Inspection Section of the Kentucky State Fire Marshal's Office as required by KRS 236.110.
    - Each boiler or pressure vessel used or proposed to be used within the state of Kentucky, except boilers or pressure vessels exempt under KSR 236.060, shall be thoroughly inspected as to their construction, installation, and condition.
- Initial Autoclave Verification
    - Calibration services shall be completed by the manufacturer on all new autoclave units. Documentation of this calibration shall be maintained with the maintenance records for each unit.
    - Calibration must be verified via biological indicator testing and enrolled in the UK AVP prior to processing potentially biohazardous waste in the unit.

Autoclave Maintenance

Autoclave operation and maintenance manuals shall be maintained by the Responsible Individual or "owner" and provided to service technicians as needed during preventive maintenance and repair activities as needed. Preventive maintenance shall be performed according to the manufacturer's suggested procedures and schedules. The Responsible Individual must maintain a log of all maintenance activities for each autoclave. Following any significant maintenance activity or repair, the autoclave must be verified by biological indicator prior to processing potentially biohazardous waste in the unit.

Autoclave Training

- For the Responsible Individual
    - For new autoclaves, model specific autoclave training from the manufacturer (or manufacturer approved contractor) must be completed by personnel responsible for autoclave maintenance. A representative from the Office of Biological Safety, Department of Research Safety, should also be present.
    - For existing autoclaves, the previous Responsible Individual will provide training to the new Responsible Individual. Alternatively, an autoclave repair technician or manufacturer approved contractor will be contracted to provide training.
    - UK AVP training will be provided by the Office of Biological Safety, Department of Research Safety.
- For Autoclave Users

- The Responsible Individual or their designee will train all users on standard operating procedures specific to the autoclave unit(s) being used.

Autoclave Operation

- Before utilizing any autoclave, verify that materials are safe for autoclaving
  - Can be autoclaved – surgical instruments, bottles, beakers, and other laboratory glassware, plastic tubes, pipettes, pipette tips, water and culture media, animal cage waste, potentially biohazardous laboratory waste such as discarded cultures, stocks, tissues, gloves, etc.
  - CANNOT be autoclaved – Volatile or corrosive chemicals, red bag waste or regulated medical waste, radioactive materials, sharps containers
- If autoclaving potentially biohazardous waste, ensure that the autoclave you are using is marked "Designated for Biohazardous Waste." Autoclaves designated for biohazardous waste are enrolled in the UK Autoclave Verification Program (AVP).
- Only CLEAR or ORANGE autoclave bags, marked with the universal biohazard symbol, should be utilized for solid biohazardous waste. Red bags are designated for Regulated Medical Waste (RMW) ONLY and should NEVER be autoclaved.
- Loading the autoclave
  - Prepare autoclave load to allow for steam penetration (bags, bottles NOT tightly sealed).
  - Leak proof tray must be utilized for transport to autoclave facility.
  - Materials for autoclaving should be placed in a secondary container capable of withstanding autoclave temperatures, such as a stainless-steel tray or polypropylene bin.
  - Do not overfill containers or bags.
  - Do not leave potentially biohazardous waste unattended.
  - Load material to allow for steam penetration. Do not crowd items in autoclave. Do not allow material(s) to be autoclaved to touch the sides or top of the autoclave chamber.
  - Clean items and biohazardous waste should be autoclaved separately.
  - Visually inspect to ensure autoclave is functioning properly before use.
  - Record information in User Log for the load you are processing.
  - For autoclaves without automated documentation, a chemical integrator must be incorporated into each load of biohazardous waste. See Autoclave Verification Program below for more information.
  - Close and properly secure door.
  - Choose the appropriate cycle for your materials.
- Biohazardous waste is typically processed at a temperature of 121°C and 15 psi steam pressure with an exposure time of 20 minutes on slow exhaust/liquid cycle. Total processing time should be 60-120 minutes.
- A combination of chemical inactivation and autoclaving is necessary for materials known or suspected to contain prions. Please contact the Biosafety Officer if you will be generating prion-containing waste in your research.
- Responsible Individuals should provide training to users on cycle parameters and what types of cycles are appropriate for various materials. Cycles can vary depending on the autoclave model.

- Unloading the autoclave
    - Allow autoclave cycle to finish before attempting to open the door or unload. Pressure gauge must read zero.
    - Verify cycle conditions were met on autoclave readout or by visualization of chemical integrator.
    - Wear appropriate personal protective equipment (PPE) including lab coat, eye protection, closed-toe shoes, and heat-resistant gloves.
    - Record cycle result in user log. If using a chemical integrator, tape the autoclaved strip to the user log.
- Autoclave malfunction
    - If the cycle did not meet appropriate operating conditions or aborted, repeat the cycle. If the cycle fails again, notify the Responsible Individual immediately so that autoclave may be repaired in a timely manner.
    - Remove waste to an alternate autoclave which has been validated with a biological indicator until repairs are complete and autoclave verified by biological indicator.

11.1.1 Autoclave Verification Program

The UK Autoclave Verification Program (AVP) is designed to ensure that all biohazardous waste processed via autoclave on UK campus is properly decontaminated prior to final deposition in landfill. It requires monthly testing of the ability of campus autoclaves designated for biohazardous waste to kill microorganisms. ALL autoclaves on UK campuses that are utilized for decontamination of biohazardous waste MUST be enrolled in the UK Autoclave Verification Program. There are two components to the UK Autoclave Verification Program – verification of load parameters for each cycle of biohazardous waste AND monthly verification via biological indicator testing.

- Autoclave Designation:
    - For autoclaves enrolled in the UK AVP and designated for biohazardous waste, signage must be posted or otherwise labeled to communicate to autoclave users that the unit is “Designated for Biohazardous Waste.” This signage/label must also list the Responsible Individual such that users know who to contact in the event of autoclave failure/malfunction.
    - For autoclaves not enrolled or designated for biohazardous waste, signage must be posted or otherwise labeled to communicate to autoclave users that the unit is not suitable for decontamination of biohazardous and/or potentially infectious waste. Glassware, liquids, and other laboratory items may be sterilized in these units, but they are not suitable for decontamination of biohazardous waste. This signage/label must also list the Responsible Individual such that users know who to contact in the event of autoclave failure/malfunction.
    - The UK Office of Biological Safety, Department of Research Safety, will provide signage upon request.
- For EVERY load of biohazardous waste:
    - Autoclaves with automated documentation of load parameters –
        - Review the printed report at end of cycle to ensure cycle parameters were met.
        - If conditions were met, initial, date, and place User Log number on the printed report.
            - Temperature between 121°C-124°C
            - Total processing time between 60-120 minutes
            - Exposure time >20 minutes
            - Pressure minimum 15 psi
    - Autoclaves without automated documentation of load parameters –

        - A chemical integrator must be incorporated into each load of biohazardous waste to provide visual confirmation (color change) that cycle parameters were met.

        - The UK Office of Biological Safety, Department of Research Safety, will provide 3M ATTEST 1243B Steam Chemical Integrators (or equivalent chemical integrators) to all Responsible Individuals, as needed for biohazardous waste.

        - Once completed, tape the autoclaved chemical integrator strip into the appropriate section of the User Log for your load.

- Monthly biological indicator testing:

    - Checks all conditions of the autoclave cycle (time, temperature, pressure) are met and verifies ability of autoclave to kill microorganisms.

    - The UK Office of Biological Safety, Department of Research Safety, will provide a 3M ATTEST Auto-reader 390 incubator, 3M ATTEST 1296 Rapid Readout Biological Indicator Steam Packs, and Biological Indicator controls (or equivalent BI testing system and supplies) to all Responsible Individuals for monthly autoclave verification.

    - Monthly verification records must be maintained by the Responsible Individual and kept for five (5) years. Records can be maintained via logbook, or preferably via enrolling in the UK Office of Biological Safety, Department of Research Safety, online record keeping system.

11.2 Biological Safety Cabinets (BSCs) and Laminar Flow Devices

Engineering controls, such as biological safety cabinets (BSCs), reduce the risk of employee exposure by removing or isolating the worker from the hazard. Biological safety cabinets (BSC) are the primary means of containment for personnel working with infectious agents and other biohazards. BSCs are only one part of an overall biosafety program that requires consistent use of good microbiological practices. The efficacy of BSCs depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory. Personnel must use appropriate practices and procedures while working in a BSC for the cabinet to contain potentially infectious splashes and aerosols, which are generated by many experimental procedures. Personnel must be adequately trained in the use of biological safety cabinets prior to use. BSC exhaust air is passed through a certified high-efficiency particulate air (HEPA) filter, which is effective at trapping particulates and infectious agents. The exhaust air from the BSC is either re-circulated back into the laboratory or exhausted out of the building. BSCs that recirculate air into the room shall not be used for work with volatile or toxic chemicals as health and safety hazards can result from the buildup of chemical vapors in the cabinet and laboratory.

Class/Types of BSCs

Class II, Type A1

- Internal fan draws room air through the front grille to maintain an average inflow velocity of at least 75 lfm (linear feet per minute) at the face opening of the cabinet.

- Supply air flows through a HEPA filter and provides particulate-free air to the work surface.

- Downward air splits as it approaches the work surface and the internal fan draws air to the front grille and the rear grille.

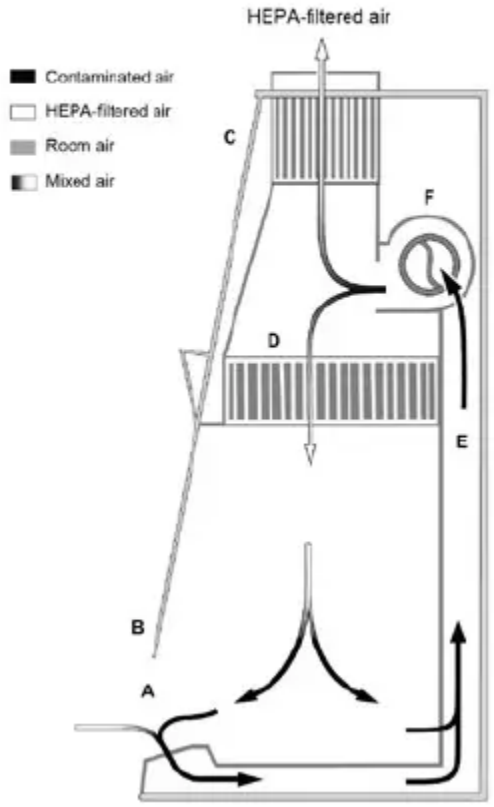
- Approximately 30% of air passes through the exhaust HEPA filter and 70% is recirculated through the supply HEPA back onto the work surface of the cabinet.

Class II, Type A2

- Differs from Class II, Type A1 in the inflow velocity; Class II, Type A2 cabinets have an inflow velocity of 100 lfm.

- Small quantities of volatile toxic chemicals may be used in a Class II, Type A2 cabinet ONLY if it exhausts to the outside via a canopy or thimble connection with an exhaust alarm.

(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) exhaust blower. Note: Since 2010 there is minimal difference between the Class II, Type A1 and Class II, Type A2 except for the inflow velocity.



Source: CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition

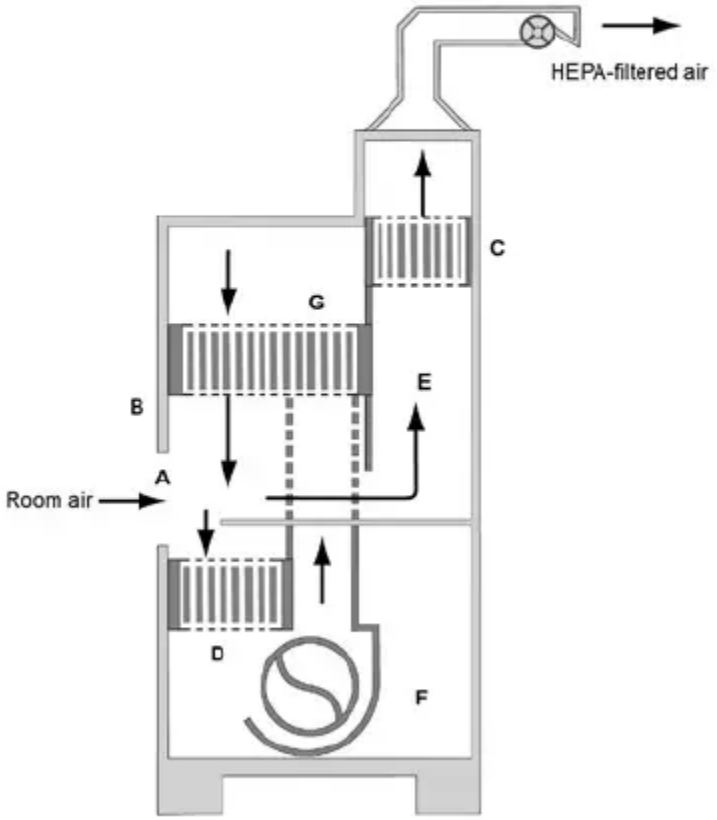
Class II, Type B1

The Class II, Type B1 cabinet is suitable for work with biohazardous materials that also require the use of small quantities of toxic volatile chemicals, including organic solvents or carcinogens.

- Supply blower draws room air and a portion of the cabinets recirculated air through the front grille and through the supply HEPA filter.
- Particulate-free air flows upward through a plenum on each side then downward to the work area.
- Room air is drawn through the face opening at an inflow velocity of 100 lfm.
- As with Type A cabinets, the downward airflow splits just above the work surface.
- Approximately 70% of the downflow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building.
    - Because the air flowing to the rear grille is discharged into the building exhaust, work with toxic volatile chemical vapors or gases should be conducted towards the rear of the cabinet.

- The remaining 30% of downflow air is drawn through the front grille, passes through the supply HEPA filter, and is recirculated.

(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. Note: The cabinet exhaust needs to be direct-connected to the building exhaust system.



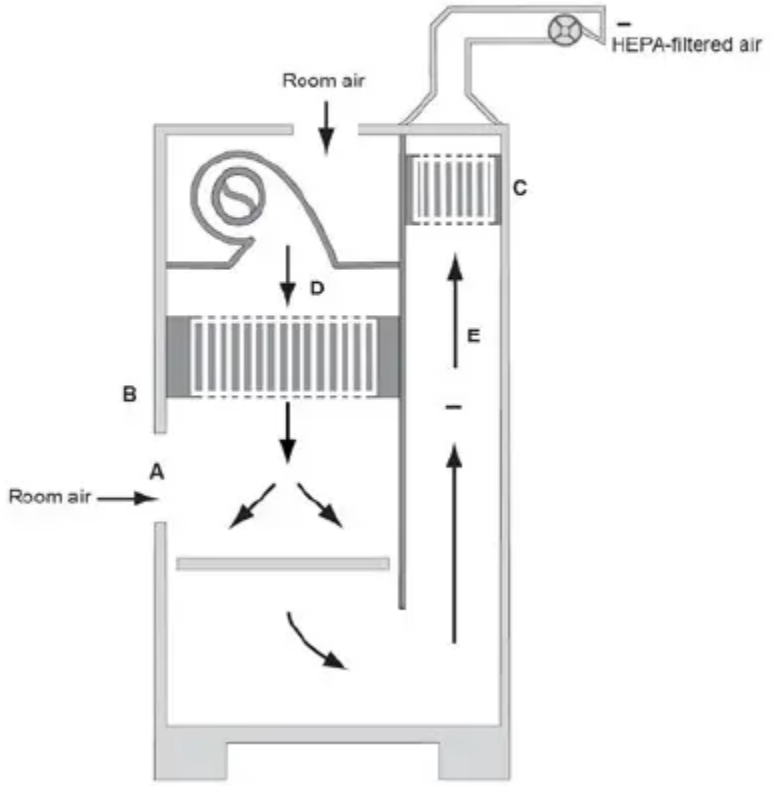
Source: CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition

Class II, Type B2

The Class II, Type B2 cabinet provides both biological and (small quantity) chemical containment.

- Supply blower draws room or outside air in at the top of the cabinet, passes it through a supply HEPA filter, and down over the work surface.
- The building exhaust system pulls air through the front and rear grilles. This captures the supply air plus room air needed to create an inflow velocity of 100 lfm.
- All air entering this cabinet is passed through an exhaust HEPA filter and exhausted via the building exhaust system.
- A Class II, Type B2 cabinet will exhaust as much as 1,200 cubic feet per minute of conditioned room air.
- Expensive to operate and maintain.

(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum. Note: The cabinet needs to be direct-connected to the building exhaust system.



Source: [CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition]

Annual Certification of BSCs

- Biosafety cabinets will be certified at least annually by NSF-certified technicians and according to NSF 49 standards.
- All BSCs and LFBs (laminar flow benches) at the University of Kentucky shall be placed in the University of Kentucky Office of Biological Safety inventory database.
- Any BSCs or LFBs not certified will be reported to the Department Chair, Executive Vice-President of Research, and Provost as a serious lab violation.
- It is the responsibility of the Principal Investigator (PI) to participate in the required annual certification process.
- The Principal Investigator (PI) is responsible for the costs associated with BSC certification, repair/service, decontamination, and moving expenses.
- Prior to certification, the user will be responsible for decontaminating the interior surfaces of the cabinet using an approved method and disinfectant specific to the agent.
- Copies of annual certification reports will be maintained by the Principal Investigator or Laboratory Manager.

Only approved vendors may be contacted to certify, repair, or decontaminate biological safety cabinets or laminar flow benches on the University of Kentucky campuses.

As of 10/01/2022, the following vendors are authorized to certify, repair, or gas-decontaminate BSCs or LFBs on UK campus:

- **Precision Air Technology (919) 812-0340**
- **SafetyPlus, LLC. (877) 821-9822**

The most up-to-date list of approved vendors is available here, under the "Annual Certification of BSCs" section.

BSC Training

All users should be trained prior to utilizing a BSC.

Online BSC training is available in the SciShield Course Directory - https://uky.scishield.com/raft/training/courses

In-person BSC training and demonstrations are available upon request. Email biosafety@uky.edu for more information.

BSC Operation

- Conduct all procedures involving the manipulation of potentially infectious materials, including aerosol-producing activities, when using aerosol-producing equipment, or when using high concentrations/large volumes of organisms, inside a biological safety cabinet.

- Storage of excessive materials or equipment inside a BSC can disrupt airflow, resulting in turbulence, cross-contamination, or breach of containment. Therefore, only materials and equipment necessary for immediate work will be placed in the BSC. Additionally, storing items on top of the BSC can interrupt exhaust airflow and can result in damage to the BSC. Place large items close to the sidewalls, rather than at the back of the cabinet where they will interfere with airflow. Blocking the intake grills in the front and rear of the cabinet will interfere with the proper functioning of the cabinet, which can cause a loss of containment of infectious organisms.

- Prior to each use examine the cabinet to ensure it is clean and in good repair.

    - Verify that the cabinet blower is on and functioning properly based upon observance of Magnehelic gauges and/or digital readouts.

    - Ensure that UV lights are disengaged prior to commencing work in the BSC.

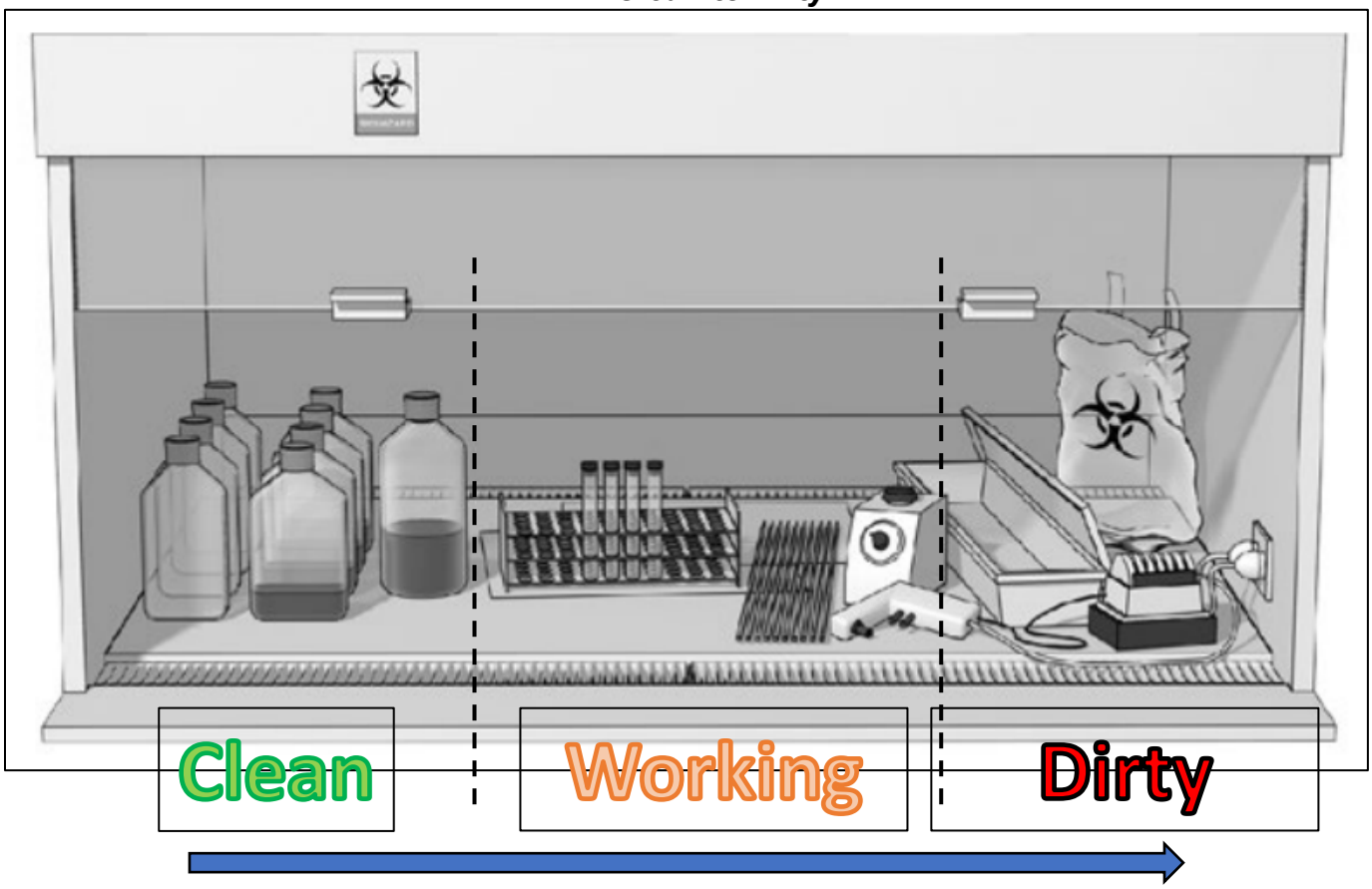
- Wipe interior of BSC (work surface, walls & interior surface of window) and items placed in the cabinet with an efficacious and approved disinfectant.

- Ensure the cabinet drain valve is closed prior to starting work, to contain spills.

- Place items necessary for the procedure (including a receptacle for waste) in BSC prior to initiating work. This will minimize the number of arm-movement disruptions to the air barrier, which can compromise the containment of the BSC.

- Arrange the materials within the cabinet in a manner to ensure good aseptic technique; e.g., all workflows from one side of the cabinet (clean) to the other side (dirty). Contaminated items never cross over uncontaminated/clean supplies.

Clean-to-Dirty



A typical layout for working from the clean to the dirty side within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded and other contaminated materials placed in the biohazard bag (right). Reverse this arrangement for left-handed persons.

Source: CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition

- Verify that the sash is set at the appropriate height. The operating height of a BSC is not variable; follow the manufacturer's recommendations for the BSC that you are using.
- Cabinets are designed for a single operator. More than one person working in the BSC at one time (even in a six-foot BSC) produces enough disturbances in the airflow to breach the containment of the BSC. Never lean into a BSC or place your head into a BSC.

BSC Surface Decontamination

The BSC must be emptied and decontaminated at the end of each procedure and/or workday. Every individual is responsible for cleaning the BSC when they have finished working.

- Remove all items from the BSC. The exterior of all potentially contaminated material must be surface decontaminated with an appropriate disinfectant prior to being removed from the unit.
- Place all biohazardous waste in an autoclave bag in the BSC and seal prior to removing.
- Wipe the interior surface of the unit (work surface, sides and back, interior of the glass) with an appropriate disinfectant after all items have been removed from the unit. Ensure adequate contact time.
- ***DO NOT*** rely on UV light as a means of decontamination.

Avoid the following Common Mistakes:

- Air currents and drafts can disrupt BSC airflows. Locate BSCs away from doors, vents/diffusers and traffic paths.
- Do not store equipment or supplies inside the cabinet.
- Make sure that items necessary for procedures are inside the BSC prior to starting work in the BSC.
- To prevent disruption of airflow and damage to the HEPA filter, do not store items on the top of the cabinet.
- Prevent damage to the cabinet by keeping all objects (i.e. paper towels, Kim Wipes, work surface diapers, etc.) from being pulled into the back, front, and side grills or slots.
- Never disengage the alarm. The alarm indicates when the cabinet has improper airflow or reduced performance, which may endanger the researcher and/or the experiment.
- Avoid rapid motions at the front of the unit and minimize movement of arms in and out of the cabinet which may disrupt the air curtain.

Routine Maintenance of BSCs

- Routine maintenance of BSCs will be performed as recommended by the cabinet manufacturer.
- All maintenance and repairs will be performed by UK approved BSC certification and maintenance contractors.
- The PI is responsible for any service or repairs that are needed and the associated cost.
- A risk assessment will be completed prior to maintenance or repairs to determine the extent of decontamination required prior to commencement of work. The Biological Safety Officer will determine the type of decontamination necessary. Typically surface decontamination is adequate, however, there are instances such as extensive repair inside the cabinet envelope or HEPA filter replacement that will require gas decontamination.
- Recertification of the BSC may be required based upon the type of maintenance or repair work that was completed. The Biosafety Officer will determine the need for recertification.
- Copies of maintenance or repair reports will be maintained by the Principal Investigator or Laboratory Manager.

Use of Natural Gas in BSCs

Biological safety cabinets (BSCs) are designed to protect personnel, their products, and their environment. Most BSCs at the University of Kentucky are recirculating. The use of natural gas or other flammable gases within a BSC presents several potential safety hazards:

- Use of natural gas, or other flammable gases, presents a potential fire and/or explosion hazard. Most BSCs at the University of Kentucky are recirculating cabinets, which may allow for flammable gases to quickly accumulate. If a gas leak occurs (i.e. a valve is left on or a tube leaks) inside a recirculating BSC, over time the gas would become more and more concentrated and could reach explosive levels. Since the gas is within a BSC, the user may not be able to detect the leak and, upon ignition, could explode.
- The high-efficiency particular air (HEPA) filters—responsible for providing the sterile environment in the cabinet—can act as a mass of combustible material during an uncontrolled fire inside the cabinet. The heat generated by a Bunsen burner can also damage the HEPA filter and/or the filter's adhesive. This could result in leaks in the filter, adverse flow patterns in the cabinet, and ultimately potential user exposure.
- The heat generated by an open flame compromises the carefully controlled airflow pattern responsible for providing containment of potentially biohazardous materials. Normal airflow in the cabinet is directed from the top down across the working surface. The addition of a Bunsen burner will produce turbulent airflow due to the heated

air rising countercurrent to the normal downward flow. This may result in the spread of contamination within the cabinet and potential user exposure.

OPEN FLAMES ARE **STRICTLY PROHIBITED** IN BSCs!

If you must use open flames within a BSC, first contact UK Biosafety (biosafety@uky.edu or 859.257.1073).

Always use the smallest quantity of flammable liquid possible. Place flammable liquids in a metal or glass container and proceed with extreme caution.

If using an alternative flame/heat source, small bottled gas or gas cylinders should be used as the fuel supply for the burner to limit the supply of fuel. Plumbed gas provides an inexhaustible source of fuel.

Plumbed gas may be used if the following design criteria and operator procedures are implemented:

- The plumbed natural gas line must have a shutoff valve outside the cabinet. An automatic burner with a foot switch or hand switch to operate the flame must be used.
- Excess flow check valves or flow limit valves designed to shut off gas flow if a pre-set limit is exceeded should be installed. These devices could prevent the flow of flammable or toxic gases into an area when other conditions have resulted in failure of point-of-use control systems. Use of these valves must be considered early in the design of the piping system.
- Regardless of gas source, users must be trained to visually inspect the gas petcock or valve and check for the odor of gas before turning on the BSC blowers. The burner should only be used in the rear of the cabinet to minimize the effects of air turbulence. At the end of burner operation, the user must turn the gas petcock or valve off and check for the smell of gas.
- The BSC should be ducted to an exhaust system with an explosion-proof roof exhaust fan.
- Butyl rubber hose should be used to connect the burner to the fuel supply. Use of yellow natural rubber or latex tubing is specifically prohibited.

The following are **NOT PERMITTED** within a BSC:

- **Bunsen Burners**
- **Alcohol burners**

Open flames are neither required nor recommended in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence that disrupts the pattern of HEPA-filtered air being supplied to the work surface. When deemed absolutely necessary and approved by the appropriate facility authorities after a thorough risk assessment, touch-plate micro burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric furnaces are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable loops should be used whenever possible.

Appendix A - Primary Containment for Biohazards, CDC Biosafety in Microbiological and Biomedical Laboratories, 6th Edition

UV Lamps in a BSC

Ultraviolet (UV) lamps should not be used as the sole disinfection method in a BSC. UV light is not a suitable method of decontamination in a BSC. *If installed, UV lamps should be cleaned regularly to remove any film that may block the output of the lamp. The lamps should be evaluated regularly and checked with a UV meter to ensure that the appropriate intensity of UV light is being emitted. Replace the bulb when the fluence rate is below 40 uW/cm2 . Unshielded UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure. If the*

cabinet has a sliding sash, close the sash when operating the UV lamp. Most new BSCs use sliding sashes that are interlocked when operating the UV lamp to prevent exposure.

**Please note that BSC users are not expected to measure a UV lamp's output.*

CDC Biosafety in Microbiological and Biomedical Laboratories, 6th Edition

Definitions & Acronyms

Aerosol-producing Activities: May include, without limitation, opening containers with non-ambient pressures; intranasal inoculation of animals; harvesting infected tissues/fluids, or embryonate eggs; transfer operations; necropsy of infected animals; changing animal cage bedding and operating aerosol-producing equipment.

Aerosol-producing Equipment: May include, without limitation, vortexers, blenders, sonicators, centrifuges, grinders, vigorous shakers, and mixers. However, equipment (e.g. sealed rotors, buckets, or centrifuge safety cups) that is designed to contain aerosols does not have to be operated in a BSC, provided that the containers (e.g. rotors or centrifuge safety cups) are opened only in a BSC.

Biological Safety Cabinet (BSC): Primary containment device which utilizes HEPA-filtered directional airflows to contain potentially infectious materials during experimental procedures. The BSC provides protection for the surrounding environment, research personnel, and research materials being manipulated.

Laminar Flow Benches (LFBs): Also referred to as clean benches. Provide an aseptic environment for experimental work by passing HEPA-filtered air across the work surface. The Department of Biological Safety actively discourages the purchase and use of LFBs since air is blown across the work surface into the face and torso of the operator. The Institutional Biosafety Committee and the Department of Biological Safety recognize that clean benches do not provide personnel or environmental protection from infectious or potentially infectious agents, allergens, chemicals, or radioactive materials. If you are using a clean bench, contact the Department of Biological Safety for a review of your procedures.

National Sanitation Foundation (NSF): Regulatory agency responsible for the development of standards associated with the certification and testing of BSCs.

Primary Containment: The engineering control which prevents the release of potentially infectious material into the laboratory or outside environment. The use of a primary containment device, such as a BSC, prevents contamination of the room.

References

NSF/ANSI Standard 49 Class II (laminar flow) biosafety cabinetry - https://d2evkimvhatqav.cloudfront.net/documents/bc_biosafety_cabinetry_testing.pdf

CDC Biosafety in Microbiological and Biomedical Laboratories, 6th Edition - https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

11.3 Blenders, Mixers, Sonicators, Vortexers, Homogenizers

Tissues, suspensions, and other materials utilized within a laboratory that contain biohazardous material sometimes need to be disrupted, thoroughly mixed, or processed into more experimentally friendly sample states. Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding, or disrupting biohazardous materials, particularly with an energetic force. Examples of devices that have the potential to produce hazardous aerosols include but are not limited to mortar and pestle, ball mills, colloid mills, jet mills, tissue grinders/homogenizers, magnetic mixers, stirrers, sonic cleaning devices, sonicators/ultrasonic cell disintegrators, French presses, vortexers, blenders, and shakers.

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

- Any open/unsealed blending, cell disruption, and grinding equipment should be operated within a Biological Safety Cabinet (BSC).
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leak-proof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline, or food dye solution is recommended prior to use to check for leaks.
    - If the blender is used with biohazardous material place absorbent material moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use.
    - Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break.
    - Before opening the safety blender bowl permit the blender to rest for at least 30-minutes to allow settling of the aerosol cloud.
- Homogenization of tissue containing infectious agents should be performed within a BSC, double bagged in a Stomacher, or in a sealed homogenizer.

11.4 Centrifuges

A centrifuge is a common piece of equipment in most research laboratories. All centrifuges, regardless of size, present a hazard to laboratory personnel if not used safely and properly maintained. Hazards include physical hazards due to mechanical failure (mechanical stress, metal fatigue, corrosion of rotor, imbalanced loads, etc.) and exposure hazards due to the materials utilized in the centrifuge (aerosolization of biohazardous, chemical, or radioactive materials).

Benchtop centrifuges are typically low speed centrifuges (up to 5000rpm) or microcentrifuges (up to 15,000rpm). High speed centrifuges (up to 25,000rpm) are generally floor model centrifuges. Ultracentrifuges, which may exceed 100,000rpm, are typically found in core (shared) equipment areas. These are the most expensive and dangerous types of centrifuge on UK's campus. Knowledgeable use, careful procedures, and preventive maintenance are all necessary to ensure centrifuge safety for all lab personnel.

Centrifuge rotors undergo tremendous mechanical forces and will show signs of metal fatigue over time. Always follow manufacturer guidance as to when to derate (permanently lower the speed) and when to retire centrifuge rotors. Although centrifuges have been designed to contain the rotor in case of failure, there have been documented incidences of rotor failure that were not contained and caused physical injury to personnel and property.

In addition to mechanical failure of the machine, centrifuge tubes may break and release aerosols into the surrounding environment. Improper centrifuge use or malfunction is often cited as the most common cause of laboratory-acquired infections (LAIs).

The following procedures will help ensure safe centrifuge operation and the longevity of the machine.

Preventative Maintenance

- Establish a preventative maintenance schedule
    - Schedule regular cleaning of the centrifuge interior, depending on frequency of use. Ensure this schedule is maintained by ALL USERS of a shared centrifuge. Refer to the operator's manual or contact the manufacturer for guidance.
- Record Log Book
    - For ALL high speed and ultracentrifuges, maintain a log book.
    - Run dates, duration, speed, total rotor revolutions and any notes regarding the rotors condition should be recorded for every run.

Proper Use

Ensure all personnel are trained PRIOR to operating the centrifuge. Users should wear appropriate Personal Protective Equipment (PPE), including a laboratory coat, safety glasses, and disposable gloves.

- Inspect the centrifuge prior to use

- Ensure tubes are rated for the intended use (speed, temperature) and free of cracks or stress marks before use.
- The rotor must be compatible with the centrifuge and seated correctly on the drive.
- O-rings should not be cracked, missing, or worn. Ensure O-rings are appropriately greased.
- Safety cups/buckets should be attached correctly and able to move freely.
- If your inspection identifies centrifuge components in need of repair or replacement, contact a qualified service technician. DO NOT use the centrifuge. Post signage on the centrifuge to communicate to other users that the centrifuge is unfit for use.

- Prepare centrifuge tubes for loading.

  - When centrifuging biohazardous materials, fill and decant centrifuge tubes/bottles inside a Biological Safety Cabinet (BSC).
  - DO NOT overfill. Follow manufacturer's limits for tubes/bottles.
  - Wipe down the exterior of tubes/bottles with an efficacious disinfectant.
  - Load tubes/bottles into safety buckets/cups or sealed rotor within the BSC.
  - Wipe down the exterior of safety buckets/cups or sealed rotor with an efficacious disinfectant prior to removal from BSC.

- Balance centrifuge and start run.

  - Monitor the centrifuge until full operating speed is reached and running safely.
  - If any unusual noise or shaking is noticed, immediately stop centrifuge
  - DO NOT exceed maximum speed or mass limits for the centrifuge rotor.
  - Ensure the centrifuge has come to a complete stop before opening.
  - Check for leaks or spills.
  - Sealed rotors or safety buckets/cups must be opened and unloaded in a BSC.
  - Using an efficacious disinfectant, wipe down centrifuge rotors, buckets, cups after use.

Emergency Response

Emergency Response In case of leaks/spill or centrifuge malfunction, follow the steps below.

1. Turn off the centrifuge and unplug the power cord.
2. Alert personnel nearby to leave the area. Post signage warning personnel of a centrifuge malfunction and Do Not Enter.
3. Allow 30 minutes for aerosols to settle.
4. Don appropriate PPE (lab coat, gloves, and face shield) prior to opening the centrifuge (carefully) to assess damage.
5. Cover all interior surfaces of the centrifuge with an efficacious disinfectant and allow appropriate contact time (ex. 10% bleach for 20 minutes).
6. Transport (carefully) centrifuge rotors/buckets/cups to the nearest available BSC to open containers.
7. Use a sturdy cart for transport.
8. Disinfect contents with an efficacious disinfectant (as described above).
9. Remove materials for proper decontamination (ex. autoclave) and disposal.
10. DO NOT use your hands to pick up any sharp materials. Use forceps to safely remove broken/damaged items.
11. Sharps materials should be disposed of in a designated sharps container.
12. Non-sharp solid materials should be disposed of in an orange/clear autoclave bag for autoclaving and disposal.

11.5 Flow Cytometers & Cell Sorters

Flow cytometers and cell sorters have become powerful tools for research and are now in common use. Flow cytometers pass cell suspensions in a narrow fluid stream through a laser beam, measuring various characteristics of each cell that passes by (cell size, volume, light/fluorescent qualities, etc.) before flowing into a waste collection container. Cell sorters operate on a similar principle, with the addition that once the flow cytometer measures a cell’s characteristics, desired cells are diverted into a separate collection vessel. This occurs when a force is applied to the cell suspension fluid stream, forming droplets containing single cells that are then physically separated (commonly via electrostatic charge) from the other cell droplets that do not meet those characteristics.

Based on this principle of operation, aerosols are readily produced during use. This poses a considerable hazard when biohazardous materials are being utilized. BSCs (Biological Safety Cabinets), sometimes custom-built for the equipment, can house flow cytometers and cell sorters to contain and help mitigate exposure to biohazardous aerosols. For example, the sorting of unfixed fluorescently labeled HEK293 cells (a human-derived cell line) must be conducted on a cell sorter that resides within a BSC to contain potentially aerosolized human cells that can harbor bloodborne pathogens. Additionally, vented flow cytometer waste containers require an appropriate filter (ex. in-line HEPA filter) on the vent. This helps to reduce the risk of biohazardous aerosol exposure from the waste container until it can be appropriately decontaminated. When appropriate engineering controls are not available for flow cytometry/cell sorting of live, biohazardous materials, additional risk assessment will be required to determine the appropriate PPE and administrative controls.

Samples that are fixed using a validated method and are no longer biohazardous may be analyzed in a flow cytometer on the open bench at BSL1. Fixed or unfixed samples containing chemical or radiological hazards have additional considerations and require extra precautions. Please contact the Office of Biological Safety at biosafety@uky.edu, the Office of Chemical and Laboratory Safety at labsafety@uky.edu, or the Office of Radiation Safety at radsafe@uky.edu if you plan to perform flow cytometry/cell sorting on materials with these hazards.

11.6 Growth Chambers

Distinctly different from greenhouses and incubators used for tissue culture and microorganisms, growth chambers are designed specifically for the growth and/or containment of plants, transgenic seeds, and plant materials infected with plant pathogens under controlled environmental conditions. Growth chambers tend to be sizable pieces of equipment, often larger than a refrigerator but are made in a range of sizes and have interior surfaces that are easily cleaned. The set environmental conditions should be checked regularly and any materials no longer necessary for research are removed and disposed of appropriately. The interior surfaces of the growth chamber should be regularly cleaned when possible and decontaminated before beginning work with different plants/agents. Special attention should be paid to any drains present to prevent the escape of materials.

11.7 Incubators

For any laboratory working with microorganisms and/or tissue cultures, incubators are common and essential pieces of equipment. If your laboratory utilizes an incubator, it is imperative to ensure it is cleaned and the set environmental conditions are checked regularly. The surfaces within the incubator should be cleaned appropriately and regularly, both to offer the best-controlled growth environment for your organisms and to ensure biohazardous organisms remain safely contained.

Avoid the following common mistakes:

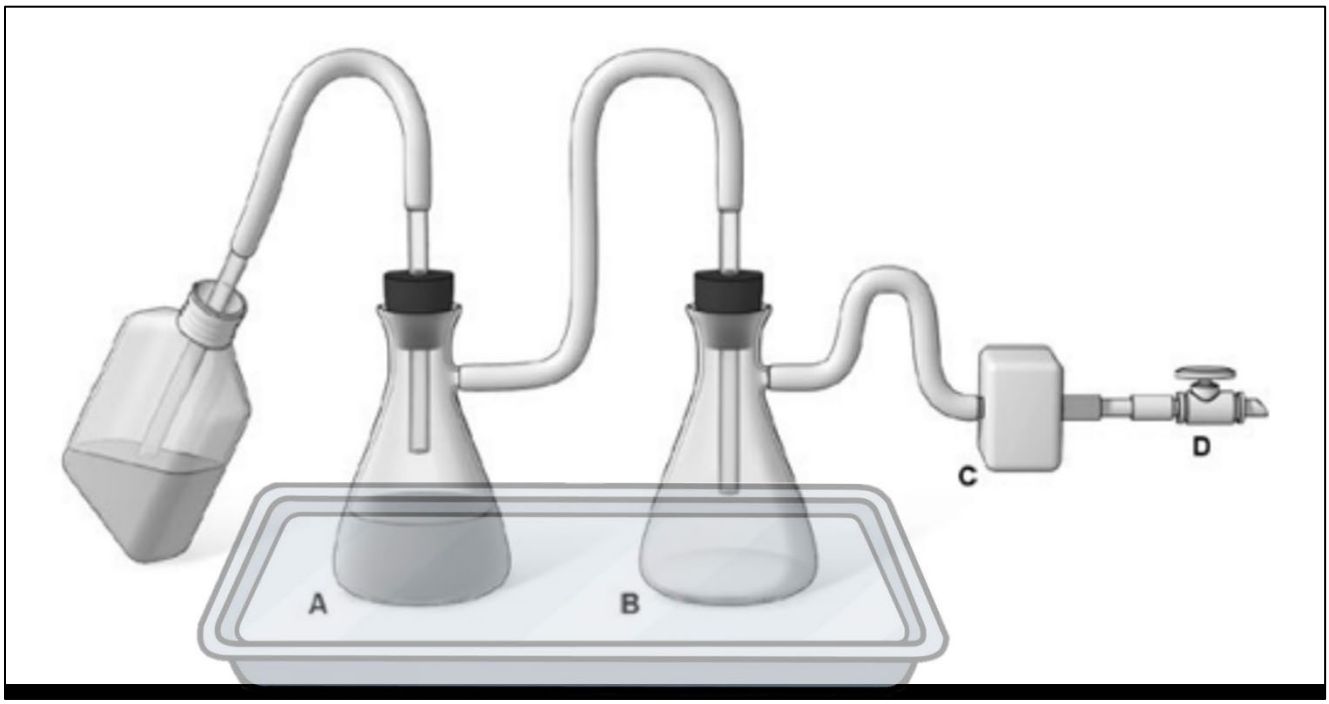
- For humidified incubators/growth chambers, pay attention to the condition of the water reservoir. This reservoir offers an excellent environment for the outgrowth of environmental organisms (ex. Molds). Water should be changed regularly, and the use of antifungal/algicides in the water should be considered.
- Ensure the unit’s door is securely closed each time after accessing the unit’s interior.
- Do not allow the unit’s door to slam when closing. This can jostle the contents and lead to spills and/or leakage of biohazardous materials from their containers within the unit.
- The exterior of the unit must be labeled appropriately for its current contents. Ex. Hazard signage
- For units with internal HEPA filters, replace these filters according to the unit manufacturer’s guidelines, often annually for best performance.
- Interior metal shelving can have sharp edges from the manufacturing process. Be mindful of these as you manipulate the contents/culture vessels when putting in and taking materials out of the unit.
- Incubators utilized with carbon dioxide (or other) gas must have the corresponding gas cylinders secured to a wall/surface or approved cylinder stand.

11.8 Microtome/Crytostat/Microstat

A Microtome/Cyrostat/Microstat is an instrument used to cut very thin slices (sections) of a block of tissue. Cyrostats/Microstats specifically differ from Microtomes in that Cryostats & Microstats operate at much lower temperatures (ex. -20°C). This sectioning of a tissue block is accomplished by precisely controlled movement of an exceptionally sharp blade affixed within the equipment. Due to the nature of the materials used with the Microtome/Cyrostat/Microstat and the sharpness of the blade, training is essential both in the use of the equipment and the hazards of the materials utilized with it. Users shall be informed of the need to prevent cuts and scrapes as well as protect the eyes, nose, mouth, and skin from exposure to the materials being used. New personnel shall be trained in the proper use and maintenance of the equipment and demonstrate proficiency prior to use. When the equipment's blade requires cleaning/handling/removal, only a user specifically trained in this procedure may do so. The equipment's blade should never be touched directly by hand, and forceps or similar tools should be utilized for blade handling. Consult the manufacturer's user manual/guide for specific instructions or contact UK Biosafety for assistance.

11.9 Vacuum Line Traps

Vacuum line trap systems are utilized in many laboratories to collect liquid biohazardous waste and prevent suction of infectious and non-infectious materials into vacuum lines (house vacuum line or vacuum pump). Setting up the vacuum line trap system correctly will allow for safe collection and decontamination of liquid biohazardous waste.



Source: CDC, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition

The diagram above details the correct setup of two vacuum flasks and an in-line hydrophobic/HEPA filter in series connected by vacuum lines to a port for house vacuum. Alternatively, a vacuum pump may be used if house vacuum is not available. Both flask **A** and flask **B** must rest inside of a non-porous leak-proof secondary container or tray, sufficient to contain any leaks or spills from the flasks.

A. Catch Flask
B. Overflow Flask
C. In-line hydrophobic/HEPA filter
D. Vacuum Source (house vacuum or vacuum pump)

Liquid waste material is drawn into catch flask **A**, which is pre-filled with an appropriate disinfectant*. Catch flask **A** is connected to Overflow flask **B**. Note that flask **B** is empty. Flask **B** provides overflow protection for flask **A**. Care must be taken to prevent overflow of flask **A**. An in-line hydrophobic/HEPA filter **C** is located between overflow flask **B** and the

vacuum port **D**. Both flasks reside in a spill tray/bin to contain any spill should the flasks be knocked over and must be non-porous, leak-proof, and autoclave safe.

When vacuum line trap flasks reside within a Biological Safety Cabinet (BSC), the work surface of the BSC will act as the secondary spill/leak containment for the flasks. The valve drain of the BSC must be closed to ensure containment of any spill should flasks be knocked over.

*Household bleach is commonly used for disinfection of liquid biohazardous waste. When utilizing household bleach for disinfection of liquid biohazardous waste, catch flask **A** should be pre-filled with fresh household bleach such that when the flask is 2/3 full, the final concentration of bleach is 10%. Ensure appropriate contact time (20 minutes for 10% bleach), then dispose of the contents down the drain while flushing with copious amounts of water.

The following considerations should be made when setting up a vacuum line trap system.

Flasks – Flasks should be constructed with heavy walls able to withstand vacuum pressure. Appropriate flasks will have a glass hose connector such that flasks may be connected in series via tubing.

Tubing – Vacuum tubing should be thick-walled such that it can withstand vacuum pressure without collapsing. Vacuum tubing should be autoclavable.

Spill Tray – Spill tray must be non-porous, autoclavable, and of a size appropriate to contain the contents of flasks in case of spill.

Filter – in-line vacuum filters must be hydrophobic/HEPA filters.

11.10 Laboratory Furniture

Within all wet research laboratories on UK campuses, laboratory surfaces must be easily cleaned. This means that porous surfaces like carpets, rugs, and other textiles/fabrics are not appropriate. Chairs utilized within the laboratory must be covered with non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant. Benchtops need to be appropriate for the anticipated uses and workloads, as well as impervious to water and resistant to heat, acids, alkalis, organic solvents, and other chemicals. Laboratories that have windows capable of opening must be fitted with screens and kept closed. This is imperative for building/room air balancing and containment.

12.0 Laboratory Surfaces & Equipment Decontamination

12.1 Introduction

A laboratory working with biohazardous materials will inevitably require certain laboratory surfaces and equipment to be disinfected or decontaminated, to mitigate the possibility of transmission of pathogens to laboratory workers, the public, and the environment. The terms cleaning, disinfection, decontamination, and sterilization are often misunderstood and incorrectly used interchangeably. This chapter will clarify and define these terms, discuss considerations for the disinfectants often utilized in research laboratories, and identify suitable methods of decontamination and disinfection for these surfaces and items.

> ***Cleaning*** – Often the first step, cleaning refers to the removal of gross contamination from a surface to the extent necessary for further processing. Cleaning can be an essential first step in the disinfection or sterilization process that removes microorganisms and other contaminants (ex. blood, soil, other organic matter) from a surface by physical means (ex. scrubbing).
>
> ***Disinfection*** – Eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (ex. bacterial spores, prions) present on an inanimate object. A less-lethal process than sterilization.
>
> ***Decontamination*** – A process by which the level of microbial contamination is reduced such that transmission of infection is prevented. Decontamination renders an area, device, item, or material reasonably free of the risk of disease transmission and therefore safe to handle. In a research laboratory setting, this is most often accomplished by sterilization via steam autoclaving.

Sterilization – A process by which an item, device, or solution is made completely free of all forms of living microorganisms, including spores and viruses. Sterilization can be accomplished by dry or moist heat, gases, and vapors (ex. Chlorine dioxide, ethylene oxide, formaldehyde, hydrogen peroxide, etc.), plasma sterilization technology, and radiation (ex. gamma).

12.2 Decontamination and Disinfectants

The appropriateness of a decontamination procedure depends on your goal. Do you wish to disinfect or sterilize? Will you be using the disinfectant on hard surfaces, in a biosafety cabinet, on instruments, or on waste? When choosing a disinfectant one should consider the organism, the item to be disinfected, the cost and ease of use of the disinfectant, and what level of disinfection will accomplish your goal.

Antimicrobial pesticides (ex. disinfectants) are classified as pesticides and are regulated by both the United States Environmental Protection Agency (EPA) and the United States Food and Drug Administration (FDA). A laboratory is responsible for selecting an appropriate EPA-registered product and for using it according to the manufacturer's specific instructions for that product. The Office of Biological Safety is available to assist laboratories in selecting an appropriate product based on a risk assessment. A list of EPA-registered disinfectants is available at https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants.

The FDA defines three types of liquid chemical germicides for processing medical devices (also applicable to laboratory equipment utilized with biohazardous materials in the case of UK research laboratories), and these germicides are regulated as auxiliary devices per the FDA 1977 Policy Manual.

Low-Level Disinfection – A lethal process utilizing an agent that kills vegetative forms of bacteria, some fungi, and enveloped viruses.

Intermediate-Level Disinfection – A lethal process utilizing an agent that kills viruses, mycobacteria, fungi, and vegetative bacteria, but no bacterial spores.

High-Level Disinfection – A lethal process utilizing a sterilant under less than sterilizing conditions (ex. 10-30 minutes contact time instead of 6-10 hours needed for sterilization). The process kills all forms of microbial life except for large numbers of bacterial spores.

Disinfectant solutions should be made and stored according to manufacturer directions for maximum stated effectiveness. It is crucial to remember that a clean surface is more effectively decontaminated than a soiled surface, the required contact time for a given disinfectant product may vary, and disinfectants are not necessarily detergents. However, some disinfectants do include a detergent or surfactant to aid in the removal of gross contamination from a surface during the disinfection or decontamination process.

There are several types of liquid chemical disinfectants and sterilants, including but not limited to household bleach (sodium hypochlorite), peroxides, quaternary ammonium compounds, aldehydes, phenolic compounds, alcohols, and iodine/iodophors. The general characteristics of these categories are outlined here.

Chlorine (Sodium Hypochlorite)
Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical disinfectant. It is normally sold as household bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. Chlorine is highly alkaline and can be corrosive to metal, which must be considered when it is used on metal clad benches and laboratory equipment (ex. Incubators, Biological Safety Cabinets, etc.). The disinfectant activity of chlorine is reduced by organic matter (ex. protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, may release chlorine gas thus weakening their disinfectant potential. Undiluted household bleach stored at room temperature in the original container has a shelf-life of approximately one year. Working bleach solutions should be prepared daily. Household bleach (typically 5.25% NaOCl, please check the label) should be diluted 1:10 to obtain final concentration of 0.5% NaOCl. Industrial solutions of bleach have a higher sodium hypochlorite concentration and must be diluted accordingly to obtain the correct concentration. Chlorine gas is highly toxic. Bleach must therefore be stored and used in well-ventilated areas only. Undiluted bleach must not be mixed with acids or other incompatible chemicals, such as ammonia containing compounds, to prevent the rapid release of chlorine gas.

Peroxides (Hydrogen Peroxide & Peracetic Acid)

Peroxides are non-chlorine based strong oxidizers with many desirable characteristics for a liquid chemical disinfectant. They are capable of a wide range of bactericidal, fungicidal, and virucidal activity, are capable of protein and lipid denaturation, and degrade into relatively harmless byproducts; hydrogen peroxide (H_2O_2) degrades into oxygen & water. Hydrogen peroxide possesses these properties but also ranges from an intermediate level of disinfection up to sterilization* depending on the concentration and contact time employed. At lower concentrations, hydrogen peroxide is not fully sporicidal and an organism's ability to produce the peroxidases (ex. catalase) can reduce its effectiveness. However, when used at the concentrations found in most peroxide-based disinfectants (typically ≥3%), peroxidases have limited impact on effectiveness. A stronger peroxide disinfectant is Peracetic acid ($C_2H_4O_3$) which is a mixture of hydrogen peroxide and acetic acid. Acetic acid acts as a strong acid catalyst enabling peracetic acid to have robust bactericidal, sporicidal, fungicidal, and virucidal properties while also maintaining activity in the presence of peroxidases. Peracetic acid offers a high level of disinfection up to sterilization* depending on concentration and contact time, and it decomposes into acetic acid and oxygen. Both hydrogen peroxide and peracetic acid can be corrosive to metal surfaces, though stainless-steel surfaces are minimally affected. Additionally, hydrogen peroxide and peracetic acid-based disinfectants do not require inactivation after application.

Quaternary ammonium compounds
Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols, or detergents. They have good activity against some vegetative bacteria and lipid-containing viruses. The germicidal activity of certain types of quaternary ammonium compounds is reduced by organic matter, water hardness, and anionic detergents. Care is therefore needed in selecting agents for pre-cleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria grow in quaternary ammonium compound solutions, requiring careful attention to storage and usage. Quaternary ammonium compounds, when properly diluted, have a low odor and are non-irritating.

Aldehyde compounds
Aldehyde based compounds (ex. glutaraldehyde, formaldehyde, paraformaldehyde) are capable of a wide range of antimicrobial activity levels, from low-level disinfection to sterilization* with the appropriate combination of concentration and contact time. These compounds are frequently found in laboratories as fixative agents for tissues and microscopy preparations. However, they are NOT recommended for the disinfection or decontamination of research laboratory surfaces or equipment due to their known chemical hazards. For example, formaldehyde is classified as a known human carcinogen with a low permissible exposure limit and there are currently no FDA cleared liquid chemical disinfectants that contain it as an active ingredient. These compounds should be reserved only for specific experimental procedure purposes.

Phenolic compounds
Phenolic compounds, a broad group of agents, were among the earliest germicides. However, more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against non-lipid-containing viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (ex. triclosan and chloroxylenol) are among the more commonly used antiseptics. Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water. They may be absorbed by latex gloves and can also penetrate the skin. Phenolic compounds can be irritating to the skin and eyes and may have an associated odor.

Alcohols
Ethanol (ethyl alcohol, C_2H_6O) and 2-propanol (isopropyl alcohol, (C_3H_8O) have similar disinfectant properties. They are active against vegetative bacteria, fungi, and lipid-containing viruses but not against spores. Their action on non-lipid-containing viruses is variable. For the highest effectiveness, they should be used at concentrations of approximately 70% (v/v) in water. Higher or lower concentrations are not as germicidal as 70% (v/v). A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. Since alcohol tends to evaporate rapidly, they are most effectively used in circumstances where items can be submerged, allowing the appropriate contact time to be achieved. A 70% (v/v) aqueous solution of ethanol can be used to soak small pieces of surgical instruments, with a contact time of ten minutes or more required. Ethanol should never be used to disinfect hands since ethanol can dry the skin, reducing its effectiveness as a barrier. Alcohol-based hand-rubs, alcohol mixed with emollients, are recommended for the disinfection of lightly soiled hands in situations where proper handwashing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores, Hepatitis B Virus (HBV), *Mycobacterium tuberculosis* (TB), and may not kill all types of non-lipid-containing viruses. Alcohol is volatile and flammable and must not be used near open flames. Do not use 70% ethanol to clean a Class II, Type A recirculating Biological Safety Cabinet (BSC). The

vapors from ethanol are flammable and the lower explosive limit (LEL) for ethanol is easily attained with the amount of ethanol required to clean a BSC. Working solutions should be stored in proper containers to avoid evaporation and prevent vapor build-up in an area. Additionally, alcohols may harden rubber and dissolve certain types of glue. Bottles with alcohol-containing solutions must be clearly labeled and must not be autoclaved.

Iodine and Iodophors
The action of these disinfectants is like that of chlorine, although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is unsuitable for use as a disinfectant. On the other hand, iodophors and tinctures of iodine are good antiseptics. Povidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodine are unsuitable for use on medical/dental devices. Iodine should not be used on aluminum or copper and can be toxic. Organic iodine-based products must be stored at 4–10°C to avoid the growth of potentially harmful bacteria in them. No liquid chemical disinfectant with iodophors as the main active ingredient has received clearance by the FDA as of 2023.

*Note: Sterilization with a liquid chemical sterilant is not considered by the FDA to produce as sterile a result as the processes of steam/vapor/gas sterilization. Sterilization is still achieved by liquid chemical sterilants but is not as readily maintained immediately after processing due to environmental factors and application.

**Healthcare/Clinic: UK Healthcare (UKHC) utilizes its own procedures for selecting appropriate disinfectants in the clinical setting. Please defer to UKHC guidance on the disinfectants required in your specific clinical setting.

**DLAR: UK Division of Laboratory Animal Resources (DLAR) utilizes its own procedures and processes for the selection of appropriate disinfectants in animal housing and DLAR procedural spaces. Please defer to UK DLAR guidance on the disinfectants required in your specific DLAR setting.

**Expired Disinfectants: If your laboratory possesses expired disinfectants, please contact UK Environmental Quality Management (EQM) for guidance on the proper disposal of these chemicals.

12.3 Lab Surfaces and Equipment

Utilizing the information earlier in this section, informed decisions can be made regarding the most effective selection and application of a liquid chemical disinfectant or sterilant in the laboratory. A decontamination procedure needs to be developed by each laboratory that corresponds to the specific work with biohazardous materials and is determined during a risk assessment. For example, a laboratory conducting work with spore forming bacterial pathogens or prions will have quite different decontamination procedure considerations than a laboratory only conducting work with uninfected human-derived cell lines. This decontamination procedure for laboratory surfaces and equipment will need to be utilized after work with biohazardous materials is completed. Your UK Institutional Biosafety Committee (IBC) protocol registration will specify the disinfectant(s) to be utilized in your laboratory.

An effective decontamination procedure is required for surfaces and equipment that have been utilized with biohazardous materials and is to be employed before the removal/surplus of any such contaminated equipment from the laboratory and a laboratory closeout. Hard, non-porous surfaces can effectively be decontaminated with surface applied liquid chemical disinfectants. Porous objects and surfaces are not recommended to be used with biohazardous materials due to the drastically increased difficulty in their decontamination.

In most cases, lab surfaces and equipment cannot be steam sterilized in an autoclave and will require liquid chemical disinfectants. In UK research laboratories, 10% (final concentration) household bleach is one of the most utilized disinfectants. After applying 10% bleach, allow a 20-minute contact time, and follow with a final wipe down of the equipment or surface with 70% ethanol or water that is needed to remove residual disinfectant.

However, as mentioned in Section 13.2 the effectiveness of household bleach is reduced by the presence of organic matter (ex. protein, tissues, bacterial cultures, etc.). Heavily soiled items or surfaces will require cleaning prior to achieving decontamination. Household bleach can also be utilized in the cleaning step of the decontamination procedure, but more than one application of household bleach may be required to sufficiently remove enough organic matter to achieve effective decontamination of the surface or item. If a surface has minimal organic matter contamination, a single application of household bleach as denoted above is sufficient (excluding prion contamination) to achieve decontamination unless otherwise determined in the risk assessment of your work with the biological agent. Please contact the Office of Biological Safety if you require guidance on the decontamination of a surface or item.

For the decontamination of spills involving biohazardous materials, please reference Section 17.5 Biological Spill SOP.

12.3.1 Decontamination Considerations for Specific Equipment

Laboratory Equipment utilized with biohazardous materials must be emptied, cleaned, decontaminated, and tagged by UK Biosafety ANY TIME equipment is moved outside of the room in which it is currently located, taken out of service, or surplused. Once laboratory equipment is empty, clean, and decontaminated, contact UK Biosafety at biosafety@uky.edu to schedule a biosafety equipment clearance appointment. Below are decontamination considerations for specific equipment:

Biological Safety Cabinet (BSC)

Contact UK Biosafety (biosafety@uky.edu or 859.257.1073) at least one-month prior to moving a BSC to determine the need for gas decontamination!

Interior surfaces of BSCs must be thoroughly cleaned and decontaminated with an appropriate disinfectant. Freshly prepared 10% bleach (20 minutes contact time) followed by 70% ethanol is most recommended, but specific recommendations may vary depending on the biohazardous material in use. Consult your current IBC protocol or contact UK Biosafety at biosafety@uky.edu for recommendations. The PI (Principle Investigator) is responsible for ensuring proper decontamination of the BSC.

- *BSCs remaining in place* – Often, for BSCs remaining in their current location, thorough surface decontamination is sufficient. Depending on the biohazardous materials used in the BSC and other factors, gas decontamination may be necessary. UK Biosafety will determine the need for gas decontamination on a case-by-case basis. *

- *BSCs moving between UK Laboratory Locations* - A thorough surface decontamination may be sufficient for BSC(s) that will move between UK laboratory locations. Depending on the biohazardous materials used in the BSC and other factors, gas decontamination may be necessary. UK Biosafety will determine the need for gas decontamination on a case-by-case basis. *

- *BSCs going to Surplus* – ALL BSCs must be gas decontaminated prior to being sent to Surplus. *

*Gas decontamination should be scheduled as early as possible to ensure the vendor will be available prior to the move date. Only UK approved vendors are allowed to certify, repair, or decontaminate BSCs or other laminar flow benches on UK campus. UK approved BSC vendors are -

- **Precision Air Technology 919-812-0340**
- **SafetyPlus, LLC. 877-821-9822**

Refrigerators, Freezers, Refrigerator-Freezer Combos

Empty materials from unit, clean and defrost freezer, as needed. Wipe down the interior, handles, and any visibly soiled area with disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol).

Freezers may be moved between UK laboratory locations without being emptied and defrosted IF the following conditions are met:

1. Freezer contents are packed with packing materials such that items cannot shift during transport.
2. Freezer door may be secured via lock or duct tape.
3. Freezer has been inspected by UK Research Safety team member and deemed safe for transport between UK laboratory locations. UK Research Safety will tag the unit with a clearance tag and tamper-evident label.

Incubators

Empty materials from unit, clean, and drain the water jacket, as needed. Wipe down the interior, shelving, trays, door, and any visibly soiled area with disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol). The exterior of all potentially contaminated materials must be surface decontaminated with an appropriate disinfectant, prior to being removed from the unit.

Growth Chambers

Empty materials from unit, including any plant debris and clean. Wipe down the interior, shelving, trays, door, and any visibly soiled area with an appropriate disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol). The exterior of all potentially contaminated materials must be surface-decontaminated with an appropriate disinfectant, prior to being removed from the unit. Any removed plant materials must be appropriately destroyed/devitalized according to the associated permits and IBC protocol, where applicable.

Microtomes/Cryostats/Microstats

The blade should be safely removed by a trained user, utilizing forceps/tools NEVER by hand, and disposed of as sharps waste appropriately (ex. blade used with biohazardous materials is disposed of in a biohazardous waste sharps container). After the blade has been safely removed, empty any remaining material and wipe the interior of the equipment and any visibly soiled area with an appropriate disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol). After blade removal, the exterior of all potentially contaminated materials must be surface-decontaminated with an appropriate disinfectant, prior to being removed from the unit. Consult the manufacturer's manual/guide for additional information.

Centrifuges, Mixers, Other Misc. Laboratory Equipment

Empty materials from unit, clean inside and outside with soap and water, and wipe down the inside and outside of equipment with disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol).

12.4 Prion Decontamination

Prions are abnormal transmissible pathogenic agents that can induce the formation of certain abnormally folded proteins, causing pathology primarily in the brain (ex. transmissible spongiform encephalopathy). Prions are notably resistant to conventional decontamination methods (ex. heat and chemical germicides), making decontamination procedures especially challenging. For guidance on effective decontamination of prion-contaminated surfaces or items, please contact the Office of Biological Safety.

13.0 Transport

Sometimes it may be necessary to transport biohazardous materials from one laboratory location to another. Whenever it is necessary to transport biohazardous materials, animals, or plants between UK laboratory locations, special care must be taken to ensure the health and safety of the UK community.

13.1 Transport of Biological Materials on Campus

When necessary to transport biohazardous materials between UK laboratory locations (for instance, transporting infectious agents from the primary lab location to DLAR for administration to animals), materials must be sealed in a primary container and placed within a **leak-proof, shatter-proof, secure-lidded, secondary container** such that if the secondary container were dropped, biohazardous materials would not be released into the surrounding environment.

Pay special attention to the path you will travel between laboratory locations, taking care to avoid public elevators, hallways, and otherwise crowded areas of campus.

Transportation of Biological Materials via Private Vehicle

The transport of certain biological materials in personal vehicles is exempt from most DOT hazardous materials regulations, as long as they adhere to the requirements of the Materials of Trade (MOT) exception (49 CFR 173.134 (a)). This exemption applies when certain Division 6.2 materials are carried for purposes such as research, diagnosis, or investigational activities. Division 6.2 materials are defined as:

a. Category A, Infectious Substances (must be transported by contracted carrier)
b. Category B, Biological Substances

c. Patient specimens may be classified as Biological Substances, Category B or exempt depending on the health status of the patient.

Examples of exempt materials include Category B biological substances, non-infectious biological materials, and human samples for routine testing (49 CFR 173.134 (b)). This exclusion **does not apply** to Category A infectious substances or other categories of Dangerous Goods.

Material types which qualify for MOT exemption:

1. A division 6.2 material, other than a Category A infectious substance, contained in a patient sample being transported for research, diagnosis, investigational activities, or disease treatment or prevention, or a biological product, when such materials are transported by a private or contract carrier in a motor vehicle used exclusively to transport such materials. (49 CFR 173.134 (b) (10)).
2. Non-infectious biological materials from humans, animals, or plants. Examples include non-infectious cells, tissue cultures, blood or plasma from individuals not suspected of having an infectious disease, DNA, RNA or other non-infectious genetic elements. (49 CFR 173.134 (b) (2)).
3. A human or animal sample (including, but not limited to, secreta, excreta, blood and its components, tissue and tissue fluids, and body parts) being transported for routine testing not related to the diagnosis of an infectious disease, such as for drug/alcohol testing, cholesterol testing, blood glucose level testing, prostate specific antibody testing, testing to monitor kidney or liver function, or pregnancy testing, or for tests for diagnosis of non-infectious diseases, such as cancer biopsies, and for which there is a low probability the sample is infectious. (49 CFR 173.134 (b) (11)).
4. Corpses, remains, and anatomical parts intended for interment, cremation, or medical research at a college, hospital, or laboratory. (49 CFR 173.134 (b) (14)).

Packaging & Transport Requirements

All biological samples must be packed according to DOT/International Air Transportation Association (IATA) regulations. This includes triple-packaging all samples, even if materials are exempt.

Components of Triple-Packaging:

1. Primary receptacle: The primary receptacle holds the biological material and must be leak-proof or sift-proof.
2. Leak-proof secondary container: The secondary container must be durable, watertight, and leak-proof. The secondary container encloses and protects the primary receptacle(s).
3. Rigid outer container: The outer container is a rigid and durable container with one side that is at least 10 cm by 10 cm (or 4 inches by 4 inches) that houses the secondary container.

All biological samples should be transported in accordance with the following requirements:

1. All required DOT/IATA labeling and marking information should be on the outside of the package.
2. The vehicle shall be driven directly from the point of origin to the intended destination without stopping at other locations on the way. Specimens should be transported directly to the lab, avoiding unnecessary additional stops/time in transit.
3. Materials needed to contain or clean-up a spill, such as absorbent pads, gloves, and eye protection, should be available in the vehicle. Spill kit supplies can be obtained by contacting biosafety@uky.edu.
4. Hazardous materials should be transported in the trunk or as far away from passengers as possible.
5. Personal vehicles must be dedicated to the purpose of transporting the specimens, not for any other purposes at the same time.

Temperature Control During Transport

If temperature control is required for transport, please consider the following. Ethanol is NOT recommended. If using dry ice, a dry ice label should be placed on the container when in use, and care should be taken to prevent off gassing. Liquid nitrogen up to 1 liter can be utilized in an appropriate container.

13.2 Shipping of Biological Materials

Environmental Quality Management Department (EQM) provides assistance to the UK community regarding the shipping of dangerous goods by providing the training required to originate such shipments. This training is required by the US

Department of Transportation (DOT) which also mandates compliance with the International Air Transportation Association (IATA) training requirements. The DOT has established regulations (link is external) for domestic transport (within the United States) of hazardous materials by rail, air, vessel (ships), and motor carrier (ground). While IATA has established guidelines exclusively for the transport of dangerous goods by air (both domestic and international). The DOT term “Hazardous Material” and the IATA term “Dangerous Good” are used interchangeably by UK.

When shipping via air (international or domestic), you must use the IATA guidelines. Each commercial carrier may also have special provisions that must be met before a package containing dangerous goods can be transported on an aircraft. In addition, when shipping internationally, some countries have specific requirements for dangerous goods. All of these are addressed in the IATA Dangerous Goods Regulations. Following IATA regulations is required regardless of the routing and whether the shipment ends up physically moving by air transportation, ground transportation, or a combination of these.

Who needs DOT/IATA Training?

Anyone who is involved in any aspect of shipping biohazardous materials, including:

- Packing
- Labeling
- Transporting
- Signing Shipping Papers

Initial Training

All UK faculty, staff and students who are involved in any aspect of shipping dangerous goods as described above must be trained in conformance with DOT/IATA. The EQM offers, free of charge to the UK community, training that is commensurate with this regulatory standard. This training is provided on regular basis in either in-person or virtual formats depending upon the specific requests received. Class size is limited and pre-registration is required. The contact information to find out more about the availability of the Initial DOT/IATA Training Course or to register for the class is provided below:

Charles Lowe
Sr. Hazardous Waste Specialist
859-257-3147
charles.lowe@uky.edu

Refresher Training

At least every two (2) years following the completion of the Initial Training course, it is required to complete DOT/IATA Refresher Training to maintain the legal ability to ship dangerous goods. This refresher certification can only be taken by individuals who have previously successfully completed the Initial Training course. If Refresher Training is not completed within two (2) years of the Initial Training date, the classroom training must be retaken.

This class is available ONLINE.

Shipping Dry Ice

Dry ice is a common and necessary preserving material accompanying many shipments of materials originating from UK. Therefore, the following basic information is provided but additional assistance can also be provided by contacting EQM:

- The box must be labeled “Dry Ice”, “UN 1845” and the net quantity of dry ice (in Kilograms, kg.).
- Carbon dioxide, solid (dry ice), when offered for transport by air, must be in packaging designed and constructed to permit the release of carbon dioxide gas and to prevent a build-up of pressure that could rupture the packaging.
- All specific dry ice requirements are in addition to the labeling and packaging requirements for Biological Substances, Category B materials.

Required Container Markings for Infectious Substances (Division 6.2)

- DOT Primary Hazard class label - must have at least one
- Two (2) orientation arrows - on opposing sides of the package

- Proper shipping name
- UN number
- Full name and address of the shipper and consignee
- Name and phone number of person responsible (NO PAGER NUMBERS PERMITTED)
- An itemized list of contents, placed between the primary and secondary packaging
- All markings must be on one side of the package
- Container must be designed for infectious substances, i.e., a marking of “Class 6.2” must be indicated

Packing Requirements for Infectious Substances (Division 6.2)

Packaging must include:

- Watertight primary container
- Watertight secondary packaging
- An itemized list of contents, enclosed between the secondary and outer packaging
- Rigid outer packaging (no envelopes)
- A container specifically designed for the transportation of infectious substances
- When the infectious substances to be transported are unknown but suspected of meeting the criteria for inclusion in Category A and assignment to UN 2814 or UN 2900, the words “Suspected Category A Infectious Substance” must be shown in the parentheses following the proper shipping name on the itemized list of contents and the Shipper’s Declaration, but not on the outer packaging.

Required Container Markings for Biological Substances, Category B (UN 3373)

- DOT Primary Hazard class label (must have at least one)
- 2 orientation arrows (on opposing sides of the package)
- Proper shipping name
- UN number
- Full name and address of the shipper and consignee
- An itemized list of contents, placed between the primary and secondary packaging
- All markings must be on one side of the package
- A name and 24 hour number of a person knowledgeable about the material being shipped must be on the container or shipping papers (It is recommended that the information also be placed on the itemized list of contents)

Packaging Requirements for Biological Substances, Category B (UN 3373)

Packaging must include:

- Primary receptacle
- Secondary packaging; and
- Rigid outer packaging (no envelopes)
- An itemized list of contents, enclosed between the secondary and outer packaging
- The completed package must be capable of successfully passing the drop test described in IATA Dangerous Goods regulations (6.6.1) except that the height of the drop must not be less than 1.2 m.
- A dangerous goods manifest is not required when shipping a biological substance, category B material. An airway bill will suffice.
- Clear instructions on filling and closing such packages must be provided by packaging manufacturers and subsequent distributors to the shipper or to the person who prepares the package (i.e., patient) to enable the package to be correctly prepared for transport. A copy of these instructions are to be kept for at least one (1) year.

13.3 Import and Export of Biological Materials

Permits for Import, Export, Transport

The receipt, import, export, and/or transport of certain biological materials may require one or more permits, depending on the material(s) and details of transport. It is the Principal Investigator’s responsibility to ensure all necessary permits are

acquired and updated, as necessary. Please read below for more information on various permit requirements for biological materials.

CDC Import Permit Program (IPP)

The CDC Import Permit Program, or IPP, regulates the importation of infectious biological materials that could cause disease in humans in order to prevent their introduction and spread into the U.S. The program ensures that the importation of these agents is monitored and that facilities receiving permits have appropriate biosafety measures in place to work with the imported agents.

Materials Requiring CDC Import Permits

- Infectious biological agents capable of causing illness in humans
- Materials known or reasonably expected to contain an infectious biological agent
- Vectors of human disease (such as insects or bats)
- Subsequent transfers of pathogens of high consequence

CDC IPP e-Tool – Do I need an Import Permit?

Source: https://www.cdc.gov/orr/ipp/index.htm

Importer Certification Statement

Any noninfectious biological agent or biological substance that is being imported must be accompanied by an Importer Certification Statement. The statement **must** confirm the material is not known to contain or suspected of containing an infectious biological agent or has been rendered non-infectious.
You can find additional information, including an Importer Certification Statement Form template, online **HERE**.

USDA APHIS Biotechnology Permit

APHIS regulates the importation, interstate movement, or environmental release (i.e., outdoor field trials) of certain organisms developed using genetic engineering (including plants, insects, and microbes) that may pose a plant pest risk.

Permit applications, which are carefully reviewed by APHIS regulatory scientists, provide details about the nature of the organism and the conditions that will be used to prevent the spread and establishment of the organism in the environment. A permit may include additional conditions to help prevent unauthorized release into the environment.

Source: https://www.aphis.usda.gov/biotechnology-permits

USDA APHIS Plant Protection and Quarantine (PPQ) Permit

Under the authority of the Plant Protection and Honeybee Acts, a Plant Protection and Quarantine (PPQ) 526 permit is required for the importation, interstate movement and environmental release of plant pests (plant feeding insects, mites, snails, slugs, and plant pathogenic bacteria, viruses, fungi, etc.), biological control organisms of plant pests and weeds, bees, parasitic plants, and Federally listed noxious weeds.

APHIS also requires a 526 permit for the importation and interstate movement of soil or other potentially infected host material for the purpose of isolating or culturing microorganisms from those materials. Those materials may include but are not necessarily restricted to; plant material, insects/arthropods, environmental samples such as water, dust, sediments, etc. If the organism is imported on/in host material, no separate permit is required for the host material if the host material is not intended for propagation.

APHIS is authorized to inspect shipments and/or facilities at any time to verify compliance with permit conditions. Receipt of a PPQ permit does not relieve the applicant from the obligation to comply with the regulations of other Federal, State, and local agencies (e.g., U.S. Fish and Wildlife Service or the Environmental Protection Agency).

PPQ 526 Permit

The PPQ 526 permit is required for the importation, interstate movement, possession, and/or environmental release of the following:

- Insects & Mites
- Bees
- Butterflies and Moths
- Biocontrol Organisms
- Pet Food, Fish Bait, and Animal Feed
- Invertebrate Pets
- Plant Pathogenic Bacteria, Viruses, Fungi, Mycoplasms, and Nematodes
- Snails & Slugs
- Federal Noxious Weeds & Parasite Plants
- Earthworms
- Soil
- Western Corn Rootworm, Diabrotica virgifera
- Plant Growth Enhancers

How To Apply for a PPQ 526 Permit

APHIS offers both the online APHIS eFile system and a manual process for application submission. PPQ strongly recommends applicants submit permit applications online, via the APHIS eFile system because the online system efficiently provides applicants electronic access to their applications and permits. Select the following link to start the application process.

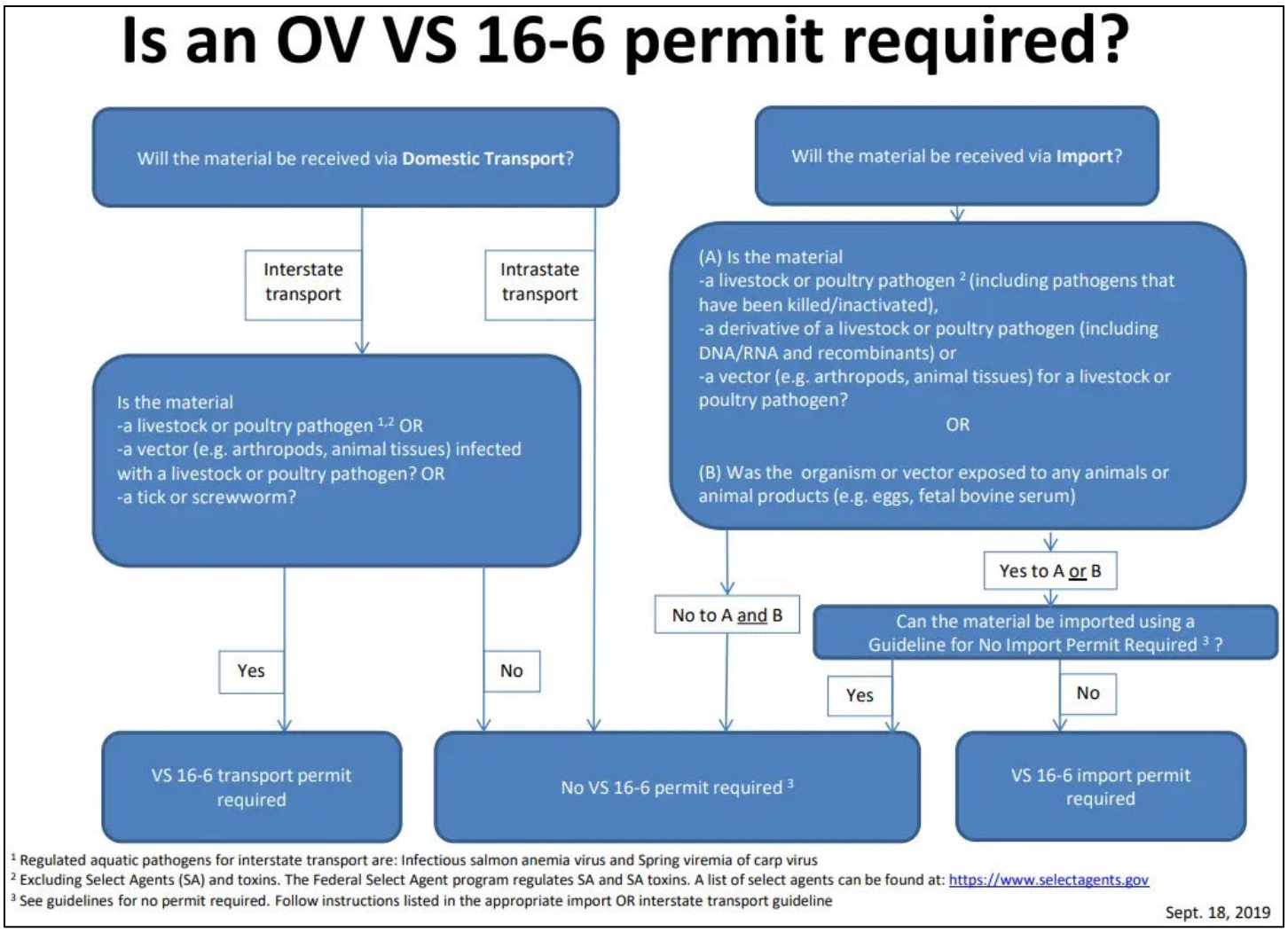
APHIS eFile Assistance

Source: https://www.aphis.usda.gov/organism-soil-imports

USDA APHIS Veterinary Services Organisms and Vectors (OV) Permit

The Veterinary Services Organisms and Vectors (OV) Permitting Unit regulates the **importation** into the United States, and **interstate transportation**, of **organisms and vectors of pathogenic diseases of livestock and poultry**.

The Code of Federal Regulations, in 9 CFR, §122.2 , mandates that “no organisms or vectors shall be imported into the United States or transported from one State or Territory or the District of Columbia to another State or Territory or the District of Columbia without a permit”.



VS-Regulated Livestock & Poultry Pathogens (Partial List)

Veterinary Services Permitting Assistant

Source: https://www.aphis.usda.gov/animal-product-import/organisms-vectors

Exporting Biohazardous Materials

Please note that export control regulations cover a wide variety of materials, technologies and information other than biohazardous material. Most research activities will fall under fundamental research exclusion in the regulations; however, it is strongly recommended that you be aware of these regulations if your research involves any international shipment or collaboration. If you are collaborating with individuals located in or visiting from a country or if you are visiting a country which the United States currently has sanctions against or is considered a State Sponsor of Terrorism, it is recommended that the regulations be consulted to ensure no violations are occurring. For example, carrying certain personal electronic devices to certain countries or training foreign nationals (individuals in the USA without a green card) from certain countries in specific methods or on specific technologies may be prohibited. Information supplied to foreign nationals may also fall under the category of deemed exports.

Export Administration Regulation (EAR)

Office of Foreign Assets Control provides a list of countries with current sanctions

U.S. Department of State Directorate of Defense Trade Controls

State Sponsors of Terrorism

Fundamental Research Exclusion

"'Fundamental research' means basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons."

Source: National Security Decision Directive 189

The funding source of a project can change the applicability of the export control regulations. If there exists any clause in the agreement with the funding agency or sponsor that would prohibit the public dissemination of the research, such as sponsor review of data prior to publication or the maintenance of confidential business information, export control regulations may then be applicable.

Commerce Control Export Licenses

Exports of materials are carefully controlled by the Department of Commerce. For shipments outside of the United States, it is crucial to contact UK Biosafety to determine if an export license is required for your material.

If a license is required, there is an online application process. There is no cost associated with the application, however, the application process can be quite lengthy.

Commerce Control List (CCL)

For questions about export control laws and your research and/or technologies, the University of Kentucky Office of Sponsored Projects Administration export compliance official's contact information is listed below and here.

John Craddock
859-253-8377
john.craddock@uky.edu

14.0 Biosafety Laboratory Inspections

Purpose

The purpose of the UK Biosafety Laboratory Inspection program is to ensure compliance with all federal, state, and local regulations, review biosafety practices outlined in relevant Institutional Biosafety Committee (IBC) protocols, identify biosafety gaps, and offer real-time, practical solutions to biosafety challenges in the research community.

What to Expect?

There are 4 different types of biosafety laboratory inspection.

1. **Biosafety Inspection (Annual)** - These inspections must be completed prior to approval of any full IBC protocol registration (New or Renewal), annually thereafter, any time there is a change of laboratory locations, or any changes in work with biohazardous materials that significantly alter the risk assessment. These inspections are scheduled in advance with the PI or their designee and focus on work with biohazardous materials as described in the corresponding IBC protocol(s).

2. **Biosafety Walkthrough (Annual)** - These inspections are completed annually by building and are unannounced. These inspections are based on visual findings observed at the time of inspection. Biosafety walkthrough inspections are scheduled by the Office of Biological Safety in coordination with other departments within UK EH&S. Biosafety Walkthrough inspections are not intended to disturb research laboratory work.

3. **Clinical Biosafety Inspection** - These inspections must be completed prior to approval of any full IBC protocol registration (New or Renewal) and annually thereafter for those UK Healthcare Clinical locations associated with UK IBC protocol registrations. Clinical Biosafety Inspections should never interfere with patient care.

4. **FSAP Select Toxin Inspection** - These inspections are completed annually for all laboratories possessing exempt quantities of Select Toxins as designated by the Federal Select Agent Program (FSAP).

How to Prepare?

Biosafety laboratory inspections are not intended to be punitive, but rather an opportunity to identify safety gaps and solutions with the Biosafety Team.

Tips & Tricks

1. Review your laboratory's profile in SciShield, our research management platform. Ensure the lab spaces, personnel, and hazards are listed correctly.
2. Ensure all lab personnel are reflected in the Lab Safety Manual's Chemical Hygiene Plan (CHP) personnel page.
3. Ensure all lab personnel have completed and documented Laboratory Specific Training.
4. Update all required online training.
5. Review and update lab door signage, if needed.
6. Review the Biosafety Inspection checklist, here.

15.0 Laboratory Exit/Closing and Relocating Laboratory Equipment

15.1 Laboratory Exit/Closing

Biohazardous Materials & Laboratory Equipment

Biohazardous materials must never be left behind upon laboratory exit and/or move. ALL biohazardous materials must either be:

1. Inactivated and disposed
    a. Biohazardous materials and waste must be inactivated and disposed of according to UK Research Safety Laboratory Waste Guidelines, available online HERE.
2. Safely transferred/transported
    a. Guidance for transport and shipping of biohazardous materials is available online HERE. If transferring biohazardous materials to a UK investigator, IBC approval must be obtained.

IBC Protocol

For researchers that are leaving the university, the corresponding IBC protocol must be:

1. Transferred
    a. If you intend to transfer your current IBC protocol to another UK investigator, follow the instructions HERE to amend your IBC protocol.
2. Closed
    a. If you do not intend to transfer your current IBC protocol to another UK Investigator, follow the instructions HERE to close your IBC protocol.

If you are moving laboratory locations within UK, your current IBC protocol must be amended. Follow the instructions HERE to amend your IBC protocol.

15.2 Relocating Laboratory Equipment for Move, Repair, or Surplus

Laboratory Equipment utilized with biohazardous materials must be emptied, cleaned, decontaminated, and tagged by UK Biosafety ANY TIME equipment is moved outside of the room in which it is currently located, taken out of service, or surplused. Once laboratory equipment is empty, clean, and decontaminated, contact UK Biosafety at biosafety@uky.edu to schedule a biosafety equipment clearance appointment.

Equipment Specific Procedures

Biological Safety Cabinet (BSC)

- Contact UK Biosafety (biosafety@uky.edu or 859.257.1073) at least one month prior to moving a BSC to determine need for gas decontamination!

- Interior surfaces of BSCs must be thoroughly cleaned and decontaminated with an appropriate disinfectant. Freshly prepared 10% bleach (20 minutes contact time) followed by 70% ethanol is most recommended, but specific recommendations may vary depending on the biohazardous material in use. Consult your current IBC protocol or contact UK Biosafety at biosafety@uky.edu for recommendations.

- **BSCs remaining in place** – Often, for BSCs remaining in their current location, thorough surface decontamination is sufficient. Depending on the biohazardous materials used in the BSC and other factors, gas decontamination may be necessary. UK Biosafety will determine the need for gas decontamination on a case-by-case basis. *

- **BSCs moving between UK Laboratory Locations** - A thorough surface decontamination may be sufficient for BSC(s) that will move between UK laboratory locations. Depending on the biohazardous materials used in the BSC and other factors, gas decontamination may be necessary. UK Biosafety will determine the need for gas decontamination on a case-by-case basis. *

- **BSCs going to Surplus** – ALL BSCs must be gas decontaminated prior to being sent to Surplus. *

*Gas decontamination should be scheduled as early as possible to ensure vendor will be available prior to move date. Only UK approved vendors are allowed to certify, repair, or decontaminate BSCs or other laminar flow benches on UK campus. UK approved BSC vendors are -

- **Precision Air Technology 919-812-0340**
- **SafetyPlus, LLC. 877-821-9822**

Refrigerators, Freezers, Refrigerator-Freezer Combos

- Empty materials from unit, clean and defrost freezer, as needed. Wipe down interior, handles, and any visibly soiled area with disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol).

- Freezers may be moved between UK laboratory locations without being emptied and defrosted IF the following conditions are met:

    1. Freezer contents are packed with packing materials such that items cannot shift during transport.

    2. Freezer door may be secured via lock or duct-tape.

    3. Freezer has been inspected by UK Research Safety team member and deemed safe for transport between UK laboratory locations. UK Research Safety will tag unit with clearance tag and tamper-evident label.

Incubators

- Empty materials from unit, clean, and drain the water jacket, as needed. Wipe down interior, handles, and any visibly soiled area with disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol).

Centrifuges, Mixers, Other Misc. Laboratory Equipment

- Empty materials from unit, clean inside and outside with soap and water, and wipe down the inside and outside of equipment with disinfectant (ex. 10% bleach for 20 minutes, followed by water or 70% ethanol).

16.0 Emergency Procedures

16.1 Emergency Response

FOR MEDICAL EMERGENCIES CALL 911 OR GO TO THE UK CHANDLER HOSPITAL (OR CLOSEST) EMERGENCY DEPARTMENT

First Aid
In case of laceration, cut, or puncture:

- Stop the bleeding by applying gentle pressure to the wound.
- Clean the wound with water.
- Apply antibiotic and cover wound with dressing.

In case of needlestick:

- Rinse area with soap and water.
- Do not attempt to squeeze blood from wound.

In case of exposure to biohazardous materials:

- If there is a splash or contact with biohazardous materials, wash area with warm water and soap.
- If there is a splash to eyes, flush area with tepid water in emergency eyewash and/or shower.
- Remove contaminated clothing or equipment.
- Exit area of exposure.

Remember:

- Stay calm! Secure any animals or infectious materials.
- Doff PPE and follow first-aid procedures.
- If an animal was involved, note its cage card, PI/owner, and status (ex. infected with infectious agent X).
- Report your injury/incident to your supervisor and, if needed, proceed to UHS for medical attention.
    - Call 911 or proceed immediately to the UK Chandler Emergency Department for life threatening injuries.
- Report incidents involving biohazardous materials to the UK Biological Safety Officer (859-257-1073).

As part of the university's Occupational Health Program, the opportunity for Laboratory Workers to receive medical evaluation must be provided under the following circumstances:

- If an employee develops any symptoms thought to arise from exposure to a hazardous chemical. Please note: Individuals exhibiting acute onset of symptoms due to known exposure should seek immediate medical attention.
- After an incident such as a spill, leak or explosion which may have resulted in exposure.

- When an overt exposure is identified through evaluation or assessment.
- There is an existing medical surveillance program in place for the work conducted.

Any medical examination required from the above-listed situations must be provided without cost to the employee, without loss of pay, and at a reasonable time and place. Records of any medical examination will be maintained at the medical facility providing service or with appropriate medical personnel at the University.

All workplace incidents and workplace-acquired injuries or illnesses sustained by UK personnel shall be reported by the Principal Investigator/Laboratory Supervisor to Workers Care by calling (800) 440-6285.

UK students may contact University Health Services (859) 323-APPT during business hours, or (859) 323-5321 after business hours, on weekends or holidays.

*UK Healthcare personnel shall follow UK Healthcare specific policies regarding accident/injury reporting.

17.2 Incident Reporting

All workplace incidents and workplace-acquired injuries or illnesses sustained by UK personnel shall be reported by the Principal Investigator/Laboratory Supervisor to Workers Care by calling (800) 440-6285.

UK students may contact University Health Services (859) 323-APPT during business hours, or (859) 323-5321 after business hours, on weekends or holidays.

Unsafe working conditions or “near-miss” incidents (incidents not resulting in injury or release of hazardous material) is encouraged to be reported to UK Occupational Health and Safety at: https://ehs.uky.edu/apps/incident

Incidents involving Biohazardous Materials
Incidents involving biohazardous materials must be immediately reported to the UK Biological Safety Officer (859-257-1073).

In compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, the University of Kentucky, as a recipient of NIH funds, must "report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents or illnesses".

This includes any "spills, accidents, or other incidents that result in breach of containment, environmental release or exposures to laboratory or other research personnel or animals to viruses, cells or organisms containing recombinant or synthetic nucleic acid molecules". These events may include "overt exposures..., the escape or improper disposition of a transgenic animal, or spills of high-risk recombinant or synthetic nucleic materials occurring outside of a biosafety cabinet". Overt exposure may include (but is not limited to): skin punctures with needles, cuts, animal bites or scratches, and direct contact with mucosal membranes (e.g. eye splash).

16.3 Incident Investigation

UK Department of Research Safety staff may reach out to you after an incident/injury with questions. Follow-up reporting to relevant regulatory agencies may be required.

16.4 Biological Spill SOP

1. Alert people in immediate areas of the spill.
2. Don appropriate protective equipment.
3. Cover the spill with paper towels or other absorbent materials.
4. Carefully pour a freshly prepared 1:10 dilution of household bleach around the edges of the spill and into the spill. Avoid splashing. Allow 20 minute contact period.
5. Use paper towels to wipe up the spill, working from the edges into the center.

6. Clean spill area with fresh paper towels soaked in disinfectant.
7. Place paper towels into a biohazard bag for disposal.

Need a Biological Spill Kit? Email biosafety@uky.edu to request a free biological spill kit!

16.5 Centrifuge Malfunction SOP

In case of leaks/spill or centrifuge malfunction, follow the steps below.

1. Turn off the centrifuge and unplug the power cord.
2. Alert personnel nearby to leave the area. Post signage warning personnel of a centrifuge malfunction and Do Not Enter.
3. Allow 30 minutes for aerosols to settle.
4. Don appropriate PPE (lab coat, gloves, and face shield) prior to opening the centrifuge (carefully) to assess damage.
5. Cover all interior surfaces of the centrifuge with an efficacious disinfectant and allow appropriate contact time (ex. 10% bleach for 20 minutes).
6. Transport (carefully) centrifuge rotors/buckets/cups to the nearest available BSC to open containers. - Use a sturdy cart for transport.
7. Disinfect contents with an efficacious disinfectant (as described above).
8. Remove materials for proper decontamination (ex. autoclave) and disposal.
9. DO NOT use your hands to pickup any sharp materials. Use forceps to safely remove broken/damaged items.
10. Sharps materials should be disposed of in a designated sharps container.
11. Non-sharp solid materials should be disposed of in an orange/clear autoclave bag for autoclaving and disposal.

17.0 Employee Health

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

17.1 Immunizations

Many biological agents that are utilized in a research laboratory environment may have an associated immunization available. Examples include seasonal influenza vaccination or hepatitis B vaccination for work with human source materials. Immunizations, when available for the biological agent(s) in use, should be made available to all laboratory personnel at no cost.

Hepatitis B Vaccination

Per OSHA 29 CFR 1910.1030, the hepatitis B vaccination series must be made available at no cost to all employees with occupational exposure to human source materials or OPIM. Employees are not required to take the hepatitis B vaccine. Employees may initially decline the hepatitis B vaccination and choose to later accept the hepatitis B vaccination.
All lab employees with exposure to human source materials or OPIM must sign the ECP Personnel Statement form to indicate their acceptance or declination of the hepatitis B vaccination. This form is available online HERE.

Obtaining the Hepatitis B Vaccination

Hepatitis B vaccination is obtained through UK Employee Health. Follow the procedure below to obtain the Hepatitis B vaccination.

- Call ahead and make an appointment with Employee Health, 323-APPT (2778), for Hepatitis B vaccination.
    - Employees must bring their UK ID badge in order to be seen by Employee Health.
    - Employees will receive documentation for services received through Employee Health, which should be returned to their supervisor.
- Speak with your Principal Investigator or departmental business manager regarding payment.
- Complete the Guarantee of Payment form PRIOR to your visit and bring with you to all appointments.

17.1.1 Vaccinia Virus Vaccine Requirements

- Vaccination **IS NOT RECOMMENDED** for researchers working with **highly attenuated** strains of vaccinia virus (MVA, NYVAC, TROVAC, ALVA), with limited exceptions.
- Vaccination **IS REQUIRED** for researchers working directly with **non-highly attenuated** strains of vaccinia virus (WR, NYCBOH, Copenhagen, Temple of Heaven, Lister), or Cowpox or Monkeypox OR animals infected with those strains.
- An unvaccinated worker **shall not** work directly with non-highly attenuated vaccinia virus OR animals infected with those strains.
- **Other factors**, such as researcher parameters, also influence vaccination recommendations.
- Documented confidential medical counseling by University Health Service **IS REQUIRED** for anyone who will be working in a laboratory where vaccinia virus (highly attenuated or non-highly attenuated) is manipulated.

Recombinant vaccinia and other pox viruses are useful microbiological research tools for expression of exogenous proteins in a variety of cultured cell types. However, their use is not without risk to laboratory personnel. As stated in the Centers for Disease Control (CDC) and National Institutes of Health's Biosafety in Microbiological and Biomedical Laboratories 5th edition (http://www.cdc.gov/od/ohs/biosfty/bmbl5toc.htm, Section VIII-E) and the Vaccinia (Smallpox) Vaccine Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001 (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010al.htm):

- Naturally or experimentally infected laboratory animals are a potential source of infection to exposed unvaccinated laboratory personnel.
- Genetically engineered recombinant vaccinia viruses pose an additional potential risk to laboratory personnel, through direct contact or contact with clinical materials from infected volunteers or animals.
- The agents may be present in lesion fluids or crusts, respiratory secretions, or tissues of infected hosts.
- Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues, are the primary hazards to laboratory and animal care personnel.
- All manipulations of vaccinia strains BSL-2 and above should be conducted in a biosafety cabinet. When work must be performed outside of a biosafety cabinet (e.g. animal surgery, microscopy), the following personal protective equipment must be used: **disposable gloves, disposable lab coat or gown, eye and mucous membrane protection (goggles which are ANSI certified and surgical mask or face shield)**. Serious ocular infections can occur even in vaccinated individuals.

Multiple strains of vaccinia virus exist with varying levels of virulence for humans and animals. Depending on the strain used, vaccinia virus presents varying levels of health risk to laboratory personnel. Strains that are highly attenuated are typically unable to replicate or replicate poorly in human cells. On the other hand, non-highly attenuated strains of vaccinia have the ability to replicate in human cells and thus pose a risk to the public health. Risk include localized skin infections and more severe, disseminated reactions to which immunocompromised individuals may be more susceptible.

Routes of transmission for occupationally acquired infections of vaccinia:

- needlestick injury
- contamination of non-intact skin, mucous membranes, eyes
- unknown exposure, possibly contamination of intact skin which was not immediately washed
- Potential routes: ingestion, inhalation, exposure to fomites (virus is stable at room temperature)

Vaccination Recommendations and Requirements:

The following information is based on national guidelines issued by the CDC in Vaccinia (Smallpox) Vaccine Recommendations of the Advisory Committee on Immunization Practices.

Vaccination is not recommended for those working with the following highly attenuated strains:

Highly Attenuated Strain	Biosafety Level*	Derived from:
MVA	2	Vaccinia virus (Ankara)
NYVAC	1	Vaccinia virus (Copenhagen)
TROVAC	1	Fowlpox virus
ALVAC	1	Canarypox virus

**Biosafety level may increase depending on the presence and characteristics of a foreign protein expressed by a recombinant vaccinia virus or other aspects of the proposed experiment.*

- Laboratory personnel who work with highly attenuated strains of vaccinia virus (e.g., MVA and NYVAC) or who work with the Avipoxvirus strains ALVAC and TROVAC do not require routine vaccinia vaccination.
- The Occupational Safety Health Board of NIH no longer requires vaccinia vaccination for personnel manipulating MVA or NYVAC in laboratories using only those strains.
- The Recombinant DNA Advisory Committee of the NIH reduced the biosafety level of NYVAC, TROVAC and ALVAC to level 1 based on accumulated attenuation data and biological properties of these strains.
- Although there is no formal surveillance system in place, there have not been any reports of laboratory-acquired infection resulting from exposure to any of the above highly attenuated strains or recombinant vaccines derived from these strains in the literature or to the CDC.
- Appropriate biosafety guidelines and infection control procedures should always be observed when working with viral material even if vaccination is not indicated.

There are some limited exceptions to this recommendation when working with highly attenuated strains, depending upon other factors which may come into the evaluation whether to vaccinate or not. Such as:

- The preparation of large volumes of high titer vaccinia virus.
- Work with recombinant vaccinia viruses that might have enhanced virulence or that express harmful proteins.
- Injection of high titer vaccinia virus into animals or other work with sharps and the vaccinia virus.
- Other potentially hazardous manipulations of the virus.

Vaccination is required for laboratory workers who directly handle a) cultures or b) animals infected with:

- Non-highly attenuated Vaccinia virus strains
- Recombinant Vaccinia viruses derived from non-highly attenuated vaccinia strains
- Other orthopox viruses that can infect humans:

	WR (Western Reserve, mouse neuroadapted derivative) NYCBOH (strain used in vaccinia vaccine)
Non-highly attenuated strains	Copenhagen Temple of Heaven Lister
Other orthopox viruses	Cowpox, Monkeypox

Advantage and Disadvantages of Vaccination:

Post-vaccination complications are possible. The risks and benefits of the vaccine have to be weighed against the exact duties of the worker. Therefore, a mandatory counseling session is required before any worker accepts or declines the vaccinia vaccination. The Principal Investigator is required to make arrangements with the University Health Service/Employee Health Service (323-582-3228) for all laboratory workers who would work with the vaccinia virus to receive this counseling. All medical information is deemed confidential and cannot be disclosed by the UHS/EHS to supervisors, etc. without the employee's written permission.

Some of the arguments for the **advantages** of vaccination:

- It confers some protection in the event of an incident. (Vaccinia immune globulin (VIG) is also available through the CDC as an Investigational New Drug (IND)).
- It may reduce the risk of seroconversion to genetically inserted material such as protein products of inserted gene material.
- It may reduce the risk of serious eye infections following accidental splash (this risk could also be mitigated by wearing proper personal protective equipment).

Some of the arguments for the **disadvantages** of vaccination:

- Risk of side-effects, which may be greater for primary rather than secondary vaccinees and for adults rather than children.
- It does not always offer full protection.
- Strict personal hygiene precautions must be followed for at least two weeks after vaccination. The vaccinia virus **can** be spread to close contacts which is especially dangerous for certain populations: infants, children, elderly, people who are immunocompromised, people with a history of eczema or atopic dermatitis, people who are pregnant or breastfeeding, people with allergies to components of the vaccine (which my be trace amount of polymyxin B, streptomycin, tetracycline, neomycin, glycerin, phenol).

Additional Considerations for Vaccination:

- Revaccination every 10 years is recommended by the CDC for people working with non-highly attenuated vaccinia strains; more frequent revaccination may be required for more virulent orthopox viruses.
- Laboratory personnel not directly handling cultures of vaccinia or animals infected with vaccinia, but working in the same lab where non-highly attenuated strains are being used should be offered medical screening for potential contraindications to vaccinia exposure.
- Other health-care workers (such as physicians and nurses) working with vaccinia virus and whose contact with these viruses is limited to contaminated materials (for example, dressings), but who adhere to appropriate infection control measures, are probably at lower risk for inadvertent infection than laboratory workers. However, because a theoretical risk of infection exists, vaccination may be considered for this group.
- A summary of published case reports of laboratory-acquired vaccinia virus infections is available in the Journal of the American Biological Safety Association, Applied Biosafety, 10(2) 2005, p.118-122 by Karen Byers.

University of Kentucky Policy Statement:

In interest of providing a safe workplace, to comply with federal regulations, and to protect the public health, the Institutional Biosafety Committee (IBC) has formulated a policy regarding immunization for workers in laboratories using vaccinia and other pox viruses. Recommendations and requirements for vaccination will be dependent upon the strain used and procedures in the proposed research. The IBC policy incorporates national guidelines set forth by the CDC as described above and as instituted by the CDC and NIH at their own facilities.

Based on these guidelines, laboratory personnel for whom vaccination is recommended or required must receive mandatory confidential medical counseling before beginning worth with the virus. These individuals must be counseled on the risks and benefits of the vaccine and medically screened for contraindications to vaccinia exposure or vaccination.

Instructions to Principal Investigators for obtaining IBC approval to use vaccinia virus and receiving vaccination:

- Principal Investigators (PI) must register research involving vaccinia virus work with the IBC
- PI's who have proposed research activities involving vaccinia must contact University Health/Employee Health Service (UHS) to arrange for counseling of all laboratory workers who would be in a laboratory where the virus (highly attenuated or non-highly attenuated) will be manipulated or where animals infected with vaccinia are held or manipulated.
- If research will involve non-highly attenuated vaccinia virus, the PI must also contact UHS to arrange for acquisition of the vaccine and vaccination of all laboratory workers who would be working directly with the virus or with animals infected with the virus, even if they think they do not want to be vaccinated.
- Following medical consultation, the individual will decide whether to receive the vaccination or not.
    - If they decide to receive the vaccination, they will sign a vaccinia vaccination consent form and receive the vaccine (This will require follow-up visits to monitor the vaccination site.) OR,
    - If they decide not to receive the vaccine, or if UHS determines that vaccination is contraindicated, they must sign a declination form, the PI will be contacted by UHS and alterations in the

individual's lab or clinical duties and responsibilities will be made such that they will not directly handle vaccinia or animals infected with vaccinia, in order to protect the health and safety of that person, their contacts, and the public health.

- If the individual does not take the vaccination, for whatever reason, and other duties cannot be found in the laboratory such as to prevent potential contact with non-highly attenuated vaccinia, the employee must resign from his/her position. UK Human Resources will attempt to help the individual find another comparable position at the University, however a position is not guaranteed.
- IBC approval may occur before all personnel listed on the IBC registration form have been counseled and vaccinated. It is the Principal Investigator's responsibility to ensure that all personnel working directly with vaccinia have received counseling and vaccination. Documentation of medical counseling of all laboratory workers. whether they will work directly with the vaccinia virus or simply work in the laboratory, must also be present in the PI's laboratory biosafety manual. Copies of this documentation must be sent to the Biological Safety Officer.

17.2 Medical Considerations

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

Pregnancy

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

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

Reproductive Biological Hazards

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

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

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

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

Other Restrictions

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

the situation.

18.0 Minors in Research

The Institutional Biosafety Committee (IBC) and UK (University of Kentucky) Department of Research Safety requires all minors (persons under the age of 18 whether student, employee, or volunteer) who work, conduct, or observe research utilizing biohazardous materials requiring IBC registration in a UK research laboratory, greenhouse, animal facility, or farm/field location outside the UK campus to abide by the UK Division of Environmental Health & Safety Minors in Research Laboratories or Animal Facilities Program (hereinafter Minors in Research Program, available online here). Minors enrolled in the program with projects within IBC purview must also be listed on the PI/Sponsor's IBC protocol registration and complete all relevant training requirements before beginning rmiwork.

The purpose of the Minors in Research Program is to document activities and safety protocols for minors working or observing research in a UK research laboratory location, including greenhouses, animal facilities, or farm/field locations, and obtain informed consent for those activities from parents and/or guardians.

Minors under the age of 14 are NOT PERMITTED inside any research laboratory, greenhouse, animal facility, or farm/field location at the University of Kentucky unless it is for a UK sponsored program which are designed for youth under the age of 14 and which have documented training and safety policies.

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

To enroll in the program, the responsible Principal Investigator (PI) or Sponsor must complete the Minors in Research Project Registration Form. Once completed, this form is submitted for review online here.

Minors enrolled in the Minors in Research Program must complete all relevant training modules before starting or being present in research laboratory space. UK EH&S (Environmental Health & Safety) online training modules are available online here, or in the SciShield course directory here.

Minors working in research laboratories or animal facilities must be provided Laboratory Specific Training by the PI/Sponsor prior to beginning laboratory work. A checklist is available online here. A copy of Laboratory Specific Training must be maintained in the PI/Sponsor's laboratory safety manual.

The Minors in Research Project Registration form will be reviewed by the UK Department of Research Safety Biosafety and Chemical/Lab Safety Teams upon submission. Once this review is completed and all requirements met, an approval memo will be sent to the PI/Sponsor. The UK Department of Research Safety will not review incomplete submissions.