University of Louisville

Biosafety Manual

Department of Environmental Health & Safety
Revised July 2023
https://louisville.edu/dehs/biological-safety

Contents

PREFACE

The University of Louisville is committed to providing a safe and healthful working and learning environment for all faculty, students, employees, and visitors. The Department of Environmental Health and Safety's (DEHS) mission is to work in conjunction with the U of L community and ensure that education, research, and health-care related activities take place in conditions that are optimally safe and healthy for students, faculty, staff, visitors, the surrounding community and general public.

The objective of the U of L Biosafety Program is to assist personnel at all levels in fulfilling the commitment to furnish a place of employment and learning that is as free as possible from recognized hazards that cause or are likely to cause harm to U of L personnel or the surrounding community.

The purpose of this manual is to provide faculty, students, and staff with general guidelines for implementing a quality and proactive safety program regarding the use of hazardous biological agents. It is not intended to be an exhaustive reference, but rather a guide for all U of L personnel to become familiar with and conduct their operations accordingly. Further advice concerning risks associated with specific processes and the development of new or unfamiliar activities when working with hazardous biological material should be obtained through consultation with your supervisor, the U of L Institutional Biosafety Committee, and/or DEHS Biosafety Program.

All users of biological hazards must be familiar with the guidelines and requirements set forth in this manual and must conduct their operations in accordance with them.

Important Contact Information

On Campus Emergencies
Emergency 911
Non-Life Threatening Emergency 502-852-6111

Campus Health Services (CHS) https://louisville.edu/campushealth
Belknap campus 502-852-6479
Cardinal Station, 215 Central Ave., Suite 110, Louisville, KY 40208

Health Sciences Center 502-852-6446
Health Care Outpatient Center, 401 E. Chestnut., Suite 110, Louisville, KY 40202

Department of Environmental Health and Safety (DEHS) https://louisville.edu/dehs
General inquiries 502-852-6670
Director's Office 502-852-6670
Biological Safety Officer 502-852-2959
Associate Biosafety Officer 502-852-1323
Radiation Safety Officer 502-852-5231

The Radiation Safety Office is located in the Library and Commons Bldg, Rms 101-102, 319 Abraham Flexner Way, Louisville KY 40202-1819; all other DEHS offices are located in the Environmental Health & Safety Bldg, 1800 Arthur Street, Louisville, KY 40208-2729. A contact list with names of individuals is maintained on the DEHS website at https://louisville.edu/dehs/contact-us

Institutional Biosafety Committee (IBC) https://louisville.edu/dehs/biological-safety
Administrator 502-852-6670

Institutional Animal Care and Use Committee (IACUC) https://louisville.edu/research/iacuc
Coordinator 502-852-7307

Biohazardous Agent Inventory Form

The Office of Science Policy (NIH) requires that all labs have manuals specific to their locations and research. The agent inventory form, below, may be used to customize this manual to individual labs.

Principal Investigator:

Location of laboratory (bldg):

(List all microbes, human-derived materials, potential carcinogens, and toxins)

Biohazardous Agent	***Room #***

Acknowledgement of Risk and Biosafety Manual Training Form

This training documentation form may be used to customize this manual to an individual lab.

We, the undersigned (faculty, staff, and students), understand that the agents listed on our inventory form are potentially hazardous to humans and the environment. We have read and understand this manual and agree to follow the stated policies and procedures presented, along with those specifically developed by the PI for this laboratory.

Name	Signature	Date

1. Biological Risk Assessment Process

The ongoing practice of biological risk assessment is the foundation of safe laboratory operations. When assessing risk it is essential to broadly engage stakeholders, including laboratory and facility staff and subject matter experts, in committee reviews of work and discussions of past studies of laboratory-associated infections (LAIs) or release into the environment and other published research. The biological risk assessment process is used to identify the hazardous characteristics of biological agent or material, if known; the activities that can result in a person's exposure to an agent or the release of the agent into the environment; the likelihood that such exposure will cause an LAI or that the release of the agent will have a detrimental impact on local natural or managed ecosystems; and the probable consequences of such an infection or release. Risk assessments provide a guide for the selection of appropriate mitigations, including the application of Biosafety Levels and good microbiological/laboratory practices, safety equipment, and facility safeguards that can help prevent LAIs or release into the environment.

The BMBL describes a six-step approach that gives structure to the risk management process and reinforces an ongoing positive culture of safety. Other methodologies may be useful, including the process described in the WHO Laboratory Biosafety Manual.

The initial factors to consider in risk assessment fall into two broad categories: agent hazards and laboratory procedure hazards. Following the assessment of the inherent risk, the Biosafety Level and any additional indicated mitigation strategies are determined. Before implementation of the controls, the risk assessment and selected safeguards should be reviewed with a biosafety professional and/or the IBC. Then, as part of an ongoing assessment of risk management, the proficiency of staff regarding safe practices and the integrity of safety equipment is evaluated and training or competency gaps are addressed. Finally, the management strategies are revisited regularly to reassess risks so that mitigations can be updated when appropriate.

1.1. Acronyms

Acronym	Definition/Comments
ABSL	Animal Biosafety Level
BBP	Blood Borne Pathogen
BL1-P	Plant Biosafety Level 1
BL2-P	Plant Biosafety Level 2
BL3-P	Plant Biosafety Level 3
BL4-P	Plant Biosafety Level 4
BMBL	CDC/NIH published guidelines "Biosafety in Microbiological and Biomedical Laboratories", 6th ed. (https://www.cdc.gov/labs/BMBL.html)
BSC	Biological Safety Cabinet
BSL	Biosafety Level
BSL-E	Biosafety Level with Enhancements
BSL-Ag	Biosafety Level with Agricultural Requirements
BSL-1/ABSL-1	Agents not known to cause disease in healthy adults; minimal potential of risk.

Acronym	Definition/Comments
BSL-2/ABSL-2	Agents post a moderate potential of hazard for personnel and the environment. Immunization or treatments may be available for these agents or they have a low morbidity and mortality.
BSL-3/ABSL-3	Agents may cause serious or potentially lethal diseases, generally as a result of inhalation exposure. Some of these agents have treatment and preventive measures available, but there are high infection rates and significant disease complications.
BSL-4/ABSL-4	Agents that are highly infectious by aerosol and cause life threatening illness for humans and agricultural animals.
CDC	The Centers for Disease Control and Prevention
HEPA	High Efficiency Particulate Air
HVAC	Heating, ventilating and air conditioning
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
Lfpm	Linear feet per minute
NIH	The National Institutes of Health
OPIM	Other Potentially Infectious Material
OSHA	The Occupational Safety and Health Administration
OSP	The NIH Office of Science Policy
PI	Principal Investigator
PPE	Personal Protective Equipment
RG	Risk Group
SOP	Standard Operating Procedure
VHP	Vaporized Hydrogen Peroxide

1.2. Classification of Biohazardous Agents by Risk Group

The Office of Science Policy (NIH) has classified biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans based on Risk Group.

Risk Group 1 (RG-1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG-2)	Agents that are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are *often* available
Risk Group 3 (RG-3)	Agents that are associated with serious or lethal human disease for which preventative or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG-4)	Agents that are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are *not usually* available (high individual risk and high community risk)

1.2.1. Other Classifications

Plants, animals, fungi and other microbes that are not associated with disease in humans are classified according to their likely impact on the environment:

- Not known to have a significant detrimental impact on local natural or managed ecosystems
- Recognized potential for low detrimental impact on local natural or managed ecosystems
- Recognized potential for medium detrimental impact on local natural or managed ecosystems
- Recognized potential for significant detrimental impact on local natural or managed ecosystems

Examples are microorganisms or plants not endemic to the local ecosystem, plants containing recombinant or synthetic nucleic acids, and plant- or animal-associated microorganisms.

1.3. Commonly Used Infectious Agents and their Biosafety Levels

Fungal, bacterial, rickettsial, viral and parasitic agents are all commonly used in the research lab. For a list of the most common agents in each category and their corresponding biosafety level, please see the table below or visit: https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

Table Comment Codes:

BMBL	The "Biosafety in Microbiological and Biomedical Laboratories 5th Edition" should be consulted for further information about this agent.
P	Plant pathogen
SA	Select Agent (see Chapter 16 for additional information)
V	Vaccination is recommended

1.3.1. Common Fungal Agents

Fungal Agent	Biosafety Level (BSL)	Animal Biosafety Level (ABSL)	Comments
Blastomyces dermatitides	2/3	2	BMBL
Blastomyces gilchristii	2/3	2	BMBL
Candida Species	2	-	BMBL
Coccidioides immitis	2/3	2	BMBL
Coccidioides posadasii	2/3	2	BMBL
Coniothyrium glycines (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)	N/A	N/A	SA, P
Cryptococcus neoformans	2	2	BMBL

Dematiaceous Molds: Bipolaris spp.; Cladophialophora bantiana; Exophiala spp; Exserohilum rostratum; Fonsecaea spp.; Pseudallescheria spp.; Rhinocladiella spp.; Scedosporium spp.; Verruconis (Ochroconis	2	-	BMBL
Dermatophyte molds: Trichophyton, Microsporum, Epidermophyton species	2	–	BMBL
Epidermophyton - pathogenic sp.	2	2	BMBL
Histoplasma capsulatum	2/3	2	BMBL
Hyaline Molds: Aspergillus spp., Fusarium spp	2	-	BMBL
Microsporum - pathogenic sp.	2	2	BMBL
Mucormycete molds: Mucor spp.; Rhizopus spp.; Rhizomucor spp.; Lichtheimia (Absidia) spp	2	-	BMBL
Paracoccidioides brasilienisis	2	2	-
Peronosclerospora philippinensis	N/A	N/A	SA, P
Sclerophthora rayssiae	N/A	N/A	SA
Sporothrix schenkii	2	2	BMBL
Synchytrium endobioticum	N/A	N/A	SA, P
Talaromyces (Penicillium) marneffei	2	-	BMBL
Trichophyton - pathogenic sp.	2	2	BMBL
Candida albicans	2	2	-
Miscellaneous Molds	2	-	BMBL

1.3.2. Common Bacterial Agents

Bacterial Agent	Biosafety Level (BSL)	Animal Biosafety Level (ABSL)	Comments
Acinetobacter calceticus	2	2	-
Actinomyces sp.	2	2	-
Anaplasma sp.	2	2	-
Aeromonas sp.	2	2	-
Arachnida propionica	2	2	-
Bacillus alvei	2	2	-
Bacillus anthracis	2/3	2	BMBL, V, SA

Bacillus cereus, Biovar anthracis			SA
Bacteroides sp.	2	2	-
Bartonella sp.	2	2	-
Bordetella sp.	2	2	-
Bordetella pertussis	2/3	2	BMBL
Borrelia sp.	2	2	-
Brucella sp.	2/3	3	BMBL, SA
Burkholderia mallei	2/3	2/3	BMBL, SA
Burkholderia pseudomallei	3	3	BMBL, SA
Camplyobacter sp.	2	2	BMBL
Chlamydia	2/3	2/3	BMBL
Clostridium botulinum	2/3	2	BMBL
Clostridium tetani	2	2	BMBL
Corynebacterium diphtheriae	2	2	BMBL
Corynebacterium equi	2	2	-
Corynebacterium haemolyticum	2	2	-
Corynebacterium pseudotuberculosis	2	2	-
Corynebacterium pyogenes	2	2	-
Corynebacterium renale	2	2	-
Coxiella burnetti	3	3	BMBL, SA
Enterobacteriaceae, all other	2	2	-
Erysipelothrix rhusiopathiae	2	2	-
Escherichia coli	2	2	-
Escherichia coli K12 derivative	1	1	-
Francisella tularensis	2/3	3	BMBL, SA
Fusobacterium sp.	2	2	-
Haemophilus sp.	2	2	-
Klebsiella sp.	2	2	-
Legionella pneumophila	2/3	2	BMBL
Leptospira interrogans all serovars	2	2	BMBL
Listeria sp.	2	2	-
Moraxella sp.	2	2	-
Mycobacterium avium	2	2	-
Mycobacterium bovis	2/3	2/3	BMBL
Mycobacterium leprae	2	2	BMBL

Mycobacterium sp.	2	2	BMBL
Mycobacterium tuberculosis	2/3	2/3	BMBL
Mycoplasma capricolum and *mycoides*	2	2	BMBL
Neisseria gonorrhoeae	2/3	2	BMBL
Neisseria meningitidis	2/3	2	BMBL
Nocardia sp.	2	2	-
Pasteurella sp.	2	2	-
Pseudomonas mallei	2/3	2/3	BMBL
Pseudomonas testoserone	2	2	-
Ralstonia solanacearum	N/A	N/A	P
Rathayibacter toxicus	N/A	N/A	SA, P
Rhodococcus (Coryne.) equi	2	2	-
Salmonella sp.	2	2	BMBL
Salmonella typhi	2/3	2/3	BMBL
Shigella sp.	2	2	BMBL
Staphylococcus sp.	2	2	-
Streptococcus sp.	2	2	-
Streptocacillus moniliformis	2	2	-
Streptomyces somaliensis	2	2	-
Treponema pallidum	2	2	BMBL
Vibrio sp.	2	2	BMBL
Xanthomonas oryzae	N/A	N/A	SA, P
Yersinia pestis	2/3	2/3	BMBL, V, SA

1.3.3. Common Rickettsial Agents

Rickettsial Agent	Biosafety Level (BSL)	Animal Biosafety Level (ABSL)	Comments
Rickettsia akari	2/3	2/3	-
Rickettsia australis	2/3	2/3	BMBL
Rickettsia canada	2/3	2/3	BMBL
Rickettsia conorii	2/3	2/3	BMBL
Rickettsia prowazekii	2/3	2/3	BMBL, SA
Rickettsia rickettsii	2/3	2/3	BMBL

Rickettsia siberica	2/3	2/3	BMBL
Rickettsia tsutsugamushi	2/3	2/3	BMBL
Rickettsia typhi (*R. mooseri*)	2/3	2/3	BMBL
Rochalimaea quintana	2	2	-
Rochalimaea vinsonii	2	2	-
Spotted Fever Group - other	2/3	2/3	-

1.3.4. Common Viral Agents

Viral Agent	Biosafety Level (BSL)	Animal Biosafety Level (ABSL)	Comments
Adenoviruses	2	2	-
Adenoviruses - animal - all	2	2	-
African horse sickness virus	3	3	BMBL, SA
African swine fever virus	3	3	BMBL, SA
Arboviruses - certain	2	2	BMBL
Arboviruses - certain	2	2	BMBL
Arboviruses - certain	3	3	BMBL
Arboviruses - certain	4	4	BMBL
Arenaviruses - certain	3	3	BMBL
Arenaviruses - certain	4	4	BMBL
Avian Erthyroblastosis Virus	2	2	-
Avian Influenza Virus	3	3	BMBL, SA
Avian Leukosis Virus	2	2	-
Avian Lymphomatosis Virus	2	2	-
Avian Myeloblasotosos Virus	2	2	-
Bovine Encephalomyelitis Virus	2	2	-
Bovine Leukemia Virus	2	2	-
Bovine Respiratory Syncytial	2	2	-
Bovine Rhinotracheitis (IBR)	2	2	-
Cache Valley Virus	2	2	-
Canine Hepatitis Virus	2	2	-
Canine Distemper Virus	2	2	-
Caprine Arthritis	2	2	-
Classical Swine Fever Virus	3	3	BMBL, SA

Coxsackie A & B Viruses	2	2	-
Crimean-Congo Haemorrhagic Fever Virus	4	4	SA
Eastern Equine Encephalitis Virus	3	3	SA
Ebola Virus	4	4	SA
Encephalomyelitis Virus	2	2	
Echovirus	2	2	-
Dengue Virus	2	2	BMBL
Encephalomyocarditis Virus	2	2	-
Epidemic Diarrhea Infant Mice	2	2	-
Epstein-Barr Virus	2	2	-
Feline Leukemia Virus	2	2	-
Feline Sarcoma Virus	2	2	-
Filoviruses	2-4	2-4	-
Flanders Virus	2	2	BMBL
Foot-and-Mouth Disease Virus	3	3	BMBL, SA
Gibbon Ape Lymphosarcoma Virus	2	2	-
Goat Pox Virus	3	3	BMBL, SA
Hart Park Virus	2	2	BMBL
Hendra Virus	4	4	BMBL, SA
Hepatitis A Virus	2	2	BMBL
Hepatitis E Virus	2	2	BMBL
Hepatitis B Virus	2/3	2	BMBL
Hepatitis C Virus	2/3	2	BMBL
Hepatitis D Virus	2/3	2	BMBL
Herpesvirus - other	2	2	-
Herpesvirus Ateles	2	2	-
Herpesvirus Saimir	2	2	-
Herpesvirus Simiae (B-virus)	3/4	3/4	BMBL
Human Herpesviruses	2	2	BMBL
Hog Cholera Virus	2	2	BMBL
Human T-Cell Leukemia Virus I/ II	2	2	-
Infectious Bronchitis Virus	2	2	-
Influenza Virus	2/3	2/3	BMBL
K (Rate) Virus	2	2	-
Kyasanur Forest Disease Virus	4	4	BMBL, SA

Lactic Dehydrogenase Elevating Virus	2	2	-
Langat Virus	2	2	BMBL
Laryngotracheitis Virus	2	2	-
Lassa Fever Virus	4	4	BMBL, SA
Lumpy Skin Disease Virus	3	3	BMBL, SA
Lujo Virus	4	4	SA
Lymphocytic Choriomeningitis Virus	2/3	2/3	BMBL
Marburg Virus	4	4	BMBL, SA
Measles Virus	2	2	-
Memingopneumonitis Virus	2	2	-
Mouse Encephalomyelitis Virus	2	2	-
Mouse Hepatitis Virus	2	2	-
Mouse Leukemia Virus	2	2	-
Mouse Pneumonia Virus	2	2	-
Mumps Virus	2	2	-
Myxomatosis Virus	2	2	-
Newcastle Disease Virus	2	2	-
Newcastle Disease Virus (VVND)	2	2	SA
Nipah Virus	4	4	BMBL, SA
Non-Defective Adenovirus 2SV40	2	2	-
Omsk Hemorrhagic Fever Virus	4	4	BMBL, SA
Papilloma Virus Shope	2	2	-
Parainfluenza Virus	2	2	-
Peste Des Petits Ruminants Virus	3	3	BMBL, SA
Poliovirus - all types	2	2	BMBL
Polyoma Virus	2	2	-
Poxvirus Alastrim	2	2	-
Poxvirus Monkey Pox	3	3	V
Poxvirus sp.	2	2	BMBL
Pseudorabies Virus	2	2	-
Rabies Virus	2/3	2/3	BMBL
Reovirus sp.	2	2	-
Respiratory Syncytial Virus	2	2	-
Retroviruses, including HIV & SIV	2/3	2/3	BMBL
Rhinovirus sp.	2	2	-

Rift Valley fever virus	3	3	BMBL, SA
Rinderpest Virus	3	3	BMBL, SA
Rous Sarcoma Virus	2	2	-
Rubella Virus	2	2	-
SARS-associated Coronavirus	3	3	BMBL, SA
Sheep Pox Virus	3	3	BMBL, SA
Simian Virus - other	2	2	-
Simian T-Cell Leukemia Virus	2	2	-
Sindbis Virus	2	2	-
Slow Viruses	2	2	-
South American Hemorrhagic Fever viruses	3, 4	3, 4	Bmbl, SA
Swine Vesicular Disease Virus	3	3	BMBL, SA
Tensaw Virus	2	2	-
Tick-Borne Encephalitis Complex Viruses	4	4	SA
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru, and related agents)	2/3	2/3	BMBL
Turlock Virus	2	2	-
Vaccinia Virus	2/3	2/3	V
Variola Major Virus (Smallpox virus)	4	4	BMBL,SA
Variola Minor Virus (Alastrim)	4	4	BMBL, SA
Venezuelan Equine Encephalitis Virus	3	3	SA
Vesicular Stomatitis Virus	3	3	BMBL
Vesicular Somatitis Virus (Lab adapted)	2	2	BMBL
Woolly Monkey Fibrosarcoma	3	3	-
Yaba Virus	2	2	-
Yellow Fever Virus 17D Strain	2	2	BMBL
Yellow Fever Virus – non17D	3	3	BMBL

1.3.5. Common Parasitic Agents

Parasitic Agent	Biosafety Level (BSL)	Animal Biosafety Level (ABSL)	Comments
Anaplasma sp.	2	2	-
Ascaris sp.	2	2	BMBL

Coccidia sp.	2	2	BMBL
Cryptosporidia sp.	2	2	BMBL
Echinococcus Granulosus	2	2	BMBL
Ehrlichia sp.	2	2	-
Entamoeba sp.	2	2	BMBL
Enterobius sp.	2	2	BMBL
Fasciola sp.	2	2	BMBL
Giardia sp.	2	2	BMBL
Haemobartonella sp.	2	2	-
Hymenolepsis nana	2	2	BMBL
Leishmania sp.	2	2	BMBL
Leukocytozoon sp.	2	2	BMBL
Naegleria sp.	2	2	-
Plasmodium sp.	2	2	BMBL
Sarcocystis sp.	2	2	BMBL
Schistosoma sp.	2	2	BMBL
Strongyloides sp.	2	2	BMBL
Taenia solium	2	2	-
Toxocara canis	2	2	-
Toxoplasma sp.	2	2	BMBL
Trichinella spiralis	2	2	BMBL
Trypanosoma sp.	2	2	BMBL

2. General Responsibilities

2.1. Deans, Directors and Department Heads

Have the primary responsibility for ensuring that this document is followed by all of their employees who have access to laboratories, work in laboratories, or assign people to work in laboratories.

Their responsibilities are:

- Support the practices outlined in this U of L Biosafety Manual to ensure the health and safety of the university faculty, staff, students, and visitors while collaborating with faculty and staff to implement this plan.
- Support in resolving DEHS personnel in resolving biosafety and laboratory assessment findings as well as regulatory agency findings.
- Ensure that all faculty, staff, and students complete the appropriate laboratory and biosafety training before beginning work in a laboratory both general training through DEHS and training that is lab-specific as given by the Principal Investigator.

2.2. Principal Investigator (PI)

PIs must submit an IBC registration before working with any biological agents!

Their responsibilities are:

- Prior to initiating research involving hazardous biological materials, conduct a risk assessment to:
    - identify hazardous characteristics of the agent and perform an assessment of the inherent risk, (risk in the absence of mitigating factors);
    - identify laboratory procedure hazards; make a determination of the appropriate Biosafety Level and select additional precautions indicated by the risk assessment;
    - review the risk assessment and plan to implement safeguards with a biosafety professional, subject matter expert, and the IBC or equivalent resource;
    - revisit regularly and verify risk management strategies to determine if changes are necessary.
- Ensure that laboratory and support personnel receive appropriate training for the potential hazards associated with the work to be performed, the necessary precautions to prevent exposures, and the exposure evaluation procedures
    - as part of an ongoing process, evaluate the proficiencies of all staff regarding safe practices and the integrity of safety equipment.
- Ensure personal protective and safety equipment is provided and properly used.
- Ensure compliance by laboratory personnel with the relevant federal, state, and local regulations, as well as institutional guidelines, and policies.
- Submit a report on the following types of incidents to any member of the Institutional Biosafety Committee (IBC), DEHS Director or Biosafety Officer :
    - Any significant research-related accident or illness;
    - Any violation of the *NIH Guidelines* such as:
        - failure to register and/or obtain approval of the IBC prior to initiation of research and/or clinical studies involving rDNA or SNA;

        - significant changes to proposed research risk without prior notification and approval by the IBC;
        - failure to renew a registration and obtain IBC approval, prior to the expiration date, for research and/or clinical studies involving rDNA or SNA (i.e. lapse in protocol approval);
    - Spills or accidents resulting in overt exposure (e.g. skin punctures with needles containing rDNA or SNA, exposure of broken skin or mucous membranes), injury, or illness at Biosafety Level 2 (BSL-2);
    - Spills or accidents occurring in Biosafety Level 3 (BSL-3) laboratories outside of a biosafety cabinet;
        - report and document near misses, laboratory accidents, exposures, unanticipated absences due to potential laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses
    - Violations of the *NIH Guidelines* containment or biosafety practices, or significant problems leading to a breach of containment (including improper disposal of rDNA or SNA materials or escape of a transgenic animal);
    - Failures in compliance with institutional and federal regulations, guidelines, and policies that result in unsafe conditions related to the use of hazardous biological agents and materials.

2.3. Laboratory Personnel

Their responsibilities are:

- Before working in the laboratory complete all relevant safety training and instruction given by DEHS, and all laboratory-specific safety training given by the Principal Investigator.
- Ensure that:
    - all known biological hazards are appropriately identified and mitigated.
    - labels on materials and equipment in the laboratory are current and accurate.
    - individuals are knowledgeable of decontamination and emergency procedures.
- Segregate and collect laboratory wastes in accordance with applicable regulations (local, state and federal) and maintain pertinent records per regulatory requirements.
- Consult with DEHS for guidance regarding best safety practices.
- Comply with the biosafety guidelines and procedures outlined in this manual and lab-specific standard operating procedures provided by the laboratory PI.
- Report all near misses, accidents, spills, or exposure incidents to your immediate supervisor.

3. General Work Practices

Many laboratory procedures can be safely performed on the open laboratory bench utilizing good microbiological techniques and appropriate personal protective equipment (PPE), provided the potential for producing splashes or aerosols is low. This type of work is restricted to RG-1 or for certain IBC approved RG-2 agents.

Work is not permitted on an open laboratory bench if the procedures may produce aerosols or splashes when working the RG-2 agent or if the work involves a RG-3 agent. Aerosol producing procedures or experiments using RG-2 agents must be carried out in biological safety cabinets. Experiments using RG-3 agents must be carried out in biological safety cabinets in special containment laboratories.

3.1. Standard Microbiological Practices

- Access to the laboratory is restricted when work is in progress. Access may be restricted by locking doors, posting warning signs or using other suitable methods as determined by the Principal Investigator and DEHS. Restriction methods will vary depending on the biosafety level of the organism.
- A biohazard sign must be posted on the entrance to the laboratory. Posted information should include the agent(s) in use, the biosafety level, the investigator's name and telephone number, and any personal protective equipment that must be worn in the laboratory. In open laboratory spaces, where multiple labs are contiguous with each other without walls or doors, signs should be posted at the entrances to the shared space.
- Procedures with a potential for creating infectious aerosols or splashes must be conducted in a certified biological safety cabinet (BSC). Aerosol producing procedures may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonated eggs.
- Persons should wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Food and drink must be stored outside the laboratory.
- Mouth pipetting is prohibited.
- All procedures are carefully performed to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated with disinfectants that are effective against the agents present upon completion of work or at the end of the day and after any spill or splash of viable material.
- Chairs and other furniture are covered with a non-fabric material, allowing easy decontamination.
- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during handling, processing and storage.
- Contaminated equipment must be decontaminated before it is sent for repair or maintenance and before removal from the laboratory.
- Spills and accidents that result in overt exposures to infectious materials are immediately reported to the Principal Investigator and DEHS.
- Animals and plants not involved in the work being performed are not permitted in the lab.
- Laboratory coats or gowns are worn while work is performed in the laboratory. Lab coats are removed before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective

clothing is either disposed of in the laboratory or laundered by an approved outside vendor. Lab coats are never taken home by personnel. Prior to sending out for laundering, contaminated coats must first be decontaminated by autoclaving or soaking in a freshly prepared 10% bleach solution for 10 minutes.

- Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised.
    - Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and should NOT be worn outside the lab. Hands are washed following removal of gloves.
- Face protection (safety glasses, goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when BSL-2 agents must be manipulated outside the BSC. When working with BSL-3 agents respiratory protection is required.

4. Safety Practices and Equipment

Proper application of biosafety results in a safe work environment for all personnel and ensures that the work being done does not adversely impact the surrounding environment. It is essential to understand that the **most important factor in biosafety is the laboratory worker**. **Good work practice, facility design, training, and protective clothing provide no benefit to individuals who are unwilling to follow proper work procedures.**

There are obvious dangers to working with infectious agents. Pathogens can infect a host through a number of routes, and it is important to be aware that a laboratory-acquired infection may not follow the same route as a naturally occurring infection. The following are some of the more common routes of exposure that can result in infection:

- Spills and splashes
- Needle sticks
- Sharp objects (including glass)
- Animal bite or scratch
- Mouth pipetting

4.1. Standard Precautions

The concept of Standard Precautions is to treat all human/primate blood and other body fluids, tissues and cells (including established cell lines) as if they were known to be infectious. Standard precautions are frequent hand washing, no mouth pipetting, no food or drink in the lab and proper disposal of medical/infectious waste as well as engineering controls and personal protective equipment (PPE).

Engineering controls are biological safety cabinets, ventilation systems, closed top centrifuge rotors, etc. – these are the primary methods to control exposure. PPE such as gloves, lab coats and eye protection must be selected and appropriately used.

4.2. Safety Engineered and Needleless Sharps

Over the last few years manufacturers have developed "engineered sharps"; these are commonly used items (e.g. scalpels, syringes, needles) that have various mechanical devices to vastly decrease the occurrence of injuries due to sharps. The Occupational Safety and Health Administration (OSHA) requires any laboratory using human or primate blood, blood products, cell lines, tissues or other potentially infectious materials (OPIM) to use needleless systems and/or engineered sharps.

4.3. Biosafety Cabinets

Biological safety cabinets (BSC) are designed to provide three types of protection:

- Personnel protection from material inside the cabinet.
- Protection for the material inside of the cabinet.
- Protection for the environment from the material inside of the cabinet.

Biological safety cabinet installation and certification must be performed by an NSF certified professional. Call DEHS if you need assistance in identifying a vendor to meet your needs. Scheduling and payment for installation, certification, and/or repairs must be scheduled by the PI or the department (some departments coordinate this effort for their researchers).

See Chapter 7 for more information regarding BSC.

4.4. Signs and Hazard Communication

All laboratories must have a sign on the outside of the door indicating that biohazardous material is used within the room. Investigators who are using RG-2 or RG-3 agents are required by the National Institutes of Health (NIH) to post a sign on the outer laboratory door that incorporates the universal biohazard symbol. The sign must include information regarding the agent name and specific requirements for entry, the PI's name and spaces for two phone numbers of laboratory staff in case emergency contact must be made. Laboratory door signs are available through DEHS.

The Bloodborne Pathogen Standard also requires that red-orange coded biohazard labels be placed on storage freezers, refrigerators, and any laboratory equipment used with BSL-2 or 3 agents, shipping containers, medical/infectious waste containers or any surface which may be reasonably anticipated to encounter surface contamination from biohazardous materials. Biohazardous labels are available through DEHS.

5. Biosafety Containment Levels

The CDC and Office of Science Policy (NIH) have established biosafety levels for work with biohazardous materials in the publication Biosafety in *Microbiological and Biomedical Laboratories* (BMBL), 6th ed. The BMBL describes combinations of microbiological practices, laboratory facilities, and safety equipment in combination with four biological safety levels for various agents infectious to humans The descriptions of biosafety levels (BSL) 1–4 in the BMBL parallel those in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Each biosafety level of containment/practices builds upon the former. Animal biosafety levels (ABSL) are also described for research involving the use of infectious

agents in laboratory animals. It is important to note that the BMBL guidelines are minimal containment requirements, and should be modified based on the risk assessment.

The Office of Science Policy has also established plant biosafety levels (BL1-P through BL4-P) for experiments using plants, including their plant-associated microorganisms and small animals, any one of which may be genetically modified. A detailed description of plant biosafety in research greenhouses can be found in "A Practical Guide to Containment / Plant Biosafety in Research Greenhouses".

No work above A/BSL 1-3 or BL1 – 2P is permitted at U of L. UofL currently does not have facilities to accommodate research that requires A/BSL-4, BL3-P, or BL4-P.

5.1. Biosafety Level 1-4

The table below describes the biosafety containment levels; more detailed information is available in the BMBL, 6th ed., and in the NIH Guidelines:

BSL	Agents are …	Practices	Primary Barrier and PPE	Facilities (secondary Barrier)
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation

BSL	Agents are …	Practices	Primary Barrier and PPE	Facilities (secondary Barrier)
				system with negative airflow into laboratory; autoclave available, preferably in laboratory
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Not permitted at UofL	Not permitted at UofL	Not permitted at UofL
	U of L: • **Does not have any laboratories certified for BSL-4** • **Does not allow possession or use of biohazardous materials requiring this level**			

The biosafety level may be:

- Equivalent to the risk group (RG) classification of the agent
- Raised or lowered based on the evaluation of risk factors

The IBC:

- Makes the final determination of the appropriate biosafety level
- Answers any questions regarding the risk assessment or appropriate containment level

5.2. Animal Biosafety Level 1-4

Below is a summary of animal biosafety levels (ABSL) for protocols using animals; more detailed information is available in the BMBL and the NIH Guidelines.

Animal Biosafety Level	Agents are...	Practices	Primary Barriers and PPE	Facilities (Secondary Barriers)

ABSL-1	Not known to cause disease in healthy human adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility: • Non-recirculation of exhaust air • Directional air flow recommended
ABSL-2	Associated with human disease Hazard: • Percutaneous exposure • Ingestion • Mucous membrane exposure	ABSL-1 practices plus: • Limited access • Biohazard warning signs • Sharps precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus: • Primary barriers • Containment equipment appropriate for animal species PPE: • Laboratory coats • Gloves • Face and respiratory protection as needed	ABSL-1 facility plus: • Autoclave available • Handwashing sink available in the animal room
ABSL-3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects	ABSL-2 practices plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols • PPE: Appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double door access • Sealed penetrations • Sealed windows • Autoclave available in facility
ABSL-4	Dangerous or exotic agents which pose high risk of life-threatening disease or aerosol transmission Related agents with unknown risks of transmission	Not permitted at UofL	Not permitted at UofL	Not permitted at UofL
	<u>U of L:</u> • **<u>Does not have any laboratories certified for ABSL-4</u>** • **<u>Does not allow possession or use of biohazardous materials requiring this level</u>**			

The animal biosafety level may be:

- Equivalent to the risk group (RG) classification of the agent,
- Raised or lowered based on the evaluation of risk factors .

The IBC:

- Makes the final determination of the appropriate animal biosafety level.

5.3. Plant Biosafety level 1-4

The table below describes plant biosafety containment levels (from "A Practical Guide to Containment / Plant Biosafety in Research Greenhouses" and NIH Guidelines); more detailed information is available in the NIH Guidelines and "A Practical Guide to Containment: Plant Biosafety in Research Greenhouses".

Biosafety Level	**Agents are ….**	**Practices**	**Primary Barriers and PPE**
BL-1P	Transgenic plants in which there is no evidence that the modified organism would be able to survive and spread in the environment and, if accidentally released, would pose no environmental risk. Sterile plants or those rendered non-propagative. DNA-modified common microorganisms, arthropods, or small associated with plants that cannot spread rapidly and are not known to have any negative effects on plants in either natural or managed ecosystems.	Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress. A record shall be kept of experiments currently in progress in the greenhouse facility. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms. Arthropods and other motile macro-organisms shall be housed in appropriate cages. If macro-organisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.	The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

BL-2P	Transgenic plants and associated organisms, which, if released outside the greenhouse, could be viable in the surrounding environment but would have a negligible impact or could be readily managed. Entire genome of an indigenous infectious agent or pathogen potentially harmful to the environment but manageable, or are exotic but have no potential for causing serious harm to managed or natural ecosystems. Plant-associated transgenic insects or small animals if they pose no threat to managed or natural ecosystems.	BL-1P plus these additional practices: • Access to the greenhouse is limited or restricted to individuals directly involved with the experiments when they are in progress • Personnel shall be required to read and follow instructions on BL2P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms • A greenhouse practices manual shall be prepared or adopted • A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility • A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility • An autoclave shall be available for the treatment of contaminated greenhouse materials.	Greenhouse floor must be composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation. BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macro-organisms in a manner that satisfies the intent of the foregoing clauses.

<table>
<tr><td rowspan="2">BL-3P</td><td>Transgenic plants, plant pathogens, or other organisms that have a recognized potential for significant detrimental impact on the environment.

Plant associated exotic infectious agents capable of causing serious environmental harm.

Transgenic plants containing genes from an exotic infectious agent in which a complete functional genome of the infectious agent could possibly be reconstituted.

Transgenic plants or organisms that contain genes coding for vertebrate toxins.

Transgenic microbial pathogens of insects or small animals that associate with plants, if the pathogen has the potential to cause harm to the local environment.</td><td>Not permitted at UofL</td><td>Not permitted at UofL</td></tr>
<tr><td colspan="3"><u>U of L:</u>
• <u>Does not have any laboratories certified for BL-3P</u>
• <u>Does not allow possession or use of biohazardous materials requiring this level</u></td></tr>
<tr><td rowspan="2">BL-4P</td><td>Certain exotic, readily transmissible infectious agents that are potentially serious pathogens of major US crops

Human pathogens or vaccines made in plants could, in some cases, cause serious human illness</td><td>Not permitted at UofL</td><td>Not permitted at UofL</td></tr>
<tr><td colspan="3"><u>U of L:</u>
• <u>Does not have any laboratories certified for BL-4P</u>
• <u>Does not allow possession or use of biohazardous materials requiring this level</u></td></tr>
</table>

The plant biosafety level may be:

- Raised or lowered based on the evaluation of risk factors.

The IBC:

- Makes the final determination of the appropriate plant biosafety level.

5.4. Space Sharing and Signage

Space in a research environment is always an issue. This leads to space sharing among investigators and agents. Space sharing occurs at all biohazard levels, in fact the higher the containment level the more space sharing occurs, due to the cost of building and maintaining such facilities.

Always read biohazard signs posted at the entrance of the laboratories. Be aware of potential restrictions (permanent or temporary) and added PPE required when entering a room. Ensure also that you have proper authorization prior to entering the room. Special restrictions can be placed on room access when certain agents are in use. These restrictions are there to protect you.

Always be cautious when entering a room as you may not know what agent or procedure is being or was performed and if there are any risks of infection. While working in the lab, make sure personnel present in the lab, or who might enter the lab are made aware of any potential hazard.

6. Personal Protective Equipment

Personal protective equipment (PPE) is used in any laboratory environment to protect the user from potentially harmful biological and chemical exposures. Proper PPE needs to be determined and approved prior to starting any type of work and will vary depending on the person conducting the research and the type of research conducted. All PPE must be selected with the goal of providing protection from a hazard.

Selection of alternate choices of PPE should be considered if the user is at risk of physiological discomfort (e.g., contact dermatitis from latex gloves, asthma from wearing a particular type of face mask). Proper training on the use and function of personal protective equipment is required PRIOR to implementation. Consultation or advice on PPE is provided by DEHS.

PPE will be provided without personal cost to all individuals who are at risk of occupational exposures. All PPE must be inspected, cleaned, or replaced as needed. PPE will be chosen based on the anticipated exposure to blood or other potentially infectious material (OPIM). The protective equipment will be considered appropriate only if it does not permit blood or other potentially infectious materials to pass through or reach the individual's clothing, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used.

All PPE shall be removed prior to leaving the work areas and placed in designated areas for disinfection or disposal. ***At no time will personnel be permitted to take home any PPE, including lab coats, for laundering or cleaning.***

6.1. Laboratory Coat

There are different types of laboratory coats that can be used for specific needs.

The lab coat is usually made of cotton material and closes in the front. Disposable coats are also available. Different types of coats are available for different types of protection and great care should be taken when ordering a coat. The coat needs to be worn closed whenever handling biological or chemical reagents, whether on a benchtop or in a biological safety cabinet or chemical fume hood. It is not recommended to wear the lab coat outside of the laboratory. This style lab coat should be used in a BSL-1 or BSL-2 laboratory.

The cover gown can be made of cotton material or disposable material and closes in the back. Again, multiple choices are available such as type of material the gown is made of, the cuffs and the way in which the gowns are tied. In BSL-3 and ABSL-3 laboratories all lab gowns need to close in the back and have tight cuffs at the wrists. The gown must be put on when entering the laboratory and removed prior to exiting. In the (A)BSL-3 only disposable cover gowns are to be used. Back closing cover gowns are used in animal housing facilities.

A full body suit may be needed for some high risk experiments. These suits are frequently made of fluid-impervious material. A fluid-impervious material suit might be worn in the cage wash facilities of a vivarium to protect clothes from getting wet, or in ABSL-3 labs to provide additional coverage protection for high consequence pathogens.

Non-disposable laboratory protective clothing should be decontaminated by autoclaving before laundering. Laundering protective clothing must be done in-house or by a professional service and must NOT be taken home for cleaning. **Disposable protective clothing should also be autoclaved.**

Animal facilities at U of L do recycle zippered Tyvek or SMS (spunbond meltdown spunbond) jumpsuits/coveralls from facilities that are ABSL-1. These facilities contain no human pathogens and jumpsuits/coveralls are used more to protect the animals, than the researchers. To support U of L's sustainability effort, gowns from these facilities are autoclaved and recycled until visibly worn or visibly contaminated.

6.2. Gloves

Gloves are used both to protect the user and the material from contamination. All personnel engaged in activities that may involve skin contact with potentially infectious agents or materials must wear gloves. Gloves are also required for laboratory workers with dermatitis or other lesions on the hands who may have direct or indirect contact with OPIM. Handwashing with soap and water must be a routine practice immediately after direct contact with potentially infectious materials and on completion of work, even when gloves are worn.

Gloves should be removed before touching common equipment (phone, doorknobs, computer, and appropriate laboratory equipment) to prevent contamination. Gloves must be replaced frequently and immediately if they become contaminated or damaged in any way.

Gloves come in many different materials (latex, nitrile, butyl rubber, neoprene, Norfoil, Viton, polyvinyl chloride, polyvinyl alcohol) and most any are suitable for biological work. The main differences will be in personal choice. The material tends to define the sensitivity and dexterity of the user. Latex gloves are the most common choice for use with biological and water-based materials. However, latex gloves are also a common cause or trigger of latex allergies. Nitrile gloves are a typical replacement for those who can't wear latex gloves. Nitrile gloves are good for work with solvents, oils, and some acids and bases. Additionally, because tears and breaks are readily identified with nitrile gloves, they are a popular choice for work in animal facilities.

In certain instances, due to the nature of the work, gloves made out of specific material might be needed, recommended or not allowed. This may include work with chemicals that might melt the gloves such as Xylene, or the use of high-risk material such as scissors or needles. For further information please discuss with project PI and/or contact DEHS.

Gloves must NOT be worn in hallways. If you carry an item (e.g., a test tube containing infectious agents) to an equipment room, the item should be placed in a secondary sturdy clean container and can be transported without wearing gloves. Gloves would then be put on at the destination to handle the item containing infectious material.

6.3. Safety Glasses/Face Shield

Protective eyewear or face shields must be worn in laboratories where it is reasonably possible that research procedures may create splashes of infectious materials or other hazardous materials.

6.4. Head Cover/Shoe Cover

Head and shoe covers are typically used by workers in animal facilities. Head covers protect the user from trapping animal-generated aerosols or other biohazardous material in their hair. Shoe covers are used to prevent transportation of hazardous bedding/material. Head and shoe covers should be donned at the appropriate entrance station and then removed prior to leaving the space.

Laboratory clean rooms may also require head and foot covers to preserve the "cleanliness" of the room and prevent outside contaminants from being introduced. In this case, items are donned at the door prior to entering the clean room and removed upon exit.

6.5. Respiratory Protection

The UofL Respiratory Protection Program applies to all university employees who are required to use or voluntarily wear respirators during work, for both routine tasks and emergencies. Major requirements of the program include hazard assessment, medical evaluation and qualification, fit testing, training and a unit-specific written respiratory protection plan.

Selecting and using respirators can be a complicated procedure requiring hazard evaluation, and an understanding of OSHA regulations. Therefore, DEHS must conduct a hazard assessment to confirm if respiratory protection is indicated. OSHA requires that exposures first be reduced through engineering and administrative controls. The use of respirators is only allowed if other controls are in the process of being installed or implemented or if these controls are inadequate or not feasible.

When it is determined respirators will be used, DEHS can assist you in choosing the type of respirator that will offer the best protection. DEHS also provides the required training and fit testing. The written plan must contain work site-specific procedures and hazard assessments addressing the hazards in the work area. DEHS is available to assist supervisors with this requirement, and has developed a template to be used to create a Respiratory Protection Plan customized to their specific needs. Employees using filtering face piece respirators (paper dust masks) in a voluntary use situation are exempt from most, but not all, requirements of the OSHA standard.

Questions regarding the Respiratory Protection Program can be directed to DEHS at 852-6670.

6.5.1. Surgical Masks

Single-use surgical masks are used mainly to protect the field of work and offers ***minimal*** protection to the user. They can be worn when non-respiratory tract infectious agents are in use. Dispose of properly and wash hands thoroughly with soap and water after handling.

6.5.1.1. N95 and N100 Respirators

N95 respirators are used to protect the user from biological agents. They offer 95% protection to the users and material and are in the N category of respiratory protection (non-oil proof). N100 respirators offer ~100% protection to the users and the material. These respirators can be tested for protection efficiency (contrary to the surgical mask). There are wide varieties of respirators available to fit each individual. These respirators are the minimum required respiratory protection for work in the ABSL/BSL-3 labs.

Prior to using an N95 or N100 respirator, you will need to be "fit tested" by DEHS to determine which type and brand of N95 is suitable for you. Facial hair, such as mustaches and beards, renders the N95 inefficient and, therefore, other respiratory protection measures will have to be determined.

6.5.2. Face Respirators

Half or full-face respirators offer even better protection (100%) to the users as the air breathed is filtered by a HEPA filter. Face-fitting respirators are in the P category (oil resistant, air purifiers). The half-face respirator will only protect the respiratory tract mucosa and protective eyewear will have to be worn to protect the ocular mucosa. Most of the facial skin is not protected by this means. This type of respirator is needed when an N95 respirator does not fit, when using highly hazardous biological and chemical agents or there is a high risk of aerosol production. The full-face respirator will protect the facial skin and mucosa against aerosols and liquid projection.

Both of these types of respirators require a medical exam by Campus Health Services of the person intending to wear them as well as the need to be "fit tested" by DEHS to ensure proper protection for each individual. As with the N95, facial hair does interfere with these systems.

6.5.3. Powered Air Purifying Respirator (PAPR)

Powered Air Purifying Respirators (PAPR) also offer 100% protection to the user due to a HEPA filter system that is battery powered. This system can be used by anyone and does not require "fit testing", but does require an annual medical review by Campus Health Services. It can be used if facial hair is a limitation for other types of protection. Proper training on the use and maintenance of the PAPR is needed and can be provided by the PI or DEHS.

6.5.4. Cleaning Respirators

Both surgical masks and N95s are for single use and should not be reused. Face respirators and PAPRs are reusable masks. In order to properly clean the respirators; remove the cartridges (full and half-face respirators) or connective tubing (PAPR) and wash the interior with a mild detergent and water. Dry the unit with absorbing material and finish by air-drying.

Verify the integrity of the unit to ensure that the rubber has not been damaged. Store the unit in a dry place away from dust. Care should be taken with the PAPR and full-face respirator to ensure that the eye shield part of the unit is not scratched or bent as this will distort your vision as well as cause unnecessary fatigue to your eyes.

7. Biological Safety Cabinets and Chemical Fume Hoods

A biological safety cabinet is used as a primary barrier against exposure to biohazardous or infectious agents as it surrounds the immediate workspace involving the agent. However, total containment is not provided by primary barrier equipment and aerosols can escape. **A PRIMARY BARRIER SUCH AS A BIOLOGICAL SAFETY CABINET MERELY COMPLIMENTS CAREFUL WORK PRACTICES.**

Biological safety cabinets contain High Efficiency Particulate Air (HEPA) filters that have 99.97% to 99.99% efficiency for ≥0.3 micron-sized particles. These cabinets operate with laminar airflow, which is the movement of air with uniform velocity in one direction along parallel flow lines, either horizontally or vertically.

7.1. Primary Containment: Biological Safety Cabinets

Biological safety cabinets (BSC's) are among the most effective and most commonly used primary containment devices in laboratories that work with infectious agents. The three general types of BSC's (Class I, II, III) that are available have different performance characteristics and applications which are described in this chapter. Properly maintained Class I and II BSC's, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (RG-2 and RG-3 agents).

Both Class I and II BSC's have inward face velocities (75-100 linear feet per minute) that provide comparable levels of containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSC's also protect the research material itself through high-efficiency particulate air filtration (HEPA filtration) of the airflow down across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the community, and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

7.1.1. Class I Biological Safety Cabinets

The Class I BSC is a negative pressure, ventilated cabinet usually operated with an open front and a minimum face velocity at the work opening of at least 75 linear feet per minute (lfpm). All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside. The Class I BSC is designed for general microbiological research with low and moderate risk agents and is used for containment of mixers, blenders, and other equipment. These cabinets are not appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet.

The Class I BSCs can also be used with an installed front closure panel without gloves, which will increase the inward flow velocity to approximately 150 lfpm. If such equipped cabinets are ducted to the outside exhaust they may be used for toxic or radiolabelled materials as an adjunct to microbiological research. Additionally, arm-length rubber gloves may be attached to the front panel with an inlet air pressure release for further protection. In this configuration, it is necessary to install a make-up air inlet fitted with a HEPA filter in the cabinet.

Class I BSC's are currently being manufactured on a limited basis. Many have been replaced by Class II BSC's.

7.1.2. Class II Biological Safety Cabinets

The Class II BSC is designed with inward airflow at a velocity (75-100 lfpm) sufficient to protect personnel, HEPA-filtered downward vertical laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Design, construction, and performance standards for Class II BSC's, as well as a list of products that meet these standards, have been developed by and are available from the National Sanitation Foundation International (Ann Arbor, Michigan). Utilization of this standard and list should be the first step in selection and procurement of a Class II BSC.

Class II BSC's are classified into two types (A and B) based on construction, airflow velocities and patterns, and exhaust systems. Class II A cabinets are sub-typed into type A1 and A2. Type A1 cabinets are suitable for microbiological research in the absence of volatile or toxic chemicals and radionuclides, since 70% of the air is recirculated within the cabinet. Type A1 cabinets may be exhausted into the laboratory or to the outdoors via a "thimble" connection to the building exhaust system. However, A1 cabinets should never be hard ducted to the building exhaust system.

Type B cabinets are further sub-typed into types B1 and B2. A comparison of the design features and applications are presented. Type B1 will recirculate about 30% air and exhaust 70% through the hard duct. Type B2 cabinets will exhaust 100% of the air.

It is UofL's policy that Class I and II BSC's be tested and certified:

- **at the time of installation in the laboratory;**
- **any time the BSC is moved;**
- **and at least annually thereafter.**

Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supersede the critical certification prior to use in the laboratory.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the BSC. Of particular note are activities that may disrupt the inward directional airflow, such as:

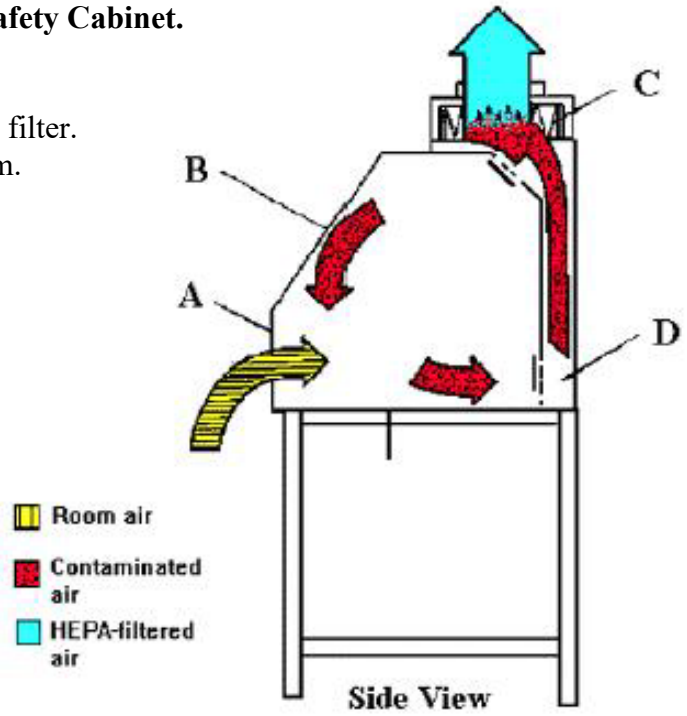
- Repeated insertion and withdrawal of the workers' arms into and out of the work chamber
- Opening and closing the door to the laboratory or isolation cubicle
- Improper placement or operation of materials or equipment within the work chamber
- Brisk walking past the BSC while it is in use

Each of these events causes the escape of aerosolized particles from within the cabinet.

Accordingly, BSC's should be located away from traffic patterns and doors. Airflow from fans, room air supply louvers and other air moving devices can disrupt the airflow pattern at the face of the cabinet. Strict adherence to recommended practices for the use of BSCs and their proper placement in the laboratory are as important in attaining the maximum containment capability of the equipment, as is the mechanical performance of the equipment itself.

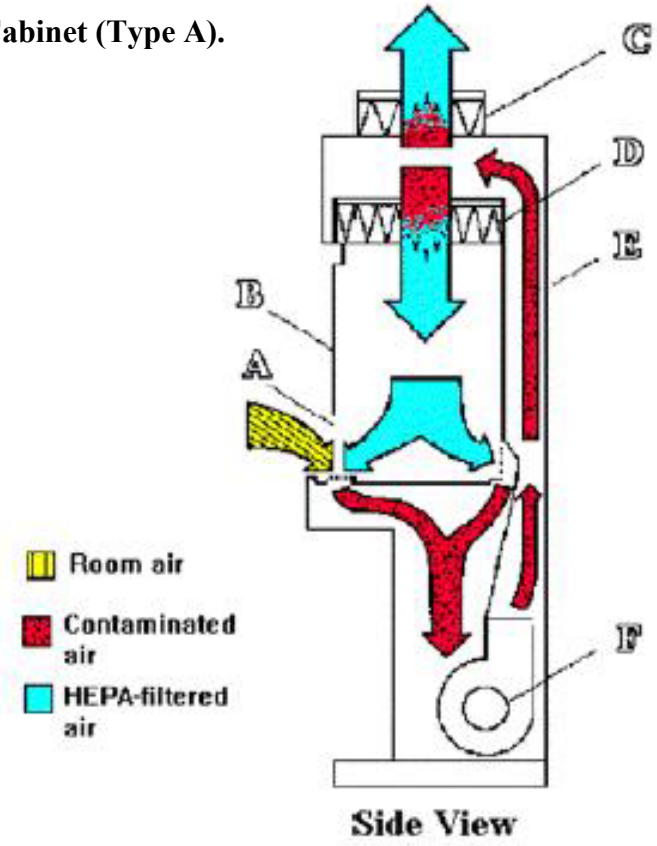
Class I Biological Safety Cabinet.

A. Front opening.
B. Sash.
C. Exhaust HEPA filter.
D. Exhaust plenum.



Class II Biological Safety Cabinet (Type A).

A. Front opening.
B. Sash.
C. Exhaust HEPA filter.
D. Rear plenum.
E. Supply HEPA filter.
F. Blower.



7.1.3. Class III Biological Safety Cabinet

The Class III biological Safety Cabinet is a totally enclosed, ventilated cabinet of gas-tight construction and offers the highest degree of personnel and environmental protection from infectious aerosols, as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4 containment.

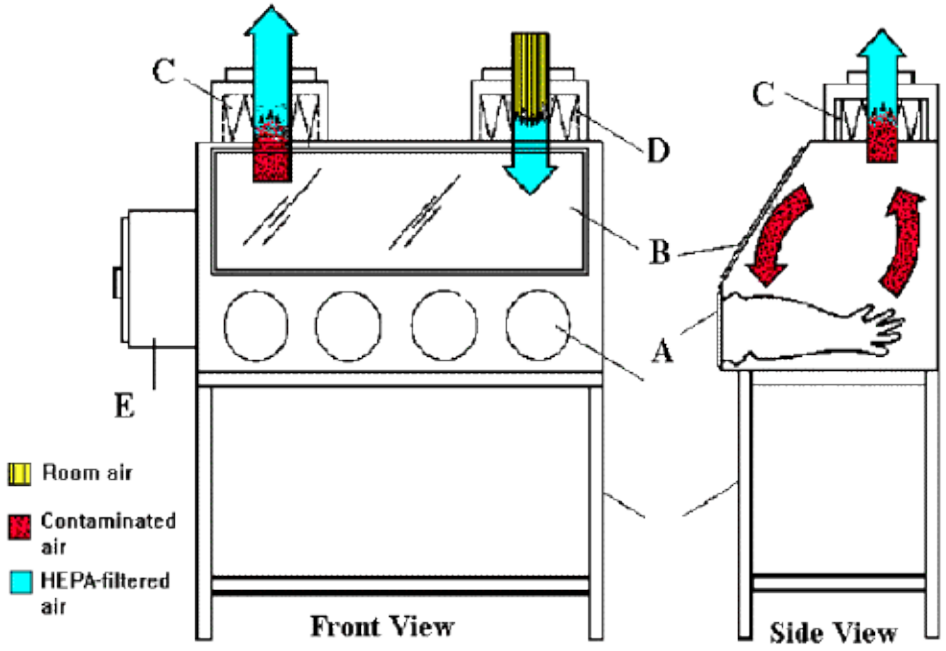
All operations in the work area of the cabinet are performed through attached arm length rubber gloves or half-suits. The Class III cabinet is operated under negative pressure. Supply air is HEPA-filtered and the cabinet exhaust is filtered through two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside of the facility.

All equipment required by the laboratory activity such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system. The Class III cabinet must be connected to a double-doored autoclave and/or chemical dunk tank used to sterilize or disinfect all materials exiting the cabinet, and to allow supplies to enter the cabinet. Several Class III cabinets are therefore typically set up as an interconnected system.

Class III Biological Safety Cabinet.

A. Glove ports with O-ring for attaching arm-length gloves to cabinet.
B. Sash.
C. Exhaust HEPA filter.
D. Supply HEPA filter.
E. Double-ended autoclave or pass-through box.

Note: A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be connected to an independent building exhaust system.



7.1.4. Comparison of Biological Safety Cabinets

Type	Face Velocity (lfpm)	Airflow Pattern	Radionuclides/ Toxic Chemicals	Biosafety Level(s)	Product Protection
Class I* Open front	75	In at front; rear and top through HEPA filter	No	2,3	No
Class II Type A1	75	70% recirculated through HEPA; exhaust through HEPA	No	2,3	Yes
Type A2	100	Same class II A1, but plena under negative pressure to room and exhaust air is ducted	Yes	2,3	Yes
Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Yes (Low levels/volatility)	2,3	Yes
Type B2	100	No recirculation; total exhaust via HEPA and hard ducted	Yes	2,3	Yes
Class III	NA	Supply air inlets and exhaust through 2 HEPA filters	Yes	3,4	Yes

* Glove panels may be added and will increase face velocity to 150 lfpm; gloves may be added with an inlet air pressure that will allow work with chemicals/radionuclides.

7.2. Biological Safety Cabinet Certification and Professional Decontamination.

BSC's are the primary barrier of protection for individuals working with biological materials. Never use a biological safety cabinet unless it has been certified to meet minimum safety specifications. Every BSC must be certified by an NSF certified professional at the following times:

- When newly installed
- After filter replacement
- After the cabinet has been moved
- Annually

Certification is required according to:

- The CDC/NIH Biosafety in Microbiological and Biomedical Laboratories 6th edition, June 2020.
- The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules April 2019.

- The NSF International Class II (laminar flow) Biohazard Cabinetry Standard 49, 2002 or later edition
- The Food and Drug Administration.
- The Occupational Safety and Health Administration. Controlling occupational exposure to hazardous drugs, 1995.
- The American Society of Hospital Pharmacists Technical Bulletin on handling cytoxic drugs in hospitals, 1990.

Biosafety safety cabinets need to be subjected to a full decontamination (sealed and paraformaldehyde or vaporized hydrogen peroxide released) prior to having repair work completed and prior to being moved to a new location. Full decontamination can be performed by your certification vendor.

Currently, each lab chooses their own certification vendor from among those contracted to the University and is responsible for scheduling the certification appointment.

U of L has a contract with the following biosafety cabinet service providers, therefore a PIs or their designees must contact one of these providers to schedule services:

Lewis Testing Services, Inc.
P.O. Box 39109 Indianapolis, IN 46239
Phone: 317-862-9387
Fax: 317-862-2397
Email: laura@lewistestingservices.com

Precision Air Technology
PO Box 46449
Raleigh, NC 27620
Phone: 919-812-0340
Fax: 801-740-3346
Email: sanderson@precisionairtechnology.com

7.3. Safety Rules for use of Class I and II Cabinets

7.3.1. UV Lights

Ultraviolet (UV) lamps should not be used as the sole disinfection method 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.

NEVER work in the BSC with the UV light on.

7.3.2. Understanding the Biological Safety Cabinet Monitors

Before any work is performed in a BSC, it is very important to check the airflow monitors to determine if work can or cannot be safely performed.

There are several aspects to understand when looking at the monitors.

- Will both you and the samples be protected from one another?
- Will the BSC protect you from the biological hazards that are inside it?
- Will the samples in the BSC be protected from the outside environment?

If the BSC is in alarm:

- If the monitor indicates negative pressure you will be protected from the biohazards, meaning that it is pulling air inside the biosafety cabinet, but on the other hand your samples are at risk of contamination.
- If the monitor indicates positive pressure you are at risk of being exposed as the air inside the cabinet is being pulled out into the room; if this should happen while working with biohazardous agents, close the sash immediately, notify co-workers present to leave the room, post DO NOT ENTER - BIOHAZARD EXPOSURE signs on the door and notify your supervisor immediately.

There are different types of monitor displays depending on the biosafety cabinet manufacturer:

1. Digital readout with green, yellow and red lights

If the green light is on, you can safely work in the BSC. Both you and the samples will be protected.

If the yellow light is on, an alarm should sound stating that there are risks present while working in the BSC. You should not work in the BSC if this is the case. If you had started working in the BSC then you but not your samples are protected; you should stop working as soon as possible.

If the red light is on, then you should not start working in the BSC. If you are working with a biohazard when the alarm goes off, stop immediately, close the sash and evacuate the room.

2. Magnehelic gauge with numeric readout

If your BSC has a numeric readout you should know in what range it is safe to use the BSC. This safe range may vary from hood to hood and should be indicated on the certification label.

7.3.3. Working in a Biological Safety Cabinet

Before starting work, verify that BSC is turned on and is functioning properly. If the BSC is not turned on, turn it on and wait 5 to 10 minutes before you proceed. Verify that the drain valve is closed (if present on the BSC model you are using). Disinfect interior surfaces using an appropriate disinfectant. See Section 11.1.2 for a list of UofL approved disinfectants.

Everything going into a BSC should be disinfected at all biosafety levels. Additionally, if you are working at BSL-3, everything coming out of the BSC must also be disinfected.

Ideally, everything needed for a complete procedure (or a section of a procedure) should be placed in the BSC before starting work. Nothing should pass through the air barrier until the procedure is completed. Avoid overloading the work area and thereby compromising the efficacy of the BSC.

Work supplies are best arranged to segregate clean from dirty materials.

Wait five minutes after all materials have been placed in the BSC before beginning work, this will enable the BSC to purge airborne contaminants from the work area. Work as far back in the BSC workspace as possible.

To avoid disturbing the airflow of the BSC, it is beneficial to place a waste container in the BSC before starting the work. Waste can be collected in that container and removed at the completion of the procedure or at a break point if needed.

If working at BSL-3, at least one waste container should contain 10% bleach to disinfect contaminated liquid or bulky items. All BSL-3 waste needs to be securely closed prior to removal from the BSC, in preparation for autoclave decontamination.

Avoid using toxic, flammable, or radioactive substances in the BSC unless a DEHS representative has approved the procedure.

At the completion of the job, decontaminate all interior work surfaces and thoroughly wash hands and arms with soapy water.

7.3.4. Do's and Don'ts of the Biological Safety Cabinet

Do:

- Use them to work with any infectious or potentially infectious agents.
- Keep work areas free of unnecessary clutter including equipment and supplies; unnecessary clutter may result in a loss of proper airflow containment.
- Organize your work time so that you do not have to rush.
- Always surface decontaminate all surfaces and material coming in and out of the BSC.
- Always change or decontaminate gloves (spraying with alcohol, Clidox or other appropriate disinfectant) when taking hands in and out of the BSC.
- Decontaminate any surface that may have become contaminated with a biological material.
- Always use mechanical pipetting aids.

Don't

- Work in the BSC if the alarm or warning lights are on.
- Cross hands while working in biosafety cabinet.
- Block front and rear intake grids.
- Reattach gowns, touch face, or get hair out of face with gloves on. In general, don't touch anything on yourself with gloves on, wait until the gloves are off.
- Touch equipment or other items with potentially contaminated gloves; disinfect the gloves prior to touching.
- Make sudden swift movement of hands in BSC or repeatedly removing arms and hands in and out of the BSC.
- Work in the BSC if certification has expired.

7.3.5. Biohazardous Spills in the Biological Safety Cabinet

In case a biohazardous spill occurs inside the BSC:

- Decontamination steps should be taken while the cabinet is operating to prevent the escape of contaminants.
- Cover the spill completely with paper towels or other absorbent material.
- Apply appropriate disinfectant to the area, saturating the paper towels and allowing the disinfectant to sit on the contaminated area for at least 10 minutes).
- Spray or wipe walls, work surface and all affected apparatuses with an appropriate disinfectant. Make sure to wear gloves while doing this.

- If a drain system is involved, make sure that BSC valves are closed and consult the BSC manufacturer's specific instructions regarding decontamination.
- Wipe the area clean with water followed by 70% ethanol.

After a spill is decontaminated, the BSC shall be thoroughly cleaned and dried. Residual materials can support the growth and multiplication of microorganisms, and can jeopardize the product protection normally provided by BSC's.

7.3.6. Maintenance of the Biological Safety Cabinet

The BSC should be properly cleaned before and after each use by surface decontamination of walls, tray and sash with the appropriate disinfectant (cavicide, 70% ethanol, etc.). **DO NOT use bleach, as it can damage the surfaces.** If bleach is needed or used accidentally, follow with alcohol or water to remove residual bleach.

Once a month the bottom tray of the workstation in the BSC should be removed from the cabinet to expose the bottom portion of the BSC. To do so you may have to remove a few screws, but generally the tray will simply lift out. While the tray is out, remove any debris or trash that may have fallen through the grids and vent system (paper towels, glass beads, tubes) and are now in the area below the tray.

Care should be taken if glass, such as Pasteur pipettes and ampoules, is used in the BSC because glass may be found in bottom of cabinet beneath the grille. Add a generous amount of disinfectant to the bottom of the BSC (the area that is below where the tray sits) and let soak. Wipe off the liquid and continue to totally clean the bottom portion of the cabinet. Clean the walls of the BSC as well as the sash and replace the tray. Surface decontaminate all exposed areas of the BSC one last time. Let the air circulate at least 30 minutes before starting to work in the BSC.

Once a year have the BSC certified by a UofL contract vendor

Proper maintenance of the BSC will insure proper functioning as well as limiting contamination of the work.

7.3.7. Biological Safety Cabinet Bottom Line

The biological safety cabinet is not a substitute for good laboratory practice.

- **Aerosols can escape**
- **The airflow is disrupted by:**
  - **Rapid movement of hands or arms in and out of BSC**
  - **Opening doors in the room**
  - **Persons walking past the BSC**
- **Decontaminate the cabinet before and after each use**

7.4. Chemical Fume Hoods

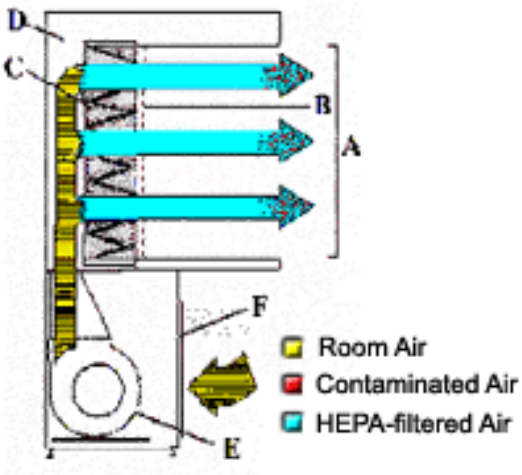
Chemical fume hoods are not to be confused with the BSC. They offer no sample protection, nor do they offer personnel or environmental protection against biological agents. Fume hoods should only be used for chemical and radioactive work. The hoods need to be certified yearly (by DEHS) and air intake is dependent on the type of work you do.

Air intake from the fume hood is not filtered and is 100% exhausted. The fume hood is hard ducted to the outside.
It is important to keep the sash open within its proper operation position.

- Verify that the chemical hood is exhausting.
- Work with the sash lowered to the 100-110 ft./min level. The sash must be below chin level.
- Work at least 6 inches inside the hood.
- Do not block the face of the hood (e.g. with shielding or large pieces of equipment).
- Do not block rear exhaust slot. Place bulky items to the rear and sides on a supporting mesh elevated at least two inches from the work surfaces to allow passage of air to the rear slot.
-

7.5. Clean Bench – Class 100 Cabinet

Clean benches should not be confused with Class I BSCs. The clean bench works in the opposite way from a biological safety cabinet. A clean bench will offer sample protection but not personnel protection. Air intake is HEPA filtered but the exhaust air is not. The airflow can be vertical or horizontal. This type of hood is typically used to prepare microbiological culture plates and for nucleic acid work.



A. front opening
B. supply grille
C. supply HEPA filter
D. supply plenum
E. blower
F. grille

8. Recommended Work Practices for High-Risk Procedures

Some equipment or procedures are considered high-risk due to the potential for aerosolization, mechanical failure and/or a significant ability to cause harm to the user.

Aerosols are liquid droplets and fine solid particles suspended in the air. An aerosol with a diameter of 5 microns or less can remain airborne for a long-period of time, spread wide distances, and can be easily be inhaled. Particles with a diameter larger than 5 microns tend to settle rapidly and can contaminate skin, other surfaces and ventilation systems.

8.1. Pipettes and Pipetting Aids

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Use the following precautions when performing work outside or inside a BSC:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.

  Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette.
- Biohazardous materials should not be forcibly discharged from pipettes. Use "to deliver" pipettes rather than those requiring "blowout".
- Do not discharge biohazardous material from a pipette at a height (make sure the pipette is inside or close to the opening of the receiving vessel). Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Autoclave the pan and pipettes as a unit before processing them for re-use or disposal.
- Discard contaminated Pasteur pipettes in sharps containers that are the appropriate size.
- When work is performed inside a BSC, all pans or sharps containers for contaminated glassware should be placed inside the BSC while in use.

8.2. Syringes and Needles

Syringes and hypodermic needles are established dangers when working biohazardous agents and thus need to be handled with extreme caution to avoid accidental infection and aerosol generation. The use of syringes and needles should be restricted to procedures for which there is no alternative. Do not use a syringe and needle as a substitute for a pipette.

Unless it is not possible, always use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe.

When using syringes and needles with biohazardous or potentially infectious agents:

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

Needles should not be bent, sheared, replaced in the sheath or guard (capped) or removed from the syringe following use. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one-handed scoop method must be used. Always dispose of needle and syringe unit promptly into an approved sharps container. Do not overfill sharps containers ($3/4^{th}$ filled = full) and place in Stericycle box.

8.3. Cryostats

Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk since freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets or infectious material. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate frequently with 70% ethanol or another disinfectant that is most suitable for the agent in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them and properly dispose of these materials during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after cutting tissue known to contain infectious agents (e.g. bloodborne pathogens, *M. tuberculosis*).
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider staining solutions that contact potentially infected frozen sections to be contaminated and dispose of appropriately as biohazardous waste.

8.4. Centrifuge Equipment

Centrifuges are considered extremely high-risk equipment for biosafety use. Hazards associated with centrifuging include mechanical failure and the creation of aerosols. Centrifuges can be either mini, bench top or floor units, and are classified as low, high or ultra speed. Additionally, the centrifuges will have either covered rotors or safety cups and buckets.

8.4.1. Mini or "Quick Spin" Centrifuges

Mini centrifuges are low or high-speed units that accommodate only very small amounts of tubes. These units are small in size and their rotors are not removable. If such units are used with biohazardous material, the unit must be placed in the BSC when in use. At the end of the work, the centrifuge should be properly surface decontaminated before being removed from the BSC.

8.4.2. Bench Top or Table Top Centrifuges

Bench top centrifuges are high speed, and are the most common centrifuges found in laboratories. They vary in size and can be refrigerated and have either removable rotors or cups with safety caps.
Units may only be used for biohazardous materials in a BSL-2 (or higher) laboratory if they have the following specifications.

- A removable rotor or swing buckets with caps
- The rotor has lids or the buckets have caps with proper seals that have been approved by the vendor for use with biohazard material.

Samples must be loaded and unloaded in the rotor or buckets inside the BSC, and then sealed with lid or safety caps**. Seals need to be thoroughly inspected for cracks and wear before starting the procedure as well as before and after each centrifugation**. In addition, the rotor/safety caps and buckets need to be surface decontaminated before being removed from the BSC.

8.4.3. Floor Unit Centrifuges

Floor unit centrifuges are usually high-speed centrifuges for large volumes or ultra-speed centrifuges for small volumes. The rotors are generally removable so they can be loaded and sealed in the biological safety cabinet.

All manufacturer's precautions must be taken when using a floor unit centrifuge, proper balancing of tubes in a centrifuge becomes crucial when using an ultra-speed centrifuge. Any differences in weight between tubes, might cause severe damage to the rotor. If the rotor breaks or a suspended bucket breaks off during centrifugation they can become dangerous for the outside environment. The suspended buckets can become missiles and go through the centrifuge wall.

In general, ultra-speed centrifuges are equipped to detect a problem before going into ultra-speed with an unbalanced rotor. However, if you hear any suspicious noises, the area should be evacuated and the centrifuge turned off. These centrifuges are usually on their own electrical circuit; therefore, it is recommended to go to the circuit breaker and shut the power off from there to avoid being close to the unit when it is not working properly.

Ultra-speed centrifuges should be maintained on a service contract. Service contractors usually change oils and HEPA filters from the centrifuge, and assure overall proper functioning. Training of each user on the use of ultra-speed centrifuges is needed and should be documented.

8.4.4. Mechanical Failure

To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users must be properly trained. Operating instructions including safety precautions should be prominently posted on the unit.

8.4.5. Aerosol Production

Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and resuspending sediment pellets. The greatest aerosol hazard is created when a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Personnel must be trained on the proper use and specification of each individual centrifuge in the laboratory. Documentation of this training should be kept in the lab.
- All centrifugation must be done using centrifuge safety cups or buckets or sealed centrifuge tubes in sealed rotors. If a small centrifuge is used and centrifuge safety buckets are not available, it must be operated in the BSC.
- Inspect the rotor or safety cap and bucket/cups for cracks and leaks after each centrifugation.
- Visually examine tubes and bottles for cracks or stress marks before and after using them.

- Use sealed tubes and safety buckets/cups that seal with O-rings. Before use, inspect tubes, O-rings, and buckets/cups for cracks, chips, erosions, bits of broken glass, etc.
- Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill all centrifuge tubes and bottles within the BSC. Wipe the outside of tubes with disinfectant before placing in safety buckets or rotors.
- Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for most centrifuge tubes is ¾ full.
- Always tightly cap tubes before spinning.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when re-suspending sediment material. Use a swirling rotary motion rather than shaking. If shaking or vortexing is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Always balance tubes, both in weight and distribution, in the rotor or safety bucket. Unbalanced tubes will damage the rotor and can cause it to break during centrifugation.
- Wipe outside of the rotors or safety caps and bucket with disinfectant before removing them from the BSC.
- Heavy or large rotors should be placed on a cart for movement to and from the BSC.
- Do not carry more than 2 buckets at a time. If buckets are placed on a cart to move more than 2 or because they are heavy, they should be double contained to prevent spills if they tip over. Styrofoam coolers work well for this.
- A HEPA-filtered respirator must be worn when opening the centrifuge to remove the rotor if risk assessment of the procedure indicates high probability of aerosols formation (specifically important for BSL-3 agents).
- If construction of the centrifuge permits, the centrifuge chamber is to be connected to a vacuum pump with a HEPA filter installed between the centrifuge and the vacuum pump.
- High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatigue resulting in disintegration of rotors, and to use proper cleaning techniques for centrifuge components. More specifically, manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

8.4.6. Biohazardous Spill in Centrifuges

- Stop work immediately. Treat the incident as a potential exposure.
- Inform all others in the area that a biological spill/aerosol may have been generated and evacuate personnel from the room.

- Notify the Principal Investigator or lab director and DEHS (502-852-6670).
- Label the lab area off limits for at least 30 minutes. DEHS will respond and determine how long of an evacuation is needed (generally 30 minutes) and whether the evacuation should be limited to a specific area of the lab.
- After the evacuation period, put on appropriate personal protective equipment and enter the room.
- If the centrifuge was properly used, any spilled liquid should be contained within the sealed rotor or safety cup.
- Remove rotor or safety bucket and place in BSC.
- Use paper towels to cover any pooled liquid within the centrifuge and then saturate the towels with disinfectant. Absorbent materials reduce the potential of generating additional aerosol due to the decontamination procedure itself. If no visible liquid is present, perform a simple wipe down with disinfectant.
- Return to the BSC and proceed to clean the rotor or safety cup. Collect any broken pieces of tubes with forceps and place the broken pieces in a sharps container.
- Decontaminate the rotor or bucket by wiping down with disinfectant and filling the tube holes with 70% ethanol. Once the tube holes are filled with disinfectant, allow the rotor or bucket to soak for at least 15 minutes, followed by a rinse with mild detergent and then water.
- Decontaminate all exposed surfaces before releasing the equipment (or room) for normal use.

8.4.7. Centrifuge Cleaning

Decontamination of the outer and inner parts of the centrifuge should be done on a regular basis and if possible at the end of the procedure. This can be done by using a cloth dampened with disinfectant.

At regular intervals, the rotor should be sprayed inside and out with disinfectant and allowed to either air dry or dried with paper towels. Occasionally, the rotor should be removed and soaked in 70% ethanol, followed by mild detergent and a water rinse.

8.5. Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers

The use of any of these devices results in considerable aerosol production. When working with biohazardous materials blending, cell disrupting and grinding equipment must be used in a biological safety cabinet.

8.5.1. Safety Blenders

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

8.5.2. Lyophilizers/freeze driers and Ampules

Lyophilization is a source of aerosol. The agent is dried into a fine powder by removing water under vacuum pressure. The highest risk of aerosol production occurs at the beginning of the process when the air is removed from the unit and at the end of the process when air is allowed back into the unit and the seal that was created by the vacuum is released.

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC.

After lyophilization is complete, all surfaces of the unit that have been exposed to the agent must be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

All the gaskets and seals should be inspected before starting the process. The tubes and means of adapting the tubes to the unit should be appropriate to each lyophilizer. Tubes that are placed in a vacuum chamber should be correctly secured to prevent them from tipping over.

At the beginning of the process, slowly apply the vacuum. Sudden removal of the air by vacuum will create an aerosol. At the end of the process, extreme care should be taken to allow the air to enter very slowly as the material can easily blow around. Once pressure in the chamber has been re-established, care should be taken when removing the tubes/vials; they should be sealed as soon as possible.

Respiratory protection should be worn when lyophilizing RG-2 agents. Additionally, limited access to the room with the lyophilizer should be maintained during the entire lyophilizing process and until the area is known to be free of any potential aerosol.

Lyophilization of RG-3 agents will be done in a limited-access room of the BSL-3 facility. The lyophilizer manifold will be maintained under negative pressure at all times during the drying procedure, until the manifold and ampules are back-filled under dry nitrogen gas and the glass ampules are flame-sealed. An accidental loss of negative pressure in the manifold will be considered a potential exposure and reported in the same manner as a spill. A HEPA-filtered respirator PAPR (e.g. 3M Racal) must be worn by personnel, to minimize exposure.

Opening ampules containing liquid or lyophilized infectious culture material must be performed in a BSC. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked gauze or paper towel, hold the ampule upright and snap it open at the nick. Reconstitute the contents of the ampule by slowly adding liquid to the dried material, careful to avoid aerosolization. Discard the towel as biological waste and the ampoule top pieces as sharps waste.

Glass ampoules, used to store biohazardous material in liquid nitrogen, have been known to explode causing eye injuries and exposures to infectious agents. The use of polypropylene tubes specifically designed for liquid nitrogen storage help to eliminate this hazard. These tubes are available dust free or pre-sterilized and are fitted with polyethylene caps that contain silicone washers. Heat sealable polypropylene tubes are also available.

8.6. Bunsen Burners

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

Continuous flame gas burners should not be used in the BSC's. These burners can produce turbulence which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

GAS FLAMES FROM BUNSEN BURNERS SHOULD BE AVOIDED – THE USE OF HOT BEAD STERILIZERS IS HIGHLY RECOMMENDED.

8.7. Vortexing

Vortexing is often required by research protocols. Although a very simple procedure, it can be a source of aerosols and should therefore only be used when appropriate. Vortexing of biohazard material should always be done in the BSC. Tubes should be inspected for cracks and stress marks before and after using the vortex. Tubes must be properly closed and should not be over filled. Care should be taken to have a firm hold on the tube during the vortexing process and the speed should be kept to the lowest possible setting to achieve the desired action.

8.8. Rocking/Shaking of Liquid Cultures

In order to minimize the risk of uncontrolled spillage of dangerous pathogens, the following procedures must be followed.

- Shaking liquid cultures of pathogenic organisms should be avoided whenever possible.
- The volumes of liquid to be shaken should be kept to a minimum and should only be allowed to reach no more than 1/3 of the height from the top of the flask.
- Flasks must be inspected for cracks and stress marks.
- Flasks must be individually and securely labeled (organism, researcher's name and date) and firmly fastened on the fixed clamp-type of orbital shaker (that grip individual flasks). The clamps must be the correct size for the flasks used.
- Flasks must be incubated only in rooms designated for similar biohazard level pathogens.
- Flasks must be checked at regular intervals.
- Mechanical shaking of RG-2 and above pathogens should only be performed in sealed orbital shakers, capable of containing a spill, with a transparent cover allowing inspection before opening.

8.9. Sonication

Sonication is the process by which cell membranes are disrupted by using sound waves. Sonication of samples can be done by means of a rod/probe or water bath.

Sonication using a rod or probe presents more hazards than using a water bath method and should be done in a biosafety cabinet for RG-2 and above agents. A rod or probe is placed in an open tube and then the rod or

probe vibrates to create the sound wave needed. Care should be taken not to have the rod or probe touch the sides of the tube, as this will cause the tube to break. Certain procedures will require the samples to be kept on ice to prevent overheating. If this is needed, the ice should be considered as potentially contaminated and should be properly decontaminated before leaving the BSC, pour bleach into it and let it soak. The tubes must be carefully checked for cracks and stress marks before and after use. The unit must be decontaminated inside and out before being removed from the BSC at the end of the procedure.

Sonication using a water bath allows having the tube sealed during the process. The tube is placed into a water bath and sound waves will be carried through the water to disrupt the cell membranes. This method could be used on the bench top for a RG-2 agent but should be used in a BSC for a BSL-3 agent. The tube should be inspected for cracks and stress marks before and after the process and should be properly sealed to prevent leaking. At the end of the process, the water should always be considered potentially contaminated and should be disinfected appropriately.

8.10. Tissue Grinders

Several types of tissues grinders are available, including manual and mechanical grinders.

8.10.1. Manual Tissue Grinder

Manual tissue grinders could be mortar and pestles or hand-held ground/etched glass tissue grindes. When using such grinding methods the following steps should be used:

- Verify the tubes for cracks and signs of stress before and after grinding.
- Make sure the tube is properly secure to prevent spills.
- Do not over fill the tube as the pestle will increase the volume present in the tube and might cause it to spill.
- Grinding infectious or suspected infectious material should be done in the biosafety cabinet.
- Grinding should be done with slow controlled movements.
- Disposal of the pestles and gloves should be done appropriately for the biohazard level agent being used.
- The tubes should be decontaminated before leaving the BSC.
- If using a powered pestle make sure the speed is properly set and that the user has received training on its use before using biohazardous material to prevent spills and splatters.
- When using a vortex system follow the proper methods described in section 8.7.

8.10.2. Mechanical Tissue Grinder

Mechanical tissue grinders can come in many different models. Each user needs to be trained on the proper use of each one.

- Tubes need to be inspected for cracks and signs of stress before and after use.
- Tubes need to be securely sealed to prevent accidental opening.
- The equipment needs to be properly maintained.
- The tubes need to be properly secured in the grinder and the grinder should have a lid.
- When using multiple tube units all tubes need to be balanced in the racks both in weight and in placement. All racks, in addition to tubes, need to be balanced in weight.

- The racks should be properly secured to the units and the units should have a lid.
- The speed used should be appropriate for the unit and the tubes used.

When using RG-2 or RG-3 agents, the unit should be placed in the BSC or other physical containment devices.

8.11. Laundry

All personal protective clothing must be laundered or properly disposed by the employer (generally Principal Investigator of a research lab) at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be collected in special hampers (labeled or color coded) or in biohazard bags. Lab coats, from labs working with infectious agents, should be autoclaved prior to regular laundry treatment. ***At no time will personnel be permitted to take home any PPE, including lab coats, for laundering or cleaning.***

8.12. Housekeeping

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

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

- Keeping the laboratory neat and free of clutter – surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes – old and unused chemicals should be disposed of promptly and properly through DEHS.
- Providing a workplace that is free of physical hazards – aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, and avoidance of the creation of electrical hazards in wet areas.
- Remaining in compliance with the laboratory specific chemical hygiene plan.
- All laboratory equipment needs to be cleaned and certified of being free of hazards via equipment decommissioning by DEHS before being released for repair or maintenance.

9. Miscellaneous Guidelines

9.1. Protection of Vacuum Lines

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. *In addition, at BSL-2 and above, a hydrophobic vacuum line filter should be used.*

9.1.1. Collection and Overflow Flasks

Collection tubes should extend at least 2 inches below the sidearm of the flask.
Locate the collection flask so the liquid level can be seen easily and the flask can be emptied before it overflows. The second flask (overflow) may be located outside of the cabinet.

If a glass flask is used at the floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.

In BSL-2 or BSL-3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

9.1.2. Vacuum Line Filter

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Gelman Vacushield or Whatman Vacuguard are available from several scientific supply companies.

9.2. Preventing the Transmission of Tuberculosis

Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of healthcare workers have died. In December 2005, CDC published its Guidelines for Preventing the Transmission of Tuberculosis in Health Care Facilities, 2005 (MMWR 2005; 54 (no. RR-17,1-141)).

The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at risk for exposure to tuberculosis. Propagation and/or manipulation of *Mycobacterium tuberculosis* and *M. bovis* cultures in the laboratory or animal room must be performed at BSL-3 and require IBC approval.

9.3. Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at BSL-2. A primary barrier, such as a BSC, should be used:

- When it is anticipated that splashing, spraying or splattering of clinical materials may occur.

- For initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis*).
- To protect the integrity of the specimen.

All laboratory personnel who handle human source materials are included in the Bloodborne Pathogens Program as outlined in Chapter 13. "Standard Precautions" need to be followed when handling human blood, blood products, body fluids or tissues.

The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the Laboratory Director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented. Additional recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards (NCCLS) or the College of American Pathologists (CAP).

9.4. Working with Tissue Culture/Cell Lines

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same risk group level as that recommended for the agent.

The CDC and OSHA recommend that all cell lines of human origin be handled at BSL-2. All personnel working with or handling these materials need to be included in the laboratory specific *Exposure Control Plan.*

Well established non-primate or non-human cell lines which are known to be NOT contaminated with potential infectious agents may be considered risk group 1 organisms and may be handled at BSL-1. Appropriate tests should confirm that these cells do not contain potential infectious agents.

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

Studies involving suspensions of HIV prepared from T-cells must be handled at BSL-3.

It should be noted that product recalls for bovine serum have raised the awareness of potential Bovine Spongiform Encephalopathy (BSE) or TSE (Transmissible Spongiform Encephalopathy) contamination of those sera.

Caution should be used when obtaining cell lines from international collaborators where regulations on serum testing, etc. may not be as restrictive as the United States.

9.5. Use of Human Subjects and Materials

Federal regulations and U of L policies require that all research involving human subjects or materials be reviewed and approved before initiation by the U of L's Institutional Review Board (IRB) to protect the rights and welfare of human subjects.
It is the responsibility of the Principal Investigator to assure that all research involving human subjects is reviewed and approved by the IRB prior to initiation. All personnel with a reasonable anticipated risk of exposure to bloodborne pathogens through contact with human blood or other human materials must be included in U of L's Bloodborne Pathogen Program and complete the annual required training.

9.6. Reproductive Hazards, Teratogenic Agents and Pregnancy

Substances or agents that affect the reproductive health (i.e., the ability to have healthy children) of men and women are called reproductive hazards. Teratogens and fetotoxins are examples of reproductive hazards. A teratogen is substance which interferes with embryonic or fetal development and women of child bearing potential should avoid exposure. A fetotoxin is a substance that can poison or cause degenerative effects in a developing fetus or embryo. Radiation, some chemicals, certain drugs (legal and illegal), cigarettes, some viruses, and alcohol are examples of reproductive hazards.

A reproductive hazard may cause one or more health effects, depending on the time and duration of the exposure. For example, exposure to harmful substances during the first 3 months of pregnancy may cause a birth defect or a miscarriage. During the last 6 months of pregnancy, an exposure to a reproductive hazard could slow the growth of the fetus, affect neurological development, or cause premature labor.

Reproductive hazards may not affect every person or every pregnancy in the same way. Whether a woman or fetus is harmed depends on how much of the hazard they are exposed to, when they are exposed, how long they are exposed and how they are exposed. It is important to note that exposures do not result just from direct contact with the powder or liquid forms of these agents. For instance, certain teratogens may be excreted in the urine or feces of treated animals, thus creating exposure risks for those who clean the animal cages.

Principal Investigators and laboratory supervisors are responsible for training and instructing laboratory personnel in the appropriate ways to protect themselves from the hazards in the laboratory. These safe practices should not be only limited to times of pregnancy, but should be used on an everyday basis to protect all workers. Students, employees, guests and visitors share the responsibility for learning about the hazards in their workplace, using personal protective equipment, and following proper work practices. Employees, students, guests and visitors should take the following steps to ensure their own safety:

- Store chemicals in sealed containers when they are not in use.
- Wash hands after contacting hazardous substances and before eating, drinking or smoking.
- Avoid skin contact with chemicals.
- Review the SDS for each hazardous chemical used in the laboratory to become familiar with any reproductive hazards.
- Consult a health care provider with any concerns about reproductive hazards in the workplace.
- Participate in all relevant safety and health education, training and monitoring programs offered by U of L.
- Discuss proper work practices with the Principal Investigator or laboratory supervisor.
- Contact the Department of Environmental Health and Safety to discuss potential improvements in engineering controls (e.g. ventilation, chemical fume hoods) or with questions about reproductive hazards.
- Use personal protective equipment (e.g., gloves, respirators, and lab coat) to reduce exposure to workplace hazards.
- Follow appropriate work practices and procedures to prevent exposures to reproductive hazards.
- If chemicals contact the skin, follow the direction for washing and decontamination as described in the material safety data sheet (SDS).
-

Research or work with chemicals or biological agents possessing teratogenic or mutagenic capabilities, such as Rubella, herpes, cytomegalovirus, or other agents that could cause fetal death such as Brucella, may pose a significant health risk. Always consider the health risks associated with any chemical or biological agent before working with the agent and discuss any related concerns with your doctor. Consult faculty members, laboratory supervisors, Principal Investigators, or safety officers if you have any questions or concerns about the research being conducted.

Below is a list of known teratogens and fetotoxins that require cautious use, particularly during pregnancy. However, it should be noted that this list is not all inclusive. There are more than 700 chemicals classified as teratogens, this list simply highlights the most notorious teratogens.

Notorious Teratogens and Fetotoxins

Type	Agent	Effect
Medications	ACE Inhibitors	Renal dysgenesis, oligohydramnios sequence, skull ossification defects
	Aminopterin and methotrexate	Pregnancy loss, hydrocephalus, low birth weight, dysmorphic facial features
	Androgens and high doses of nor-progesterones	Masculinization of external female genitalia
	Antithyroid drugs	Hypothyroidism, goiter
	Carbamazepine	Neural tube defects
	Cocaine	Pregnancy loss, placental abruption, retardation, microcephaly
	Diethylstilbestrol	Vaginal adenosis/ adenocarcinoma, cervical erosion and ridges
	Hydantoin	Dysmorphic facial features, hypoplastic nails, growth, and developmental retardation
	Isotretinoin	Pregnancy loss, hydrocephalus, other CNS defects, small or absent thymus, microtia/anotia, conotruncal heart defects
	Lithium	Ebstein anomaly
	Penicillamine	Cutis laxa
	Streptomycin	Hearing loss
	Tetracycline	Stained teeth, enamel hypoplasia
	Thalidomide	Limb reduction defects, ear anomalies
	Trimethadione	Developmental retardation, dysmorphic facial features
	Valproic acid	Neural tube defects, dysmorphic facial features
	Warfarin	Nasal hypoplasia, stippled epiphyses, CNS defects
Chemicals	Lead	Pregnancy loss, CNS damage
	Methylmercury	Cerebral atrophy, spasticity, mental retardation

	Polychloronated biphenyls (PCB's – ingested)	Low birth weight, skin discoloration
Biologicals	Brucella	Bacteremia
	Coxsackie virus Type B	May cause infections like meningitis or sepsis
	Cytomegalovirus	Growth and developmental retardation, microcephaly, hearing loss, ocular abnormalities
	Herpes (active)	Vertical transmission at delivery
	Herpes (primary)	Pregnancy loss, growth retardation, eye abnormalities
	HIV	Possible transmission to fetus
	Human parvovirus B19	Miscarriage
	Listeria monocytogenes	Intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion ($2^{nd}/3^{rd}$ trimester) or stillbirth
	Syphilis	Abnormal teeth and bones, mental retardation
	Toxoplasma gondii	Hydrocephalus, blindness, mental retardation
	Varicella	Skin scarring, limb reduction defects, muscle atrophy, mental retardation
	Venezuelan Equine Encephalitis	CNS damage, cataracts, pregnancy loss
	Rubella Zika	Disrupt fetal growth and cause birth defects Microencephalopathy

9.7. Nanotechnology

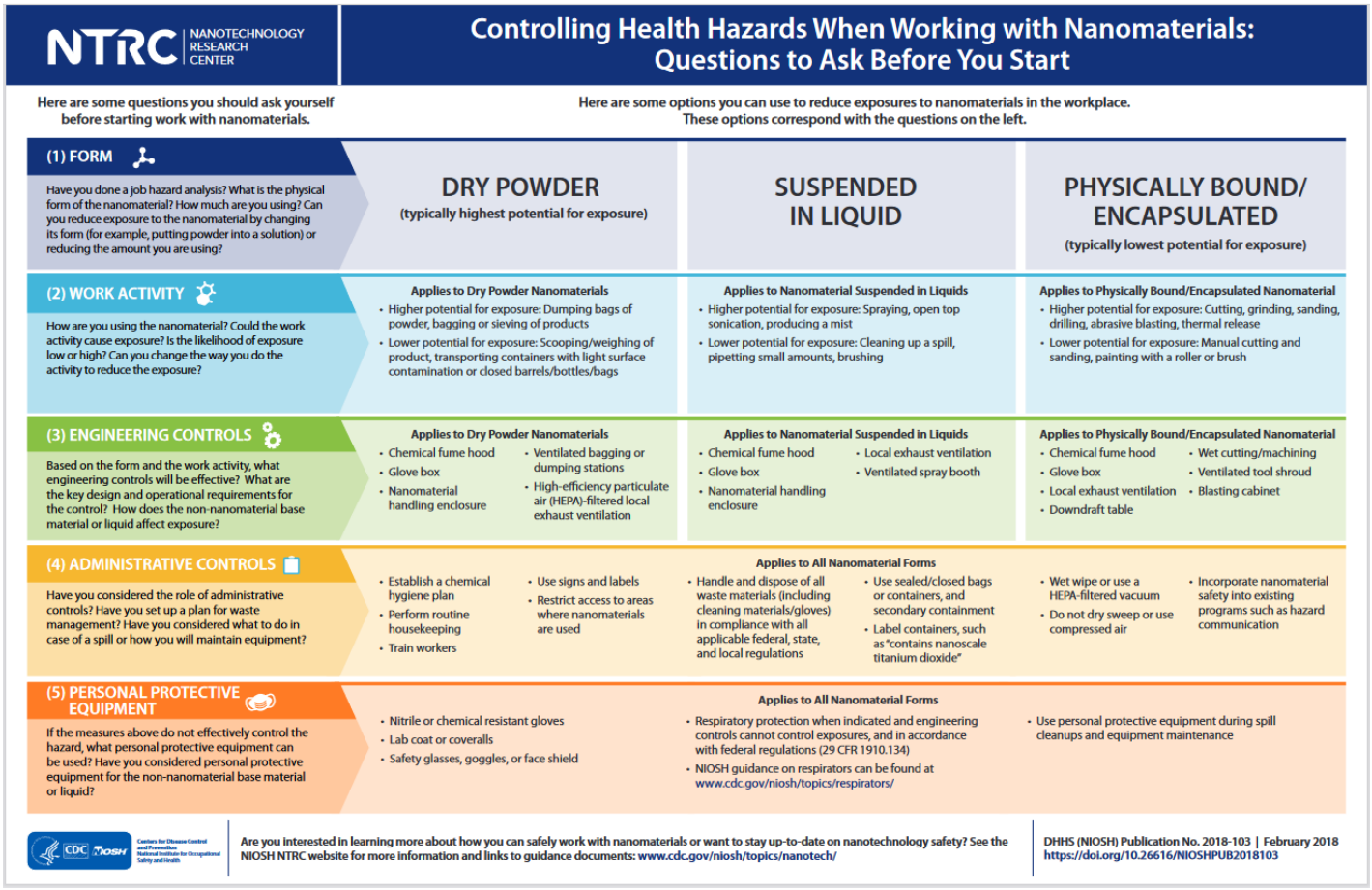
Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials and devices. The technology promises scientific advancement in many sectors such as medicine, consumer products, energy, materials and manufacturing. Nanotechnology refers to engineered structures, devices, and systems. Nanomaterials have a length scale between 1 and 100 nanometers. At this size, materials begin to exhibit unique properties that affect physical, chemical, and biological behavior. Researching, developing, and utilizing these properties is at the heart of new technology.

Workers within nanotechnology-related research may experience exposure to uniquely engineered materials. This includes novel sizes, shapes, and physical and chemical properties. Occupational health risks associated with manufacturing and using nanomaterials are not yet clearly understood. Minimal information is currently available on dominant exposure routes, potential exposure levels, and material toxicity of nanomaterials.

Studies have indicated that low solubility nanoparticles are more toxic than larger particles on a mass for mass basis. Particle surface area and surface chemistry are strong indicators for observed responses in cell cultures and animals. Studies suggests that some nanoparticles can move from the respiratory system to other organs. Research is continuing to understand how these unique properties may lead to specific health effects.

Controlling Health Hazards When Working with Nanomaterials: Questions to Ask Before You Start

Poster from https://www.cdc.gov/niosh/docs/2018-103/default.html



The health effects associated with nanomaterials are not yet clearly understood, so it is important for producers and users of engineered nanomaterials to reduce employee exposure and manage risks appropriately. In 2013, the National Institute for Occupational Safety and Health (NIOSH) published a compendium of control approaches for nanomaterial production and use processes entitled Current Strategies for Engineering Controls in Nanomaterial Production and Downstream Handling Processes. This Workplace Design Solutions document provides guidance on exposure control options for protecting workers during the handling of nanomaterial: Protecting Workers during the Handling of Nanomaterials

More information regarding working safely with nanomaterials can be found at https://www.cdc.gov/niosh/topics/nanotech/default.html

10. Waste

10.1. Medical/Infectious Waste

Medical/Infectious waste is defined as any waste materials that are capable of producing a disease by an organism likely to be pathogenic to humans, such as the examples listed below. U of L contracts with Stericycle Corporation for the disposal management of regulated medicals waste. All regulated medical waste must be disposed of in red bags (i.e. bags labeled with the universal biohazard symbol) that are secondarily contained in vendor provided biohazard cardboard boxes or plastic totes. The vendor provided medical waste container is properly marked and labeled to identify universal biohazard precautions and for DOT highway transportation. The vendor provided container must be the primary outer waste container. University personnel must not deface, cover or obliterate any markings or labels on the medical waste containers.

Examples of Medical/Infectious Waste

- Items saturated or caked with human blood or body fluids that would release blood/body fluid in a liquid or semi-liquid state if compressed, or would flake if handled.
- Human tissue, human cell lines, or anatomical wastes.
- Animal carcasses, animal tissues, animal cells lines or anatomical wastes.
- Sharps (needles, syringes with needles attached, scalpel blades, razor blades, etc.).
- Any residue that results from the clean-up of a spill of infectious waste.
- Any waste contaminated by or mixed with infectious waste.

Medical/Infectious Waste Segregation

The disposal of regulated medical/infectious waste is both highly regulated and very costly. University of Louisville faculty, staff and students must use the utmost care to segregate all waste materials properly. Medical/infectious waste is segregated by disposal treatment by either vendor medical waste incineration or vendor autoclave [steam-sterilization].

Medical and biological waste that must be separated from all other lab biological and medical waste and be destroyed via vendor medical waste incineration include the following:

- Dried preserved specimens (no liquid formalin, alcohol, etc.)
- Animal carcasses
- Human parts, organs, recognizable tissues
- Trace chemotherapeutic materials in IV bags and tubing, syringes, gowns and gloves, sheets and pads

Unless directed by the University Biosafety Officer or as prescribed in an Institutional Biological Safety Committee (IBC) protocol, all other medical and biological waste will be properly contained and collected as red bag waste for off-site vendor steam sterilization.

To obtain details about the University's medical/infectious waste program visit the DEHS web-site link at https://louisville.edu/dehs/biological-safety/biological-safety-files/biohazardous-waste-management-guide or contact the DEHS Hazardous Waste Coordinator at 852-2956. To obtain at no cost, vendor provided medical waste cardboard boxes or plastic totes and red bag liners, contact University Custodial Services at HSC Campus call 852-7174 at Belknap Campus call 852-8200. Labs and clinics must purchase their own red sharps containers from the University's preferred scientific supply vendor.

10.2. Biohazardous Liquid Waste

Durable leak proof containers will be used to receive liquid biohazardous waste which must not contain any other liquid regulated chemicals. Liquids are decontaminated by adding bleach to a final concentration of 10% or other appropriate disinfectant to a final concentration recommended by manufacturer. Mix well and allow to stand for fifteen minutes or recommended time, whichever is greater. Pour decontaminated liquid into the sink and flush down the drain with copious amounts of cold water. Liquid waste that is not compatible with disinfectant must be autoclaved for at least 30 minutes, using slow exhaust before sink disposal.

10.3. Sharps Waste

Sharps waste means any device having rigid corners, edges or protuberances capable of cutting or abrading the skin. Sharps include, but are not limited to the following:

- Hypodermic needles and razor blades.
  Important: DO NOT clip, bend, shear or separate needles from syringes and do not recap needles – these are the times that you are most likely to get injured.
- Glass (broken or whole) = Pasteur pipettes, slides, broken flasks and beakers, etc.
- Plastic items = serological pipettes, syringes without needles, tubing, Petri dishes, pipette tips.

All sharps waste must be placed in an approved sharps container

- Traditional sharps container = rigid, hard red plastic with fixed lid and labeled with the universal biohazard symbol. Needles and razor blades **must** be disposed in this container.
- Lined biohazard box = biologically contaminated pipettes, tubes, Petri dishes with agar.
- Lined glass waste box = non-contaminated pipettes, tubes, broken glass.

Sharps containers must be placed as close to the work area as possible, to prevent unnecessary transportation to the waste container. Sharps containers must not be overfilled. When sharps container is ¾ full, the lid must be securely closed. Discard securely closed sharps container inside a red bag lined medical waste container. Loose sharp containers are never to be set out for pick up by custodial service personnel.

10.4. Commingled Biohazardous Waste

Waste can often involve a mixture of medical and non-medical waste. Commingled biohazardous waste is categorized as medical waste EXCEPT for the following:

- A mixture of medical/infectious waste and hazardous chemical waste is categorized as hazardous chemical waste and is subject to the statutes and regulations applicable to hazardous chemical waste.
- A mixture of medical/infectious waste and radioactive waste is categorized as radioactive waste and is subject to the statutes and regulations applicable to radioactive waste.
- A mixture of medical/infectious waste, hazardous chemical waste, and radioactive waste is categorized as mixed radioactive waste and is subject to the statutes and regulations applicable to radioactive waste.
- Bulk chemotherapeutic drugs (unused and partially-filled containers) must be managed as chemical/hazardous waste and submitted for DEHS pick up as chemical/hazardous waste.

All waste must be properly identified and labeled with appropriate waste tags. Tags and information are available from DEHS (502-852-6670). Information on the Waste Pickup program, including on-line request for pickup, can be found at: https://louisville.edu/dehs/waste-disposal.

10.5. Animal Carcasses

After proper euthanasia of laboratory animals, animal carcasses shall be placed in red bags labeled with the universal biohazard symbol that are secondarily contained in vendor provided biohazard cardboard boxes or plastic totes and, depending on the animal facility, placed in the appropriate holding freezer. The ultimate disposal of animal carcasses is through medical waste incineration.

10.6. Drosophila Fly Disposal

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules requires the containment of organisms containing recombinant DNA. Appendix G states that:

> *The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory and the environment to organisms containing recombinant DNA molecules.*

Transgenic flies must be treated as biohazardous waste, and therefore must be treated either by autoclave, incineration or chemical prior to release into the general waste stream. To the untrained eye, it is impossible to differentiate genetically altered flies from their wild-type counterparts. It is for this reason that the disposal of **ALL** flies on campus require special disposal procedures. Non-transgenic flies are not exempt.

Flies must be treated by one of the following ways:

- Via drowning in bleach or ethanol. If using bleach, decant bleach in sink. If using ethanol, collect waste ethanol in container marked as "Hazardous Waste – Ethanol. Full waste ethanol containers must be submitted to DEHS for chemical waste pick up, If packaging for placement in the garbage, label bag as 'sterilized waste'.
- Via autoclave: make sure to place flies in autoclavable biohazard bag, and once autoclaved, discard into red bag lined medical waste container. The ultimate disposal of deceased transgenic flies is through medical/infectious waste incineration

11. Decontamination

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

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

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

Physical and chemical means of decontamination fall into four main categories: *Liquid Chemicals, Heat, Vapors and Gases, and Radiation.*

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapor and gases (e.g. ethylene oxide), radiation and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time.

11.1. Liquid Chemical Decontamination

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

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

11.1.1. Resistance to Chemical Disinfectants

	Organism	**Example**
Least Resistant	Lipid or Medium-size viruses	Herpes simplex virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus

		Human immunodeficiency virus
	Vegetative Bacteria	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella choleraesuis*
	Fungi	*Trichyphyton* sp. *Cryptococcus* sp. *Candida* sp.
	Nonlipid or small viruses	Polio virus Coxsackie virus Rhino virus
	Mycobacteria	*Mycobacterium tuberculosis* *Mycobacterium bovis*
Most Resistant	Bacterial Spores	*Bacillus subtilis* *Clostridium sporogenes*

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

11.1.2. UofL Approved Disinfectants

11.1.2.1. Selection criteria

Per OSHA interpretation in the United States (U. S.), in the case of human blood/serum, disinfectants must be either:

- Approved as a sterilant by the FDA;
- Registered as a tuberculocide with the EPA;
- Registered as a sterilant with the EPA; or,
- Registered with the EPA as effective against Hepatitis B virus and HIV-1.

In general, disinfectants suitable for work with human-sourced materials will be appropriate for working with most risk group (RG) 2 and RG 3 agents, with the exception of spore-forming organisms. Work with prions requires unique treatment with disinfecting agents to denature the prion proteins.

The product must be approved for the specific use and must be used according to the manufacturer's directions. In the U.S., it is illegal to use disinfectants except as specified by the manufacturer.

All disinfectants used for decontamination purposes must be approved by the BSO if not included in this manual.

11.1.2.2. Cautions

Many disinfectants contain harsh chemicals. Suitable personal protective equipment (PPE) must be worn. Refer to MSDS sheets (hard copies or on line access required).

Preparations must be discarded after the expiration date established by manufacturer.
NOTE: Chlorine bleach solutions must be prepared on the day they are used unless:

- Documentation of available free chlorine levels (with < 5% drop in levels) over time is generated by the laboratory, and,
- The testing is specific for the brand of bleach, water (distilled, deionized, tap) and containers that will be used.

11.1.2.3. Labeling

Disinfectants placed in a secondary container must be labeled with the following:

- Product name;
- Concentration;
- Expiration date; and,
- Relevant hazard data.

11.1.2.4. Approved disinfectants

The following are approved disinfectants for use where blood, serum, tissues and/or infectious viral, parasitic and/or bacterial agents may be present.

Surface disinfectants:

A. 0.1% Sodium Hypochlorite (Chlorine Bleach)
NOTE: OSHA has approved a 1:100 or stronger dilution of household chlorine bleach containing 5.25% sodium hypochlorite as a tuberculocidal disinfectant. This is equivalent to 500 ppm of available chlorine.

It is recommended to use a 2% bleach (1:50, 1000 ppm chlorine) solution, prepared daily, as follows:

- Check the bleach label for the concentration of sodium hypochlorite.
- Concentrations of household bleach may range from 5.25–8.25%

Use the following formula to calculate how much bleach to add to a given volume of water:
Final concentration/Concentration of bleach x Total Volume = Volume of bleach to add

- To prepare a 1 liter or 1000 ml of a 0.1% Sodium Hypochlorite solution from common household bleach containing 5.25% sodium hypochlorite, the proportions are: 19 ml of full strength bleach out of the bottle, add water to 1 liter. [0.1% / 5.25% X 1000 ml = 19 ml]

B. Cavicide or Envirocide (EPA Reg.: 46781-6; Metrex Research Corp., Romulus, MI)

C. Caviwipes (EPA Reg.: 46781-8; Metrex Research Corp., Romulus, MI)

D. CiDecon Detergent Disinfectant (EPA Reg.: 3862-179-56753; Decon Labs, King of Prussia, PA)

E. (Clorox Healthcare Bleach Germicidal Cleaner (formerly “Dispatch”) (EPA Reg.: 56392-7; Clorox Professional Products Co., Oakland, CA)

F. Clorox Healthcare Bleach Germicidal Wipes (EPA Reg.: 67619-12; Clorox Professional Products Co., Oakland, CA)

G. Conflikt Detergent (EPA Reg.: 1839-83; Decon Laboratories, Inc., King of Prussia, PA)

H. MB-10 Tablets (formerly "Aseptrol") (EPA Reg.: 70060-19-46269; BASF, Florham Park, NJ)
NOTE: Prepare at a 200 ppm solution as directed by manufacturer. Store in a tightly covered container and use within 7 days.

I. Micro-Chem Plus (formerly "NP 4.5") (EPA Reg.: 1839-95-2296; National Chemical Laboratories, Inc., Philadelphia, PA)

J. LYSOL Brand I.C. Quaternary Disinfectant Cleaner (formerly "FORMULATION HWS-256") (EPA Reg.: 47371-129-675; Reckitt Benckiser LLC, Parsippany, NJ)

NOTE: Use at a 1/256 dilution as directed by manufacturer.

K. Peroxiguard (EPA Reg.: 74559-9; Virox Technologies Inc., Oakville, Ontario L6H 6R1)

L. Super Sani-Cloth Germicidal Disposable Wipe (EPA Reg.: 9480-4; PDI, Nice-Pak Products, Inc., Orangeburg, NY)

M. Sani-Cloth Plus Germicidal Disposable Cloth (EPA Reg.: 9480-6; PDI, Nice-Pak Products, Inc., Orangeburg, NY)

N. Sporicidin Brand Disinfectant Solution (pump spray and refill) (EPA Reg.: 8383-3; Contec, Spartanburg, SC)

O. Sporicidin Brand Disinfectant Towelette (EPA Reg.: 8383-7; Contec, Spartanburg, SC)

Liquid Disinfection for bulk liquids (non-radioactive):

A. Dilute waste with bleach or sodium hypochlorite to achieve an approximate final 0.5% sodium hypochlorite solution (5000 ppm, ~ 10% bleach in waste).

Treat for a minimum of 30 minutes prior to disposal down the drain with the water running. These quantities can be approximated (measuring via a pipet is not recommended).

NOTE: Check the label of the bleach you are using for the concentration of sodium hypochlorite. Concentrations of household bleach may range from 5.25– 8.25%

NOTE: Commercial bleach has a shelf-life of about 1 year <u>from the date of manufacture</u>, NOT the date of purchase. Date of manufacture can be determined from a code on the bottle that shows the manufacture site, year, and day of year (see references below for detail). Use the following formula to calculate how much bleach to add to a given volume of water:

Final concentration/ Concentration of bleach X Total Volume = Volume of bleach to add

If the bleach contains ~6% sodium hypochlorite, one liter of waste liquid would require ~83 ml of bleach to achieve a 0.5% hypochlorite solution. [0.5% / 6% X 1000 ml = 83 ml]

After appropriate treatment, the material can be disposed of down the drain, with the water running.

B. Wescodyne (EPA Reg: 4959-16-1043, Steris Corp., St. Louis, MO). Add to waste in the ratio of ~10 ml of Wescodyne into 1 liter liquid waste. Mix gently and treat for a minimum of 2 hours prior to disposal down the drain with the water running.

   NOTE: Do not use a metal container to collect bulk liquid for treatment with Wescodyne, as it will corrode metal.

   NOTE: Wescodyne's active ingredient is iodine – Do not use if allergic to iodine.

11.1.2.5. Cleaning solutions

These solutions are appropriate for reducing environmental contamination in the laboratory and for removing disinfectant residual from equipment (e.g., after using bleach on stainless steel or centrifuge rotors). They are not appropriate for disinfection of work surfaces (when used alone) after handling potentially infectious materials.

A. 70% alcohol solutions, either prepared in the laboratory or purchased (e.g., Septihol Ready To Use, 70% USP grade, non-sterile isopropyl alcohol solution filtered to 0.22 micron (Steris Corporation)).

B. Laboratory cleaning solutions such as Alconox, Luminox, Liqui-Nox, SoluJet, 7-X are all acceptable for cleaning laboratory equipment and surfaces and as the aqueous detergent used in the first step of decontaminating a contaminated instrument or cleaning up a biological spill (for removal of proteins before application of the disinfectant).

11.1.2.6. Recommended disinfectants for use when working with bacterial spores

A. Spor-Klenz Ready to Use (EPA Reg.: 52252-7-1043, Steris Corporation, St. Louis, MO)

B. Spor-Klenz Concentrate (EPA Reg.: 52252-4-1043, Steris Corporation, St. Louis, MO)

C. 0.5% Sodium Hypochlorite solution (~10% bleach or 5,000 ppm chlorine)

D. Clorox Healthcare Bleach Germicidal Cleaner and Wipes (see above)

11.1.2.7. Prion Disinfection

Prions are difficult to destroy and require special decontamination procedures. Incineration is the preferred method of disposal of all contaminated material or equipment. Use the following procedures to prepare prion waste for incineration by DEHS.

- For Surface Wipe Down: Use disposable pads that can be incinerated. Clean surface area with detergent solution. Wipe or flood area, if possible, with a minimum of 5.25% sodium hypochlorite or 2 N sodium hydroxide. Apply pads or toweling to keep area moist. Minimum contact time of 1 hour is needed; gather up pads or toweling for incineration; rinse area with water.

- For Liquid/soak treatment: Dilute waste to a final dilution of 1N sodium hydroxide or a final minimal dilution of 2.5% sodium hypochlorite for 1 hour.

- Waste Treatment – Depending on protocol requirements, autoclaving at 134-138°C for a minimum of 18 minutes; and/or incineration. Use of only disposable equipment (pipets, beakers, etc.) that can be incinerated is recommended.

11.1.3. Disinfection of a Surface

Lab benches should be wiped down with disinfectant at the end of the work day or session, and sooner if known contamination occurs. Disinfectant contact time is not necessarily critical during general bench wipe downs because you are relying on the mechanical gesture of wiping to remove potential debris as opposed to actually killing organisms.

If a known contamination occurs, place paper towels over the contaminated surface, then add liquid disinfectant; this will prevent spread of contamination. Allow sufficient contact time after applying the disinfectant. If the contact time is too brief, the surface will not be thoroughly disinfected. When cleaning a spill of concentrated material or if the disinfectant must act on an uneven surface, allow extra contact time.

Avoid using concentrated or undiluted solutions of your disinfectant to "speed up" the inactivation process. The surface that is being disinfected may be adversely affected by strong chemicals. This is especially significant when working with bleach, which is a very strong corrosive. Some disinfectants will leave a residue of chemicals behind. Rinse the cleaned area with distilled water to avoid adverse effects on your experiment. This is especially important in tissue culture rooms where a cell line can be wiped out by disinfectant residue left on equipment.

11.2. Heat Decontamination

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

11.2.1. Autoclaves

A steam autoclave is a device designed to sterilize cultures, media, surgical instruments and medical waste. Autoclaves will sterilize on the basis of:

- Length of time in the cycle
- Temperature
- Contact
- Pressure
- Steam

An autoclave is suitable for the treatment of certain types of medical waste but not all types. The following items of medical/infectious waste must **NOT** be autoclaved:

- Items of medical/infectious waste which are commingled with chemical liquids or radioactive materials (this waste must be handled as either chemical waste or radioactive waste).

The following items of medical waste can be autoclaved:

- Microbiological waste such as cultures of human or animal specimens from medical or pathological laboratories.
- Cultures and stocks of microbiological specimens.
- Waste contaminated with biohazardous materials such as contaminated paper towels or contaminated surgical gloves.

Considerations for effective autoclaving:

- Do not overload the autoclave bag. The autoclave steam and heat cannot penetrate to the interior of an overloaded bag. If overloaded, the outer contents of the bag will be sterilized but the inner part of the bag will essentially be unaffected by the autoclave cycle.
- Do not place sharp objects, such as broken glass, in a bag since this can puncture the bag.
- Do not overload the autoclave.
- Do not mix autoclave bags and other items to be autoclaved in the same autoclave cycle.
- Liquid media requires a shorter cycle, often 15-20 minutes while autoclaveable medical waste requires a minimum of 30 minutes in order to be effectively sterilized.
- To help insure non-variability of sterilization, try to use a consistent loading pattern of materials within the autoclave (amount of material and location within autoclave).
- Validate autoclave effectiveness once every 40 working hours with biological indicator vials.

Safety considerations for autoclave users:

- Wear personal protective equipment to include heat-resistant gloves, goggles or safety glasses and a lab coat.
- Use caution when opening the autoclave door. Allow superheated steam to exit.
- Use caution when handling a bag in case sharp objects have been inadvertently placed in the bag. Never lift a bag from the bottom of the bag to load into the chamber. Handle the bag from the top and hold away from the body.
- Watch out for pressurized containers. Superheated liquids may leak from sealed containers. Never seal a container of liquid with a cork that may cause a pressurized explosion inside the autoclave.
- Agar plates will melt and the agar will become liquefied. Avoid coming in contact with this molten liquid. Absorbent packs or material should be placed in the bottom of these bags.
- Glassware may crack or shatter if cold liquid comes in contact with this superheated glassware. If glass breaks in the autoclave, use tongs, forceps, or other mechanical means to recover the fragments; make certain that the autoclave has been cooled down to avoid surface burns. Using a secondary tray will contain broken glass and make it easy to remove.
- Always use a secondary container. The secondary container will catch liquid leaking from bag, broken glass or melting plastic – making for easy clean up. Never put autoclave bags or glassware directly in contact with the bottom of the autoclave.
-

To autoclave waste, follow these procedures:

- Place waste as generated in an orange bag approved for use within an autoclave.
- Put autoclave tape loosely around the top of the bag and place the bag in a secondary container such as an autoclave pan.
- Set the appropriate preprogramed cycle.
- After autoclaving, appropriately dispose of the waste.

11.3. Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most commonly used gases are formaldehyde and ethylene oxide; hydrogen peroxide vapor is gaining popularity. Applied in closed systems under controlled conditions (e.g., humidity) these agents achieve sterility.

11.3.1. Paraformaldehyde and formaldehyde

Common use: To decontaminate large pieces of laboratory equipment, such as biosafety cabinets **(but only by professionals!**).

Effective against: Vegetative bacteria, fungi, lipid and non-lipid viruses, HBV, TB, *Coxiella burnetii*, and bacterial spores

Formaldehydes are registered carcinogens and are very toxic to use without the accessibility of a vented fume hood and/or personal protective equipment. DO NOT ATTEMPT TO USE PARAFORMALDEHYDE OR FORMALDEHYDE IN THE LAB TO DECONTAMINATE EQUIPMENT. This method of decontamination must be performed by a trained professional. The approved BSC contractor will use paraformaldehyde to decontaminate your BSC prior to changing the HEPA filters. Be sure to avoid the room in which the BSC is located while this procedure is taking place.

11.3.2. Ethylene Oxide

Common use: Often used to disinfect hospital instruments

Effective Against: Vegetative bacteria, fungi, lipid and non-lipid viruses, HBV, TB, *Coxiella burnetii*, and bacterial spores

Ethylene oxide is a carcinogen and is very toxic to use without mechanically generated ventilation exhaust and personal protective equipment. DO NOT ATTEMPT TO USE ETHYLENE OXIDE IN THE LAB TO DECONTAMINATE EQUIPMENT. THIS LEVEL OF DECONTAMINATION MUST BE PERFORMED BY A PROFESSIONAL.

11.3.3. Hydrogen peroxide

Common use: Biocontainment rooms, BSC's, CO_2 incubators and small freeze dryers

Effective Against: Bacteria (including anthrax), viruses, fungi and molds

The most beneficial aspect of hydrogen peroxide is that the lab can remain in use while equipment is being decontaminated. Additionally, the vapor phase is ideal for equipment with complex shapes. THIS ACTIVITY MUST BE PERFORMED BY A PROFESSIONAL OR TRAINED INDIVIDUAL.

11.4. Radiation

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

11.5. Frequency of Disinfection for Common Equipment

Frequent disinfection of lab equipment helps to maintain equipment over long periods of time and prevent contamination of work specimens. The frequency of disinfection should be based on the recommendation of equipment manufacture in conjunction with a risk assessment of the biological agents being used.

11.5.1. Centrifuges

Decontamination of the outer and inner parts of a centrifuge should be done on a regular basis and if possible at the end of each procedure. This can be done by using cloth dampened with disinfectant.

The rotor should be sprayed inside with disinfectant at the end of each procedure and allowed to air dry. The outside of the rotor should be sprayed each time the rotor is removed from the BSC.

11.5.2. Bench Tops

Bench tops should be decontaminated with proper disinfectant at the end of each working day and each time a potentially contaminated item comes in contact with it. The surface should be sprayed with the disinfectant and then wiped down.

11.5.3. Incubators

Incubators should be decontaminated on a regular basis (monthly if possible) to prevent contamination and loss of samples inside. Consult manufacturer's guide for specific instructions and suggested disinfectants to use, to avoid damaging equipment.

11.5.4. Biological Safety Cabinets

BSC's need to be surface decontaminated prior to any work and at the end of the work period. This includes the tray, the back and side panels as well as the glass sash (both on the inside and outside).

Complete decontamination of the cabinet should be performed on a monthly basis at a minimum. This includes removing the bottom tray and properly cleaning and decontaminating all surfaces of the BSC.

Professional decontamination where the unit is sealed and exposed to paraformaldehyde or vaporized hydrogen peroxide is required if the BSC is to be moved to a new location or is being subjected to repair work.

11.5.5. Chemical Fume Hoods

Chemical fume hoods should be cleaned once a month even if they are not being used on a regular basis. To do so, surface decontaminate all sides of the fume hood including the sash.

11.5.6. Floors

Floors should be swept and mopped at least once a week. If the lab is special in nature, such as a BSL-3 lab, mopping should be performed by the lab occupants, as opposed to housekeeping staff. Dilute bleach is generally a good mop solutions for laboratories.

12. Lab Deactivation & Equipment Disposal

When laboratories are to be relocated, renovated, vacated or closed, all chemical, radioactive, biological or other hazardous materials must be removed and disposed, in accordance with applicable EPA, OSHA, NIH, CDC and other regulations. Equipment and items that may pose a potential danger to people or the environment must be removed and properly disposed.

Principal Investigator, Researcher, Instructor, Laboratory or Clinical Manager or Other Applicable Individual must notify the intention to close the laboratory by completing the University of Louisville's Laboratory Close-Out Notification Form, and submitting it to DEHS at least 30 days prior to closure.

12.1. Lab Close Out Process and Procedures

- The UofL Lab Safety Close-Out Procedures (pdf) is a detailed checklist for ensuring proper close-out of laboratories. The list should be reviewed well in advance of the target date for vacating the laboratory.
- DEHS should be notified of the upcoming lab close-out by submitting the Lab Close-Out Notification web-form. Several week notice should be allowed so that DEHS can make provisions for staff availability and to ensure that one has time to complete the lab close-out checklists. Checklist items that do not apply to the lab should be noted "not applicable" (n/a).
- The Principal Investigator (PI) (or designee) must contact the Lab Safety Assessment Specialist at 852-6670 to confirm the date and time of the final inspection so DEHS can certify proper lab close-out. DEHS will review the completed checklists with the PI and complete a walk-through of the lab space. If there a no remaining issues DEHS will issue a Lab Close-Out Certification Form. Copies of the signed form will be sent to the PI vacating the lab and the Department Head.
- If there are questions about any items on the checklists contact the appropriate DEHS staff member. Contact information is provided in the Lab Close-Out Guide.
- Note that radioactive, biological or hazardous chemical waste MAY NOT BE MOVED to a new location. Be sure to request waste pick-ups in advance so that all any regulated waste is out of the lab prior to the final walk-through.

13. Training and Medical Surveillance

DEHS offers numerous safety training courses and materials for employees of all levels and backgrounds. A complete listing of available training programs can be found at https://louisville.scishield.com/

13.1. Laboratory Safety and Hazardous Waste Training/Basic Biosafety

Laboratory safety training, mandated by the OSHA Laboratory Safety Standard (29 CFR 1910.1450), is required for all U of L personnel that work in a research laboratory. This training provides a general overview of how to safety use hazardous chemicals in the work area, how to access safety and health information about hazardous biological materials and chemicals, and the measures employees may take to protect themselves from them. In addition, U of L's standard operating procedures for handling, storing, and disposing of hazardous materials in the laboratory are also covered. Autoclave training provides a general overview of the proper safety and operational procedures that personnel should follow in order to prevent accidents and personal injury while operating an autoclave. Initial training is in class but subsequent refresher training is available online at the link below. Live training is available for groups upon request by calling 502-852-6670.

Online safety training link: https://louisville.scishield.com/

13.2. Radiation Safety Training

Radiation safety training is mandatory for all employees who work in any lab that is authorized to use or possess radioactive material. Prior to using or working in a lab with radioactive material, classroom orientation training is required. Refresher training is required annually and is available online. Radiation safety training covers the fundamentals of radiation safety, exposure limits, and personnel exposure monitoring. Training also covers detection techniques, survey and waste handling requirements, emergency response, and radioisotope inventory management. Depending on the work assignments, personnel may be required to take additional online training, such as, X-ray or laser safety training. The online courses are available at the link listed above. Live training is available for groups upon request by calling 502-852-5231.

Irradiator training, hands on training and security clearance is required in order to gain access to U of L irradiators. Clearance packets and instructions may be obtained from the Radiation Safety Office.

13.3. Bloodborne Pathogen Training

In 1993, OSHA published the Bloodborne Pathogens Standard (29 CFR, Bloodborne Pathogens. - 1910.1030); the fundamental premise of this rule is an approach to infection control termed Standard Precautions. Standard Precautions assumes that all human blood, blood products, and certain body fluids are contaminated with HIV, HBV, HCV, or other bloodborne pathogens and that these materials be handled accordingly.

Annual training is mandatory for individuals who may be exposed to bloodborne pathogens through work with human blood, bodily fluids, tissues and cell lines. Find this training at https://louisville.scishield.com

13.3.1. Bloodborne Pathogen Standard

The Bloodborne Pathogens Standard (29 CFR, Bloodborne Pathogens. - 1910.1030) applies to all occupational exposure to blood or other potentially infectious materials. Blood means human blood, human blood

components, and products made from human blood. Bloodborne pathogens means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, Hepatitis B virus (HBV), Hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

Additionally, "Other Potentially Infectious Materials" (OPIM) are included under this standard. OPIM means (1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any bodily fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); (3) primary or established human cell lines and (4) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

The following are specific actions U of L has taken to minimize exposures to bloodborne pathogens: https://louisville.edu/dehs/biological-safety/biological-safety-files/university-of-louisville-bloodborne-pathogens-exposure-control-plan– describes how to eliminate or minimize exposure of all U of L personnel to human/primate blood or blood products that might contain bloodborne pathogens. All work at U of L that has the potential to contain bloodborne pathogens will be carried out using Standard Precautions.

Standard Precautions is an approach to infection control whereby all human/primate blood and other human/primate body fluids, tissues and cells are treated as if known to be infectious for HIV, HBV, HCV, and other bloodborne pathogens (BBP's).

PIs/supervisors are to complete an Exposure Plan based on the nature of the work being carried out in their facilities. The PI/supervisor indicates procedures and materials in the laboratory that have the possibility of exposing personnel to BBP's. Once completed, the plan remains on file in a central location within the laboratory/work place.

A copy of the U of L's Bloodborne Pathogens Exposure Control Plan template and other information about the Bloodborne Pathogens program can also be accessed on the DEHS website at: https://louisville.edu/dehs/biological-safety

13.3.2. Bloodborne Pathogen Training Program

All University of Louisville faculty, staff, and students who have potential exposure to blood or blood products must be trained annually through the DEHS online refresher course.

The online refresher course can be accessed at the following link: https://louisville.scishield.com/

To help determine if a worker is at risk for contact with BBP, please use the questions listed below.

Will the person:

- Handle human blood products, such as whole blood, plasma, serum, platelets, or white cells?
- Handle human body fluids such as semen, cerebrospinal fluid, vaginal secretions, joint fluid, pleural fluid, peritoneal fluid, pericardial fluid, or amniotic fluid?

- Work with animals, such as primates OR perform tasks where such animals are housed?
- Handle unfixed human tissue or organs (Tissues and organs soaked in chemical preservatives such as alcohol or formaldehyde are fixed)?
- Work with Hepatitis B virus or other bloodborne pathogens or with preparations, such as liquid solutions or powders containing the Hepatitis B virus?
- Handle blood, blood products, body fluids or unfixed tissues or organs of animals infected with the Hepatitis B virus or other bloodborne pathogens?
- Handle sharp instruments such as knives, needles, scalpels, or scissors which have been used by others working with human blood or other potentially infectious materials to include human organs, tissue or body fluids OR used by others working with similar body parts and fluids from animals infected with the Hepatitis B virus or other bloodborne pathogens?
- Enter areas where other individuals work with human or animal blood, body fluid, tissues or organs which are infected with the Hepatitis B virus or other bloodborne pathogens AND perform tasks where any of the aforementioned body substances may come into contact with the laboratory worker's unbroken skin, broken skin, or mucous membranes?
- Perform tasks which may potentially result in the lab worker's exposed skin or mucous membranes coming in contact with human or animal blood, body fluids, organs, or tissues which are infected with the Hepatitis B virus or other bloodborne pathogens?

If the answer to **ANY** of the above questions is yes, then the worker is considered to be at occupational risk of contracting Hepatitis B or other bloodborne pathogens. All workers at risk must complete Bloodborne Pathogen Training annually. All personnel listed on U of L IBC registration that involve work with potential bloodborne pathogens must be shown to have completed the appropriate training. All employees receive initial training at the time of hire, refresher training is then completed online or through specially scheduled departmental/lab sessions.

Additionally, in-lab training should be conducted by the PI or laboratory supervisor. This training will be a combination of the U of L Exposure Control Plan and of individualized training suitable for each individual. In a laboratory environment the type of experiments being conducted, nature of the material used, and the equipment used would determine the required types of training. Written documentation of all in lab training must be recorded and retained by the PI.

13.4. Hepatitis B Vaccine Program

The University (by means of funding provided by the department, PI or college) must make the Hepatitis B vaccination available to those employees who have the potential for occupational exposure. This must be done at no cost to the employee. While U of L encourages employees to be vaccinated, accepting vaccination is not a condition of employment. Employees that are offered the vaccine are required to either accept the vaccine or sign a declination form. For more information please call Campus Health Services (502-852-6479 or 502-852-6446).

13.5. Medical Surveillance

Medical surveillance may be required for both those workers who use biohazardous agents as well as any animal handler who must tend to animals inoculated with etiologic agents. Some animals may be naturally infected with agents not related to the research, such as pregnant sheep whose body fluid may contain *Coxiella burnetii***,** the causative agent for Q-fever. The Research Resource Facilities (RRF) will work with DEHS and Campus Health Service (CHS) to identify animal handlers who may be at risk for occupational exposure to infectious microorganisms in the course of their duties.

Procedures for receiving a medical examination

- Each department shall enroll eligible employees in the Medical Surveillance Program. The supervisor (aided by the IBC and IACUC) shall identify employees who may be at risk for occupational exposure to biological agents. The Biosafety Officer can also assist the supervisor in determining if a medical examination is appropriate.

- The employee will schedule the medical appointment with CHS (502-852-6479 or 502-852-6446). Note: If a scheduled appointment cannot be kept, then it is the responsibility of the employee to notify CHS and reschedule the appointment.

- Upon completion of the medical examination, the participant will be notified by the examining physician to review the results. Appropriate referrals will be made at this time in the event of abnormal findings.

- The requesting department or committee will receive notice of medical clearance form from the CHS. This form will describe the participant's ability to work with biological agents, work in the particular environment or other condition that initiated the examination.

- If there is a restriction indicated on the clearance form that inhibits an individual's ability to complete a job, then the supervisor shall contact DEHS to discuss a remedial course of action.

- Medical records will be kept at the CHS for the duration of the individual's participation in the Medical Surveillance Program at U of L.

13.5.1. Vaccinations

Vaccinations will also be made available for many etiologic agents used in the laboratory. The Biosafety Officer in conjunction with the IBC will make the recommendation for the use of vaccinations on a case-by-case basis. However, depending on the nature of the research, the PI may be responsible for any vaccine costs.

14. Recombinant or Synthetic Nucleic Acid Molecules: Regulations and Guidelines

The use of recombinant or synthetic nucleic acid molecules is regulated by the National Institutes of Health (NIH). The guidelines can be found in the publication *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*.

These guidelines are the official guide to all recombinant or synthetic nucleic acid molecule work done at University of Louisville. It is important to realize that following these guidelines is the responsibility of **all** investigators at University of Louisville and not solely investigators that are funded by NIH. The guidelines specify a number of different classification categories for recombinant or synthetic nucleic acid molecules.

NIH Guideline Classification	
III-A	Deliberate transfer of drug resistance to microorganisms.
III-B	Cloning of toxin molecules with LD_{50} < 100 ng/kg and experiments that have been Approved (under Section III-A-1-a) as Major Actions under the NIH Guidelines.
III-C	Deliberate transfer of recombinant or synthetic nucleic acid molecules into one or more human research participants.
III-D	Risk Group 2 or 3 agents as host-vector systems or DNA from risk group 2 or 3 agents. Infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus. Recombinant or synthetic nucleic acid molecule related experiments involving whole animals (i.e., gene therapy), excluding the generation transgenic rodents. Generation of transgenic animals (non-rodent species, rodents requiring ABSL2 or 3). Experiments involving whole plants using BL2-P and BL3-P containment. Experiments involving more than 10 liters of culture. Experiments with influenza viruses generated by recombinant or synthetic methods.
III-E	Formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3rd of the genome of any eukaryotic virus. Experiments involving whole plants. Generation of transgenic rodents (ABSL1 only).
III-F	Synthetic nucleic acids that can not: • replicate or generate NA that that can replicate in living cells • integrate into DNA • produce toxin lethal to vertebrates with an LD50 less than 100 ng/kg

	Not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes. Consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature. Consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means. Consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host. Consist entirely of DNA segments from different species that exchange DNA by known physiological processes. Genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA. No significant risk to health of the environment (as determined by the NIH Director • Recombinant or synthetic nucleic acid molecules containing < ½ of any eukaryotic viral genome that are propagated and maintained in cells in tissue culture • E. coli K-12, *Saccharomyces*, *Bacillus subtilis* or lichenformis host vector systems • Extrachromosomal elements of gram positive organisms • Purchase or transfer of transgenic rodents • Crossbreeding of certain transgenic lines (ABSL-1 only)

14.1. Human Gene Transfer

Protocols involving the use of recombinant or synthetic nucleic acid molecules for gene transfer into humans, whether done directly in the subject or *in vitro* and subsequently put into the subject, must be submitted to both the IBC and relevant Institutional Review Board (IRB). Current Federal Regulations call for submission of the protocol to the Recombinant DNA Advisory Committee (RAC) and The Food and Drug Administration (FDA) prior to submission to the local institutional panels. For additional information concerning IRB panels, please call 502-852-5188, send email to hsppofc@louisville.edu, or visit the IRB website.

14.2. Transgenic Plants

Experiments using recombinant or synthetic nucleic acid molecule-containing plants, plant-associated microorganisms, and small animals

require registration with the IBC (see NIH Guidelines). To prevent release of transgenic plant materials to the environment, the guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants.

Information Systems for Biotechnology (ISB) has released the following publication: A Practical Guide to Containment: Plant Biosafety in Research Greenhouses. The publication can be accessed at the following link: https://vtechworks.lib.vt.edu/handle/10919/78423 or

The publication is excellent and generally can answer any plant related question you may have, so we will not rewrite information for the purposes of this manual.

14.3. Recombinant or Synthetic Nucleic Acid Molecule Exposure

Purpose: The University of Louisville is required to report spills or accidents in laboratory to the NIH/Office of Science Policy (OSP).

Guideline: The U of L Procedure for Investigating and Reporting Potential Violation(s) of *National Institutes of Health Guidelines (NIH Guidelines) state that*

The following incidents will be reported immediately to the NIH/OBA by the DEHS Director and BSO via email to NIHGuidelines@od.nih.gov and a subsequent investigation will be conducted if deemed an exposure. More detailed follow-up reports, if needed, will be sent within 30 days to NIH/OBA at the above address.

- Spills or accidents in BSL-2 laboratory resulting in an overt exposure, injury, or illness.
- Spills or accidents occurring in high containment (BSL-3) laboratory resulting in an overt or potential exposure, injury, or illness.

It is recommended that the following incidents be reported as soon as possible to the NIH/OBA by the DEHS Director and BSO via email to NIHGuidelines@od.nih.gov.

More detailed follow-up reports, if needed, will be sent within 30 days of incident occurrence to NIH/OBA at the above address.

- Release of a Risk Group 2 or 3 agent/genetic material from a primary containment device (e.g. biological safety cabinet, centrifuge, or primary container into the laboratory).
- Spills or accidents that lead to personal injury or illness or breach of containment (e.g. aerosols released outside of containment).

Overt exposures are dependent both on quantity (how large of a spill it is) as well as the method of exposure. Skin punctures (needle sticks, cuts, and abrasions) are considered overt exposures. A spill that occurs outside of containment (outside of a biological safety cabinet) that cannot be covered by one paper towel is also considered an overt exposure. Overt exposures can be to the recombinant or synthetic nucleic acid molecules themselves, or, to viruses or organisms containing the recombinant or synthetic nucleic acid molecules.

Recombinant or Synthetic Nucleic Acid Molecule Overt Exposures that MUST BE reported to the NIH via the IBC:

SPILLS OUTSIDE OF BIOSAFETY CABINET (BSC)

- Cleaning instructions
  - Clearly warn others in the area.
  - Wearing gloves, lab coat and other appropriate personal protective equipment (PPE), wipe up the spill with absorbent material and place in a biohazard bag.
  - Apply appropriate disinfectant to spill area and wipe clean.
- Reporting instructions:
  - Immediately report the incident to CHS. They will advise on whether or not you need to be seen for follow-up surveillance and/or treatment. CHS will have 1 business day to report the incident to the IBC.
  - Immediately contact the Biosafety Officer, DEHS Director, IBC Chair or any IBC member.
  - The appropriate institutional official will report the incident to OSP as described in the U of L Procedure for Investigating and Reporting Potential Violation(s) of the NIH Guidelines

Recombinant or Synthetic Nucleic Acid Molecule Incidents that DO NOT require reporting to the NIH:

SPILLS INSIDE BSC

- Cleaning Instructions:
  - Make sure the BSC is turned on while decontaminating to prevent the escape of contaminants.
  - Wearing gloves, lab coat, and other appropriate PPE, wipe up spill with absorbent material, place in a biohazard bag.
  - Flood affected area with appropriate disinfectant, and let stand 15-30 minutes.
  - Spray and wipe walls, work surfaces, and all affected apparatuses with appropriate disinfectant.
  - Wipe area clean with water, followed by 70% ethanol.

14.4. Viral Vectors and Transgenes

It is important to realize that obtaining a cloning/expression vector from a commercial source does not mean it is automatically exempt, or a BSL-1 agent.

Not all vectors carry the same potential biological hazard, and more importantly, the type of gene insert can change potential hazardous risk and the biosafety level of the construct**.** The following chart discusses commonly used viral vectors.

14.5. Common Viral Vectors

Vector System	Risk Group	Biosafety Level	Biosafety level with oncogenic insert
Adeno-associated viral (AAV) vector without helper virus	RG-1	BSL-1, ABSL-1	BSL-2 ABSL-2
Adeno-associated viral (AAV) vector with helper virus	RG-1	BSL-2, ABSL-2	BSL-2 ABSL-2
Adenoviral vector	RG-2	BSL-2, ABSL-2 (ABSL-1 72 hours after administration)	BSL-2 ABS_2
Herpes virus Vector nonlytic	RG-2	BSL-2, ABSL-2	BSL-2+ BSL-3
MMLV (Ecotropic)	RG-2	BSL-1, ABSL-1	BSL-1 ABSl-1
MMLV (Amphotopic, VSV – G pseudotyped)	RG-2	BSL-2	BSL-2+/BSL-3
Lentiviral vector, $1^{st}/2^{nd}$ or unknown generation	RG-3	BSL-2	BSL-2+
Lentiviral vector, $3^{rd}/4^{th}$ generation	RG-3	BSL-2	BSL-2
Alphavirus based – SFV, SIN	RG-3	BSL-2, BSL-2+, BSL-3	
Baculovirus based	RG-1	BSL-1	BSL-2
Poxvirus based – canarypox, vaccinia	RG-2	BSL-2, BSL-2+/BSL-3	

14.6. U of L IBC Guidance for the Use of Human Cells in Animals

The University of Louisville Institutional Biosafety Committee must approve any procedure involving human cells before an animal research protocol can be approved UofL IACUC. The following chart discusses commonly used practices when using human cells animals.

	MATERIAL	BIOSAFETY LEVEL (applicable to injections / necropsy)	ANIMAL BIOSAFETY LEVEL (applicable for animal housing / cage changes)	HOUSING REQUIREMENTS	CAGE CHANGES	PRE / POST-INFECTION MANIPULATIONS	IMAGING
HUMAN CELL LINES ***(* "Retroviral" is inclusive of lentiviral vectors)***							
1	**Unmodified (established) Human cell lines inserted into an immunocompromised mouse**	BSL2	ABSL1	No special requirements	**No special requirements** - cages can be changed on benchtop. Bedding does not need autoclaved prior to disposal / cages do not need autoclaved prior to washing	No special requirements	No special requirements
2	**Human cell lines transduced with retroviral* vector then inserted into an immunocompromised mouse**	BSL2	ABSL2 for 1 week / then ABSL1	Microisolator technique for 1 week post-dosing; after 1 week, no special requirements	**For first week**: 1. ALL cage changes in BSC; 2. bag and autoclave bedding; 3. bag and autoclave caging before wash; After one week, resume normal cage change.	All injections and necropsies in BSC for DURATION of project (following BSL2 practices)	Imaging - all manipulations inside BSC where feasible. Maintain BSL2/ABSL2 practices where feasible, especially during the first week.
3	**PRIMARY human tissue transplant from healthy donor with known health status into mice**	BSL2	ABSL1	No special requirements	**No special requirements** - cages can be changed on benchtop. Bedding does not need autoclaved prior to disposal / cages do not need autoclaved prior to washing	No special requirements	No special requirements
4	**PRIMARY human tissue transplant from donor of unknown health status into mice**	BSL2	ABSL2	Microisolator technique for DURATION of experiment	1. ALL cage changes in BSC; 2. bag and autoclave bedding; 3. bag and autoclave caging before wash	All injections and necropsies in BSC for DURATION of project (following BSL2 practices)	Imaging - all manipulations inside BSC where feasible. Maintain BSL2/ABSL2 practices where feasible

14.7. U of L IBC Guidance for the use of Adenoviral Vectors in Animals

The University of Louisville Institutional Biosafety Committee must approve any procedure involving replication defective adenoviral vectors in animals before an animal research protocol can be approved by U of L IACUC.

- Animals inoculated with Adenoviruses are to be isolated for 72 hours under BSL 2 conditions following inoculation. The inoculation should be performed in a class II Biosafety cabinet in the animal facility whenever possible. These animals are to be housed in micro-isolator caging.
- If Principal Investigators will be injecting the adenovirus in the animals in a laboratory, the investigator must document in the application the plan for containment in case of spillage. Protective garments (lab coat, gloves, head cover, shoe covers) must be worn while working in the lab, protective garments and any non-animal waste should be placed in an appropriately labeled biohazard container, and all laboratory personnel must be instructed by the PI on the safe handling of both the agent and the animals. The animals may be moved within the same building but not between buildings. If the animals are being transported, the microisolator should be wiped down with an appropriate approved disinfectant, placed in an enclosed bin, then the bin wiped down and taken to the laboratory. The reverse of this process should be used when returning animals to the housing area. If using rabbits, the animal should be moved directly from the surgery area in a disposable cage to a room that is set up for BSL-2 conditions.
- The investigator must also document in the IBC application/registration a plan for containment should an accident occur during transport of the animals i.e. a cage falls off a cart or is otherwise damaged in transport.
- At 72 hours post inoculation, the animals may be removed from their cages, placed in new cages and returned to general housing in their source room (ABSL-1 conditions). Plan for the disposal of contaminated caging and bedding should be handled per plan developed between the PI and RRF and documented in the use application and animal research protocol. Animal care personnel will dispose of the contaminated material specific to the accommodations of each facility.

14.8. U of L IBC Guidance for the use of HIV-1 and HIV-2 Based Lentiviral Vectors

The University of Louisville Institutional Biosafety Committee must approve any procedure involving HIV-1 and HIV-2 lentiviral before an animal research protocol can be approved UofL IACUC.

- Animals inoculated with lentiviruses are to be isolated for 72 hours under ABSL-2 conditions following inoculation. The inoculation should be performed in a class II Biosafety cabinet in the animal facility whenever possible. These animals are to be housed in micro-isolator caging.

- If Principal Investigators will be injecting the lentivirus in the animals in a laboratory, appropriate PPE (fro example lab coat, gloves, head cover, shoe covers) must be worn while working in the lab. PPE and any non-animal waste should be placed in an appropriately labeled biohazard container, and all laboratory personnel must be instructed by the PI on the safe handling of both the agent and the animals. The animals may be moved within the same building but not between buildings. If the animals are being transported in their cage, the cage must be wiped down, placed in an enclosed bin, then the bin wiped down and taken to the laboratory. The reverse of this process should be used

when returning animals to the housing area. If using rabbits the animal should be moved directly from the surgery area in a disposable cage to a room that is set up for ABSL-2 conditions.

- At 72 hours post inoculation, the animals may be removed from their cages, placed in new cages and returned to general housing in their source room (ABSL-1 conditions). Contaminated caging and bedding should be autoclaved before disposing it. according to ABSL-2 practices.

15. UofL's Institutional Biosafety Committee

15.1. Institutional Biosafety Committee Charge

The NIH has mandates an Institutional Biosafety Committee (IBC) for all organizations that conduct research at or sponsor research that receives any support for recombinant or synthetic nucleic acid research from NIH .

The IBC is appointed by and reports to the Executive Vice President for Research and Innovation and shall be made up of at least five members with expertise in general issues of laboratory biosafety, use of infectious materials, and recombinant DNA technology. Individuals on the committee include faculty and staff, the Biosafety Officer (BSO), two members from the local community not otherwise affiliated with U of L, and any others invited to serve when their expertise is required. *Ex officio* and *ad hoc* members shall include representatives of the Department of Environmental Health and Safety, a veterinarian and other experts/consultants. The BSO is responsible for the day-to-day operation of the biosafety program and reports to the Director of the Department of Environmental Health and Safety.

The IBC reviews all U of L research involving the use of biohazardous materials such as:

- Risk Group 2 and Risk Group 3 organisms
- Synthetic and recombinant nucleic acid as defined by the NIH Guidelines
- Use of transgenic plants such as Arabidopsis lines created by T-DNA insertion
- Select agents and toxins (as defined in 7 CFR Part 331, 9CFR Part 121, and 42 CFR Part 73)
- Other biological materials such as microorganisms, plants or animals with a recognized potential for significant detrimental impact on local managed or natural ecosystems
- Biological toxins
- Prions
- Materials of human or primate origin
- Established human cell lines
- Materials that could be considered Dual Use Research of Concern

For specific details regarding the review process see the IBC procedures manual. Through the review, the committee ensures that the activities and facilities are in compliance with applicable UofL policies/procedures, local, state, and federal regulations, and best safety practices.

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The IBC also assesses suspected or alleged non-compliance in the context of approved projects, external regulations, and/or U of L policies/procedures involving biohazardous agents and/or materials. Serious or continuing non-compliance may result in a suspension of research by the IBC.

In the event of suspected or alleged non-compliance the IBC will immediately notify the involved PI that an investigation has been initiated by a designated subcommittee composed. Information related to the alleged non-compliance will be requested from the PI and, if necessary, a meeting with the PI will be scheduled. If the PI does not respond or cooperate with an IBC investigation of an alleged non-compliance within 2 business days of initial notification, the incident will escalate up the chain of command (i.e. Department Chair, EVPRI, etc.) until the investigation can be completed.

Upon completion of an investigation, the investigating subcommittee will determine if a violation of the *NIH Guidelines* occurred. The subcommittee will prepare an incident report using the NIH/OBA "Incident Reporting Template" as guidance and, if needed, the DEHS Director or BSO will notify the appropriate federal oversight agency. Copies of the incident report and correspondence with the NIH/OBA will be provided to the PI, IBC chair, EVPRI, DEHS Director, BSO, IBC, Department Chair, and University Counsel.

Upon request, the IBC will review and comment on proposed local, state, or federal biosafety regulations. When appropriate, the IBC will formulate draft policies and procedures for approval by the appropriate U of L bodies. Appeals in cases of dispute with respect to procedures or decisions of the committee may be made to the Biosafety Officer who will facilitate mediation.

15.2. The IBC Review Process

Registration applications are submitted using online forms via the iRIS website: https://iris.louisville.edu/ and reviewed by the IBC review process which is described in the IBC procedures manual: https://louisville.edu/dehs/biological-safety/biological-safety-files/university-of-louisville-ibc

In brief, the work flow starts with a registration describing the use of hazardous biological materials being submitted to the IBC using iRIS. The IBC will review the registration at its next scheduled meeting and either approve, return with modification in order to secure approval, table, or disapprove the registration.

16. Select Agents and Toxins

The Federal Select Agent Program oversees a collection of designated infectious agents and toxins that, by their nature, have the potential to pose a severe threat to public health and safety.

This threat has resulted in the creation of a number of legislative acts. "The Antiterrorism and Effective Death Penalty Act of 1996," which became effective on April 15, 1997, established the first list of select agents and required registration for transfer of said agents.

The "Uniting and Strengthening America by Providing Appropriate Tools Required to Intercept and Obstruct Terrorism Act (USA PATRIOT Act) of 2001" established provisions that regulate the possession, usage, or transfer of hazardous agents, and required the Department of Health and Human Services to issue rules to implement these provisions.

The Patriot Act specifically addressed the issue of possession of select agents by certain “Restricted Persons”, Section 817(b), and criminalized possession of select agents except for bona fide purposes. Additionally, the “Public Health Security and Bioterrorism Preparedness and Response Act of “2002” expanded the list of select agents, required registration for possession of select agents, and required security measures to prevent access to agents. The list of select agents is constantly changing. The present list of Select Agents can be found in its most updated form at: http://www.selectagents.gov/SelectAgentsandToxinsList.html

If a PI decides that use of a viable select agent or a select toxin is required, the PI must contact the Biological Safety Officer at 502-852-6670 prior to obtaining the material. Obtaining amounts of select agent and toxins above the non-exempt limit requires prior approval by the CDC or USDA. The procedure for receiving approval by the IBC to obtain and work with exempt quantities is outlined below.

16.1. U of L Requirements for Possession of CDC Select Agent Toxins

This document outlines UofL’s institutional requirements on possession of **permissible toxin amounts** of Centers for Disease Control and Prevention Select Toxins. These requirements have been established to ensure:

- Safe laboratory handling, use, and storage procedures
- Effective tracking and security of the regulated toxins
- Compliance with federal regulations

16.1.1. Exempt Quantities of CDC Select Agent Toxins

Per the federal regulations, each PI may possess exempt quantities of Select Toxin(s) without CDC registration and approval; IBC approval must still be obtained. The following quantities of Select Toxins represent the maximum amount a PI may possess at any given time (seehttp://www.selectagents.gov/PermissibleToxinAmounts.html)

Exempt Quantities of CDC Select Agent Toxins	
Toxin	**Maximum Quantity (per PI)**
Abrin	100 mg

Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins A, B, C, D, and E	100 mg
Tetrodotoxin	500 mg
T– 2 toxin	10,000 mg

Additionally, the following toxins are exempt

- Any Select Toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
- Non-functional or inactivated Select Toxins.
- An animal inoculated with or exposed to a Select Toxin.

It is important to ensure that the total amount of Select Toxin per PI in a laboratory is maintained below these permissible quantity limits at all times for exemption from Select Agent and Toxin regulation and registration. Due to the severe penalties associated with non-compliance with the Select Agent and Toxin rules, it is imperative that each laboratory using and storing Select Toxins maintains current and accurate inventory information for these toxins. DEHS will contact these labs annually to verify inventories.

Warning: Failure to register a select agent is a criminal offense, punishable by up to five years in prison and/or $500,000 in fines per the Public Health Security & Preparedness Response Act of 2002.

16.1.1.1.U of L Requirements for Possession of Permissible Quantities of Select Toxins

The Principal Investigator is responsible for ensuring that the following items are in place before obtaining and/or using Select Toxins:

- Obtain approval from the Institutional Biosafety Committee.
- Develop Standard Operating Procedures for the use of select toxin.
- Provide initial lab-specific safety training to staff on toxin-involved processes, with updates as necessary. Best practices recommend that the PI maintains documentation of training. The following topics should be covered during the training:
    - Toxin-associated hazards
    - Engineering controls used to minimize exposure (e.g., fume hood use)
    - Personal protective equipment (PPE) to be used when handling toxin
    - Safe handling and storage
    - Proper decontamination, inactivation and disposal
    - Administrative requirements (recordkeeping, inventory, security)
- Provide appropriate personal protection (e.g., gloves, safety glasses, lab coat or disposable lab coat). NOTE: If respirators are necessary, contact at 502-852-6670 for appropriate fit-testing and training.
- Use of fume hood, biological safety cabinet or glove box with toxin-associated procedures.
- Use accepted inactivation procedures. Contact DEHS if you need to discuss inactivation methods.

Chemical Inactivation of Toxins

Toxin	2.5% NaOCl + 0.25 N NaOH	2.5% NaOCl	1% NaOCl	0.1% NaOCl
T-2 mycotoxin	Yes	No	No	No
Tetrodotoxin	Yes	Yes	Yes	No
Saxitoxin	Yes	Yes	Yes	Yes
Ricin	Yes	Yes	Yes	Yes
Botulinum	Yes	Yes	Yes	Yes
Staphylococcal enterotoxin	Yes (?)	Yes (?)	Yes (?)	Yes (?)

*30 minutes exposure to various concentrations of sodium hypochlorite with and without sodium hydroxide.
Key: Yes- complete inactivation; Yes(?)-assumed inactivation.

Wannemacher, 1989.

- For secure storage the toxins must be:
    - Stored with compatible materials in a sealed container with secondary containment.
    - Under at least one layer of physical security (e.g., Select Toxin should be secured within a locked freezer, or secured within a locked storage device).

- List of PI-Approved Users: A documented list shall be maintained of PI-approved toxin users (including those having access to toxin materials). The lab must keep track of who uses the stock (and who has access to the freezer). Before becoming an approved user, the PI must ensure that each person has received training, as discussed above.

- Inventory Maintenance: Inventory must be kept on the Select Toxin Inventory and Usage Log which can be requested from the Biosafety office at 852-6670. To prevent inadvertently exceeding exempt quantity levels of select toxins, researchers are required to promptly update inventories after every container of select toxin is:
    - Acquired (by purchase/intra-campus transfer)
    - Depleted (by consumption/intra-campus transfer)
    - Inactivated

- Documented Security Inspection: Self-Inspections should be performed annually and documentation of inspections must be kept for one year or the duration of Select Toxin possession, whichever is longer. Inspection should include:
    - Review of approved users list to verify authorized access to toxins
    - Verification of appropriate labeling, storage, secondary containment, security measures
    - Comparison of physical inventory with what is accounted for on the usage log

In addition, DEHS will perform at a minimum annual laboratory visits to review compliance with institutional requirements on possession of select toxins.

16.1.1.2. Possession of Select Agent Toxins Above Exempt Quantities

For possession of select agent toxin quantities above permissible toxin amounts, approval from the IBC and Department of Environmental Health and Safety must be obtained prior to registering with the Centers for Disease Control. For any questions regarding CDC Select Agent possession at U of L, contact the Responsible Official at 502-852-6670.

17. Toxins

17.1. Guidelines for Work With Biological Toxins (Other than Select Agent Toxins)

Biological toxins are toxic substances produced by microorganisms, animals, and plants that have the capability of causing harmful effects when inhaled, ingested, injected or absorbed. The health effects of exposure can vary greatly depending on the toxin, the amount, and the route of exposure, ranging from minor (skin or eye irritation, headache, nausea) to severe (respiratory distress, muscle weakness, seizures, death).

All research projects involving a biological toxin with a mammalian LD50 equal to or less than 100 µg/kg body weight should be registered with the IBC using the iRIS IBC registration form (https://iris.louisville.edu/). The PI should also develop lab-specific Standard Operating Procedures (SOPs) for work with biological toxins at the time of submission. Research should not commence with the toxin until the project has been reviewed and approved by the IBC.

The biological toxin table shown below lists numerous toxins that require registration based on known LD50 values (obtained from a variety of sources). Please note that this list is not exhaustive and there may be other biological toxins with LD50 values < 100 µg/kg body weight that will require registration. If you need additional guidance, please contact the Biosafety Office at 502-852-6670 or biosafe@louisville.edu.

Toxin	LD50 (µg/kg body weight)	Species	Route
Aerolysin	7*	Mouse	IV
B-Bungarotoxin	7.8 10	Mouse Mouse	IP IV
Caeruleotoxin	40	Mouse	IV
Cereolysin	40-80	Mouse	IV
Ciguatoxin 2	1	Mouse	IP
Ciguatoxin 3	0.9	Mouse	IP
Clostridium difficile enterotoxin A	0.5	Mouse	IP
Clostridium perfringens alpha toxin, lecithinase	3	Mouse	IV
Clostridium perfringens theta toxin, perfringolysin O	13-16	Mouse	IV
Clostridium perfringens enterotoxin	81	Mouse	IV
Clostridium perfringens beta-toxin	4.5 0.31	Mouse Mouse	IP IV
Clostridium perfringens delta-toxin	5*	Mouse	IV
Clostridium perfringens epsilon-toxin	0.1*	Mouse	
Crotoxin	82	Mouse	IV
Diphtheria toxin	0.01 6.5	Mouse Hamster	IV IP
Listeriolysin	3-12*	Mouse	
Maitotoxin	0.6	Mouse	IP
Modeccin	1.3	Rat	IP
Nematocyst toxins	33-70	Mouse	IV
Notexin	5	Mouse	IV
Palytoxin	0.089 0.05	Rat Mouse	IV IP
Pertussis toxin	18	Mouse	IP
Pneumolysin	1.5	Rabbit	IV
Pseudomonas aeruginosa exotoxin A	14 3	Mouse Mouse	IP IV
Scaritoxin	50	Mouse	IP
Shiga toxin	0.25 0.0022	Mouse Rabbit	IP IV

Shigella dysenteriae neurotoxin	1.3 0.45	Mouse Mouse	IP IV
Streptolysin O	8	Mouse	IV
Streptolysin S	25	Mouse	IV
Staphylococcus aureus alpha toxin	0.04-0.06	Mouse	IV
Taipoxin	2	Mouse	IV
Tetanus toxin	0.003	Mouse	SubQ
Viscumin	2.4	Mouse	IV
Volkensin	1.38	Mouse	IP
Yersinia pestis murine toxin	10	Mouse	IV

IV = intravenous; IP = intraperitoneal; SubQ = subcutaneous
*Lowest dose reported to kill at least one subject (minimum lethal dose, MLD)

In recognition of the growing number of microbiological and biomedical laboratories working with biological toxins, the following is provided as a guideline for working with these toxins.

The material below is adapted from the Biological Defense Safety Program, Technical Safety Requirements (DA Pamphlet 385-69) and Appendix A of the United States Department of Labor Occupational Safety and Health Association rule "Occupational Exposure to Hazardous Chemicals in Laboratories".

Laboratory managers and facility safety officials are encouraged to utilize the references listed below and to consult with subject matter experts before using any toxin, to ensure that appropriate facilities, containment equipment, policies and procedures, personnel training programs, and medical surveillance protocols specific to the toxin and the laboratory, are in place.

General
The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. If both toxins and infectious agents are used, both must be considered when containment equipment is selected and policies and procedures are written. If animals are used, animal safety practices must also be considered.

Standard Practices
Standard practices listed under BSL-2 and BSL-3 in the CDC/NIH Publication *Biosafety in Microbiological and Biomedical Laboratories* should be reviewed and incorporated as appropriate into protocols for work with toxins.

Special Practices
Special practices listed under BSL-2 and BSL-3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

- Training specific to the toxin(s) used should be required and documented for all laboratory personnel working with toxins, before starting work with the toxin and at intervals thereafter.
- An inventory control system should be in place.
- Toxins should be stored in locked storage rooms, cabinets, or freezers when not in use.
- Access to areas containing toxins should be restricted to those whose work assignments require access.
- Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the safety officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.
- The user should verify inward airflow of the hood or biological safety cabinet before initiating work.
- All work should be done within the operationally effective zone of the hood or biological safety cabinet.
- When toxins are in use, the room should be posted to indicate "Toxins in Use Authorized Personnel Only." Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.
- All high risk operations should be conducted with two knowledgeable and trained individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
- Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Toxins should be transported only in leak/spill-proof secondary containers.
- Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g., used gloves) should be disposed of as toxic waste. Materials contaminated with infectious agents as well as toxins should also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.
- The interior of the hood, glove box, or cabinet should be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet should be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

Safety Equipment

The safety equipment guidelines list for BSL-2 and BSL-3 laboratories should be reviewed and incorporated as appropriate into protocols for work with toxins.

When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered. Eye protection should be worn if an open-fronted containment system is used. Other protective equipment may be required, depending on the

characteristics of the toxin and the containment system. For example, use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.

- When handling dry forms of toxins that are electrostatic:
    - Do not wear gloves (such as latex) that help to generate static electricity
    - Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.
- When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.
- Consider both toxin and diluent when selecting gloves and other protective clothing.
- If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

Laboratory Facilities

Laboratory facility recommendations listed under BSL-2 and BSL-3 in the CDC/NIH Publication *Biosafety in Microbiological and Biomedical Laboratories* and OSHA standards should be reviewed and incorporated as appropriate into protocols for work with toxins.

Vacuum lines When vacuum lines are used with systems containing toxins, they should be protected with a HEPA filter to prevent entry of toxins into the lines. Sink drains should be similarly protected when water aspirators are used.

17.2. Toxin Safety Plan

All investigators working with toxins of biological origin (not limited to those on the select agent list) should complete a laboratory specific **Toxin Safety Plan** that includes:

- Name and quantity of toxins
- Known hazards (attach SDS if available)
- Safety equipment and PPE
- Protocols and training requirements
- Toxin inactivation and disposal and inventory control
- Security measures
- Emergency procedures (spills, exposure management)

17.3. Sources and Mechanisms for Various Toxins and Venoms

Sources and Mechanisms for Various Toxins and Venoms		
Toxin or Class	**Source**	**Mechanisms of action**
Small molecules		

Tetrodotoxin	Puffer fish, octopus salamander	Na+ channel blocker
Saxitoxin	Shellfish contaminated with dinoflagellates	Na+ channel blocker
Ciguatoxin	Large tropical fish contaminated with dinoflagellates	Actions on Na+ channel
Cardiac glycosides	Toad skin	ATPase inhibitor
Batrachotoxin	Frog skin	CNS toxin
Palytoxin	Sea anemone	Lanophore
Proteins and polypeptides		
α Bungarotoxin	Elapid snakes (kraits)	Nicotinic receptor blocker
β Bungarotoxin	Elapid snakes (kraits)	Presynaptic cholinergic nerves
α Conotoxin	Coneshells	
μ Conotoxin	Coneshells	Skeletal muscle Na+ channel blocker
w Conotoxin	Coneshells	N-type Ca2+ antagonist
Cardiotoxin	Elapid snakes	Direct-acting cardiotoxin
Phospholipases	Many snakes	Cell membrane destruction
Bacterial toxins		
Botulinum toxin	*Clostridium botulinum*	Synaptin in nerve endings
Cholera toxin	*Vibrio cholerae*	Activation of G_s protein
Pertussis toxin	*Bordetella pertussis*	Inactivates G_o/G_s protein
Endotoxin	Gram-negative bacteria	Cell membranes
Tetanus toxin	*Clostridium tetani*	Cell membrane ionophore
Staphylococcal toxin	*Staphylococcus* sp.	Enterotoxin
Pseudomonas exotoxin A	*Pseudomonas aeruginosa*	Inhibits protein sythesis
Diphtheria toxin	*Corynebacterium diphtheriae*	ADP-ribosylation of elongation factor 2

Walker, 1997

17.4. Chemical Inactivation of Toxins

Chemical Inactivation of Toxins

Toxin	2.5% NaOCI + 0.25 N NaOH	2.5% NaOCI	1% NaOCI	0.1% NaOCI
T-2 mycotoxin	Yes	No	No	No
Brevetoxin	Yes	Yes	No	No
Microcystin	Yes	Yes	Yes	No
Tetrodotoxin	Yes	Yes	Yes	No
Saxitoxin	Yes	Yes	Yes	Yes
Palytoxin	Yes	Yes	Yes	Yes
Ricin	Yes	Yes	Yes	Yes
Botulinum	Yes	Yes	Yes	Yes
Staphylococcal enterotoxin	Yes (?)	Yes (?)	Yes (?)	Yes (?)

*30 minutes exposure to various concentrations of sodium hypochlorite with and without sodium hydroxide.
Key: Yes- complete inactivation; Yes(?)-assumed inactivation.

Wannemacher, 1989.

17.5. Heat Inactivation of Toxins

Heat Inactivation of Toxins*					
Toxin	**Autoclaving**	**200 ˚F**	**500 ˚F**	**1,000 ˚F**	**1,500 ˚F**
T-2 mycotoxin	No	No	No	No	Yes
Brevetoxin	No	No	No	No	Yes
Microcystin	No	No	Yes	Yes	Yes
Tetrodotoxin	No	No	Yes	Yes	Yes
Saxitoxin	No	No	Yes	Yes	Yes
Palytoxin	No	No	Yes	Yes	Yes
Ricin	Yes	Yes	Yes	Yes	Yes

Botulinum	Yes	Yes	Yes	Yes	Yes
Staphylococcal enterotoxin	Yes (?)	Yes (?)	Yes (?)	Yes (?)	Yes (?)

*autoclaving or 10 minutes of exposure to dry heat at various temperatures.
Key: Yes-complete inactivation; Yes(?) – assumed inactivation.

Wannemacher, 1989.

18. Transportation

18.1. Shipment of Biological Materials

The federal government, in its shipping and transportation standards, defines etiologic agents as microorganisms that cause disease in humans including the following: bacteria, bacterial toxins, viruses, fungi, rickettsia, protozoans, and parasites. These disease-causing microorganisms may also be referred to as infectious agents or infectious substances and the materials, such as body fluids and tissues that contain them, are referred to as infectious materials. Organisms such as mosquitoes that might transmit infectious diseases to other humans are called vectors.

When a package of infectious material is being imported into the United States, it may require an CDC importation permit and/or USDA permit. It is important to obtain a CDC permit and/or USDA permit PRIOR to requesting an etiologic specimen from a source outside the United States.

Transport of biohazardous materials off of U OF L property requires training and certification prior to shipping. Federal (FAA, 49 CFR) and international agencies (International Air Transport Association - IATA) have numerous regulations for shipping of dangerous goods by surface or air. Training is mandatory for the shipper (person sending out the package) and handlers (people who transport the package) and is based on these regulations; **the training certificate must be renewed every two years.** Noncompliance with these regulations can result in a fine and/or imprisonment.

18.2. Shipping Training

According to current US DOT requirements anyone who handles, offers for transport, or transports biological materials must be trained to perform the duties required by their employer. There are three required areas of training recognized by all regulatory agencies:

1. General Awareness / Familiarization Training
2. Function-Specific Training
3. Safety Training

The Department of Environmental Health and Safety offers shipping training through SciShield. The training can be accessed at https://louisville.scishield.com/

18.3. Permits

18.3.1. CDC Permits

The Centers for Disease Control and Prevention's Import Permit Program (IPP) regulates the importation of infectious biological materials that could cause disease in humans in order to prevent their introduction and spread in the U.S.A.

18.3.1.1. Materials that require a CDC permit

Materials requiring import permits fall into three categories:

- 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)

Infectious biological agent - A microorganism (including, but not limited to, bacteria (including rickettsiae), viruses, fungi, or protozoa) or prion, whether naturally occurring, bioengineered, or artificial, or a component of such microorganism or prion that is capable of causing communicable disease in a human.

Infectious substance - Any material that is known or reasonably expected to contain an infectious biological agent.

Vector - Any animals (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal products (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are known to transfer or are capable of transferring an infectious biological agent to a human.

Animals: Any member of the animal kingdom except a human including an animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws).

Arthropods – Any living insect including crustaceans, spiders, scorpions, etc. capable of being a host or vector of human disease.

Snails: Any freshwater snails (phylum Mollusca, class Gastropoda) capable of transmitting schistosomiasis.

Bats: All live bats. See below for further information on obtaining an import permit for live bats. Bats may also require a permit from the U.S. Department of Interior, Fish and Wildlife Service. For additional information, see http://www.fws.gov/permits/importexport/importexport.shtml

Non-human primate material – all non-human primate material (e.g. blood, plasma, tissue, urine, feces) requires an import permit, unless it has been specifically treated and rendered non-infectious.

Consult the CDC IPP website for more information on whether you need a permit at
https://www.cdc.gov/cpr/ipp/etool.htm

CDC Import permit application can be accessed on the CDC Etiologic Agent Import Program web-site at http://www.cdc.gov/od/eaipp/importApplication

18.3.1.2. Materials that do NOT require a CDC import permit

- A Select Agent listed in 42 CFR Part 73 and its importation has been authorized in accordance with 42 CFR 73.16 or 9 CFR 121.16.
- Diagnostic specimens not known by the importer to contain, or suspected by the importer of containing, an infectious biological agent and is accompanied by an importer certification statement confirming that the material is not known to contain or suspected of containing an infectious biological agent, or has been rendered noninfectious.
- Animal or animal products being imported for educational, exhibition, or scientific purposes and *accompanied by documentation* confirming that the animal or animal product is not known to contain (or suspected of containing) an infectious biological agent or has been rendered noninfectious.
- Nucleic acids that cannot produce infectious forms of any infectious biological agent and the specimen is accompanied by an importer certification statement confirming that the material is not known to contain or suspected of containing an infectious biological agent.
- Animal or animal products listed in 42 CFR Part 71 and their importation have been authorized in accordance with 42 CFR §§ 71.52, 71.53, or 71.56.
- A product that is cleared, approved, licensed, or otherwise authorized under any of the following laws:
    - The Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), or
    - Section 351 of the Public Health Service Act pertaining to biological products (42 U.S.C. 262),
    - The Virus-Serum-Toxin Act (21 U.S.C. 151-159).

If you are not certain if an agent you intend to use requires a CDC importation permit, please call the Biosafety Officer at 502-852-6670 for assistance making the determination.

18.3.2. USDA/APHIS Permits

Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for import transport or export of organisms infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum).

Information on USDA transport or import permits is available at
https://www.aphis.usda.gov/aphis/ourfocus/importexport

Permits are also required from the USDA/APHIS for interstate movement, importation, or release into the environment (i.e., field tests) of genetically engineered organisms that are plant pests, or that contain portions (plasmids, DNA fragments, etc.) of plant pests.

An application should be made in advance of the anticipated release or shipment date. Information and applications may be obtained at: https://www.aphis.usda.gov/aphis/resources/permits

18.3.3. Facility Registration for Receipt of Select Agents

Facility registration and completion of the CDC Form 1 are required by the CDC and USDA/APHIS prior to transfer of select agents and toxins (42 CFR Part 73). Select agents are discussed in Chapter 16. Please contact the Biological Safety Officer at (502-852-6670) if your work includes any of the agents listed in Chapter 16.

18.3.4. Department of Commerce License

A validated license is required by the Department of Commerce for export of certain microorganisms and toxins to all destinations except Canada. Information may be obtained by calling the Department of Commerce Bureau of Export Administration at 202-482-4811 or through the internet at: https://www.bis.doc.gov/index.php/licensinghttp://www.cdc.gov/Other/disclaimer.html.

When these substances derive from or are involved in "fundamental research" as defined by the export control regulations, they may be eligible for the "No License Required" (NLR) provisions of the Export Administration Regulation or other special treatment. To determine NLR or other export exemptions status, please contact UofL Export & Secure Research Compliance at 502-852-2454.

18.4. Packaging

Various carriers (FedEx, UPS, Postal Service or others) have different requirements for packaging and labeling infectious substances. In addition, various agencies such as the IATA and the Department of Transportation (DOT) have developed guidelines and procedures to facilitate the safe shipment of infectious substances or genetically modified organisms. Therefore, it is important to check with the carrier you have chosen to determine specific requirements for shipment of infectious agents.

In addition to the materials listed above which require permits, the following materials are likely to require special packaging and/or labeling:

- Infectious Substance is a viable microorganism, or its toxin, which causes or may cause disease in humans.
- Exempt human/animal specimen is any human or animal material including blood, tissue, and tissue fluids, which is not known to contain an infectious agent.
- Nonregulated biological Product is a product for human or veterinary use, such as vaccines and investigational new drugs.

The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

- A primary container that contains the specimen
- A secondary container that contains the primary container and packaging capable of absorbing the specimen
- An outer rigid shipping container that contains the secondary container and other material

18.5. Genetically Modified Microorganisms

The NIH Guidelines (Appendix H) for Experiments Involving Recombinant or Synthetic Nucleic Acid Molecules (2019) states that:

- Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant or synthetic nucleic acid molecules, if they are regulated as human pathogens by the Public Health Service or as animal pathogens (42 Code of Federal Regulations, Part 72) or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

- Host organisms or viruses will be shipped as etiologic agents if they contain recombinant or synthetic nucleic acid molecules when:
  - the recombinant or synthetic nucleic acid molecule includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest.
  - the recombinant or synthetic nucleic acid molecule codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage.
  - the recombinant or synthetic nucleic acid molecule codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage.

18.6. Human Blood and Tissue

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color coded. Various carriers may have additional requirements.

18.7. On-Campus Transport Between Laboratories or Buildings

Since UofL labs are located throughout the Health Science Campus, Belknap Campus and ShelbyHurst Campus there is a need to occasionally transport biological specimens from one building to another building on the same campus in a safe manner.

Guidelines:

1. The specimen must be contained in a **primary container** (test tube, specimen cup, etc.); it is recommend that the closure of primary container be secured with secondary or positive means (e.g. tape, parafilm, etc.) and placed in a sealable bag that contains sufficient absorbent material to absorb the entire volume of the primary container.
2. The primary container is then placed in a **secondary container** (e.g. Styrofoam shipping box or hard-sided individual cooler) that contains sufficient absorbent material (e.g. bench diapers) to absorb the entire volume of the primary container should leakage occur.
   - The secondary container must be closeable, sturdy enough to remain closed should the container be dropped and must conceal the primary container (e.g. a 50 ml tube would not serve as a secondary container to a 1.5 ml tube – because you are able to see the specimen in the secondary container).
3. The outside of the **secondary container must be labeled** with the following information:
   - Biohazardous sticker if applicable
   - PI name and phone number, U of L/Department
   - DEHS recommends creating a permanent secondary container that can be used multiple times as needed. Simply attach a biohazardous sticker to the container and label the PI name, phone number, U of L and department in permanent ink.
4. Walk the material to the intended destination.

Definitions:
Transport = Carry by hand (walking)

Shipping = Send through a 3rd party carrier (e.g. UPS or FedEx)

Biological Specimen = including, but is not limited to, cell lines, tissue/blood samples, DNA/RNA, bacteria/viruses/parasites

RG-2 and RG-3 agents may not be transported using private vehicles or shuttles. For transport of RG-2 and RG-3 organisms contact DEHS (852-502-6670) or http://louisville.edu/dehs/training/dehs-shipment-request-form-for-biological-samples) for assistance;

RADIOACTIVE SAMPLES, LIVE ANIMALS AND SPECIMENS THAT CONTAIN MORE THAN 100 ML OF FIXATIVE OR DENATURING AGENT ARE NOT ALLOWED TO BE TRANSPORTED. Contact DEHS (852-502-6670) for assistance.

19. Inactivation of RG-3 Agents for Removal from a BSL-3 laboratory

19.1. Introduction

Inactivation refers to treatment of biohazardous agents to make them safer to handle, while maintaining characteristics needed for a research study. This section describes UofL policies applicable to RG-3 agents that need to be inactivated for transfer out of a BSL-3 facility for study at a lower level of biocontainment. All inactivation procedures must be reviewed and approved by the UofL Biosafety Office *prior to use*.

19.2. Applicability

Chapter 19 applies to RG-3 agents requiring BSL-3 containment unless they are classified as Select Agents by the Federal Select Agent Program, in which case a more stringent standard is applicable. See Chapter 16 and selectagents.gov for more information about Select Agents and Toxins.

19.3. Definitions

BSO/ABSO - Biological Safety Officer or Associate Biological Safety Officer.

Risk Group 3 (RG-3) agents - Organisms associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available (i.e., high individual risk but low community risk).

Inactivation - Rendering RG-3 pathogens non-viable, or nucleic acids non-infectious, while maintaining essential characteristics needed for research.

> "Non-viable" means an infectious pathogen is no longer capable of growing, replicating, infecting, or causing disease;
>
> "Non-infectious" means pathogen-derived nucleic acids are not capable of regenerating infectious forms of the pathogen;

Inactivation SOP (Standard Operating Procedure) - A document detailing the methods used to inactivate a specified RG-3 agent. Inactivation SOPs must be validated by the PI or a designee *and* approved by the BSO/ABSO for use in a specific location or facility on campus.

Validation - A document describing the performance characteristics of an Inactivation SOP along with the objective evidence needed to show that the Inactivation SOP is likely to perform as intended for a specified RG-3 agent.

Verification - Demonstration the Inactivation SOP continues to perform as intended each time it is used. Verification is required every time an approved Inactivation SOP is performed.

19.4. Responsibilities of the PI

- Prior to removal of a RG-3 agent from BSL-3, the PI is responsible for communicating to the BSO/ABSO that an Inactivation SOP will be needed. The PI (or designee) will then collaborate with the BSO/ABSO to develop an appropriate Inactivation SOP. The PI is responsible to validate the Inactivation SOP if needed prior to final approval by the BSO/ABSO.
- The PI is responsible for ensuring that laboratory personnel receive Inactivation SOP training before using an approved Inactivation SOP in the BSL-3.

- The PI is responsible to maintain records of Inactivation SOP validation, staff Inactivation Sop training, inactivation verifications, and sample removals in a manner that allows details to be reviewed upon request for three years.
- The PI is responsible for reporting to the BSO/ABSO if an inactivation verification failed regardless if samples were removed from the BSL-3.

19.5. Steps for Development, Approval, and Use of an Inactivation SOP

1. Consult with the BSO/ABSO prior to developing an Inactivation SOP. Prior consultation avoids duplication in the event that an approved SOP is already available.
2. If Inactivation SOP validation is required, submit results of validation to the BSO/ABSO for review.
    - The BSO/ABSO will assist in making the determination if an Inactivation SOP needs to be validated prior to approval.
3. The BSO/ABSO will provide written notification when the Inactivation SOP is approved for a specified agent. The approved Inactivation SOP will then be added to the PI's laboratory safety plan or manual.
4. Provide training to the laboratory personnel on use of the Inactivation SOP
    - Training records must be maintained by the PI or designee.
5. Perform and document a verification every time the Inactivation SOP is used. Documented PI approval of inactivation is required prior to removal of RG-3 agent from BSL-3. Details to be recorded include:
    i) Identity of the Inactivation SOP and the RG-3 agent,
    ii) Date the inactivation was performed and by whom,
    iii) Number of samples tested,
    iv) Results of verification sample(s),
    v) PI approval to remove samples,
    vi) Date the samples were removed from the BSL-3 facility and by whom,
    vii) Additional details if specified in the approved Inactivation SOP.

19.6. Amending an Approved Inactivation SOP

Any modification of an approved Inactivation SOP requires review and approval by the BSO/ABSO, and possibly re-validation, before being implemented. Modification of an Inactivation SOP may also require additional training for laboratory personnel.

19.7. Resources

See the following link "Approval Process for Inactivation Methods for Infectious Material" for an example of practices employed by the NIH Biorisk Management Program, including suggested guidelines for validation of inactivation methods and an example of an Inactivated Sample Removal Log: https://ors.od.nih.gov/sr/dohs/Documents/SOP900InactivationMethodReviewProcessIBC508.pdf

REFERENCES

The following publications were used as informational resources in the preparation of this document.

1. The Centers for Disease Control and Prevention and National Institutes of Health published guidelines "Biosafety in Microbiological and Biomedical Laboratories, 6th Edition". U.S. Government Printing Office, Washington: 2020. https://www.cdc.gov/labs/BMBL.html

2. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. 2019. https://osp.od.nih.gov/wp-content/uploads/2019_NIH_Guidelines.htm

3. World Health Organization: Laboratory Biosafety Manual. 4th Edition, 2020. https://www.who.int/publications/i/item/9789240011311

4. Public Health Agency of Canada: Pathogen Safety Data Sheets https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html