GUIDELINES FOR HANDLING PATHOGENIC MICROORGANISMS AND OTHER POTENTIALLY INFECTIOUS MATERIALS AT BIOSAFETY LEVEL 2 (BSL2)

Biohazard Recognition and Control

Institutional Biosafety Committee
Office of Biological Safety
University of Chicago

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COVER EMBLEM—UNIVERSAL BIOHAZARD SYMBOL
Signifies actual or potential contamination of equipment, rooms, materials, or animals by viable hazardous agents.

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I. INTRODUCTION

This booklet seeks to increase awareness of biological hazards frequently encountered in research, clinical, and teaching laboratories at the University of Chicago and to provide guidance on recommended practices. Biological hazards include infectious or toxic microorganisms (including viral vectors), potentially infectious human substances, and research animals or their tissues, from which transmission of infectious agents or toxins is reasonably anticipated. Campus investigators contemplating research involving biological hazards or recombinant DNA are required to register their research protocol with the Institutional Biosafety Committee (IBC) at http://ibc.uchicago.edu/.

The objective of safety awareness and practice is to assure laboratory personnel that—with proper precautions, equipment, and facilities—most biohazardous materials can be handled without undue risk to themselves, their associates, their families, or the environment.

This document is intended not only for trained microbiologists, but also for individuals handling human clinical materials in other laboratory disciplines, such as biochemistry, genetics, oncology, immunology, and molecular biology. Persons who have little microbiological training might not realize the potential hazard involved.

The safety principles described are based on sound safety practices, common sense, current data, good housekeeping, thorough personal hygiene, and a plan for responding to accidents. Laboratories that are well organized and procedurally disciplined are not only safe, but also effective scientifically.

II. CODE OF CONDUCT AND CULTURE OF RESPONSIBILITY

All scientists are accountable for the establishment of a culture of responsibility in their labs and at their institutions. Fundamental to this culture of responsibility are scientific integrity and adherence to ethical codes of conduct. For the individual scientist, an ethical code of conduct centers on personal integrity. It embodies, above all, a commitment to intellectual honesty and personal liability for one's actions, and to a range of practices that characterize the responsible conduct of research, including:

- Intellectual honesty, accuracy, fairness, collegiality, transparency in conflicts of interest or potential conflicts of interest, protection of human subjects in the conduct of research, humane care of animals in the conduct of research and adherence to the mutual responsibilities between investigators and their research teams.

In the realm of research involving pathogens and toxins additional responsibilities include:

- Awareness of and adherence to all safety protocols.
- Knowledge and awareness of spill and exposure protocols.
- Knowledge of and adherence to reporting requirements related to spills, exposures, or potential releases.
- Knowledge and awareness of all emergency response protocols (e.g., fire, tornado, inclement weather).
- Completion of all classroom training requirements.
- Completion of all proficiency training requirements.
- Completion of all Occupational Health requirements, including documentation of required physicals, medical clearances, and/or vaccinations.
- **Immediate reporting to the Principal Investigator of any situation that compromises an individual's ability to perform as required in a BSL2 or ABSL2 laboratory, including physical or psychological issues.**
- Immediate reporting to the Principal Investigator and the UC, where appropriate, of behavior or activities that are inconsistent with safety and security plans.
- Awareness of and adherence to security protocols.

From the institutional perspective, the establishment of support systems for the individual scientist is essential to the development of a culture of responsibility. At the individual level, one such support system is the University of Chicago Staff and Faculty Assistance Program (SFAP- http://hrservices.uchicago.edu/benefits/healthwelfare/sfap.shtml). The SFAP is a confidential service that provides support, counseling, referrals, and resources for issues that impact your life and potentially compromise your ability to perform safely in the laboratory, such as child/elder care, family or marriage counseling, financial or legal advice, stress, alcohol and/or drug abuse, etc. You may call for help at 1-800-

456-6327 or may seek help online. Please contact the Benefits Office (773-702-9634) for login information. Registered students may also seek mental health care free of charge through the university Student Counseling Service (http://counseling.uchicago.edu).

Another important mechanism essential to the development of a culture of responsibility is the establishment of formal, confidential reporting mechanisms in instances of non-compliance with established safety and/or security policies established for the UC and for your particular laboratory. At the UC, multiple pathways exist whereby behaviors of concern can be confidentially reported, depending upon the particular situation at hand. Included among these options are: (1) Reporting to your PI/supervisor; (2) Reporting to your Department Administrator and/or Chair; (3) Reporting to the UC Whistleblower Hotline (1-800-971-4317, see Appendix 2); (4) Reporting to the Office of Biological Safety/Institutional Biosafety Committee (see Appendix 2); (5) Reporting to the Department of Environmental Health and Safety/Office of Risk Management. Depending upon the nature of a given situation, reports of concerning behavior may involve the UC Institutional Biosafety Committee as described in Appendix 2.

III. General Biosafety Principles

A. Risk Assessment

To apply biological safety principles rationally while handling a potential pathogen, one must perform a risk assessment, which considers:

1. The agent's biological and physical natures.
2. The sources likely to harbor the agent.
3. Host susceptibility.
4. The procedures that may disseminate the agent.
5. The best method to effectively inactivate the agent.

Globally, numerous government agencies have classified microorganisms pathogenic for humans into risk groups (RG) based on the transmissibility, invasiveness, virulence or disease-causing capability, lethality of the specific pathogen, and the availability of vaccines or therapeutic interventions. Risk groupings of infectious agents usually correspond to biosafety levels (BL or BSL), which describe recommended containment practices, safety equipment, and facility design features necessary to safely handle these pathogenic microorganisms. The list of pathogenic microorganisms includes bacteria, viruses, fungi, parasites, and other infectious entities. The scheme ascends in order of increasing hazard from Risk Group 1 (RG1) agents, which are nonpathogenic for healthy human adults, to RG4 agents, which display a high morbidity and mortality and for which treatments are not generally available.

The risk group listing of the NIH *Guidelines* is an accepted standard even when recombinant DNA technology is not used. It can be accessed electronically at: http://oba.od.nih.gov/oba/rac/Guidelines/APPENDIX_B.htm.

The American Biological Safety Association also provides a comprehensive risk group listing, which references global agencies. This list is accessible at: http://www.absa.org/riskgroups/index.html.

Another reliable source of information about human pathogens is available from pathogen safety data sheets posted by Health Canada:
http://www.phac-aspc.gc.ca/msds-ftss/.

Microorganisms that are RG1 require standard laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities. Many of the agents likely to be handled experimentally at the University of Chicago are RG2 or RG3 pathogens, designated as moderate and high hazard, respectively. These agents typically require more sophisticated engineering controls (e.g., facilities and equipment) than standard laboratories, as well as special handling and decontamination procedures.

Risk Group 1 agents are not associated with disease in healthy adult humans. Examples: *E. coli* K-12, *Saccharomyces cerevisiae*.

Risk Group 2 agents are associated with human disease that is rarely serious, and for which preventive or therapeutic interventions are *often* available. Examples: *E. coli* O157:H7, *Salmonella*, *Cryptosporidium*

Risk Group 3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available (high individual risk but low community risk). Examples: *Yersinia pestis, Brucella abortus*, *Mycobacterium tuberculosis.*

Risk Group 4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk). Examples: Ebola virus, Macacine herpesvirus (formerly Cercopithecine herpesvirus 1, also called Herpes B or Monkey B virus).

Microorganisms classified as RG2 or higher have been reported to cause infection and disease in otherwise healthy adults. Many have been associated with laboratory-acquired infections. The progression from invasion to infection to disease following contact with an infectious agent depends upon the route of transmission, inoculum, invasive characteristics of the agent, and resistance of the person exposed, whether innate or acquired. Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. It is prudent to assume virulence and handle such agents at the prescribed biosafety level.

B. Routes of Infection

Pathogens are transmitted via several routes of infection, depending on the pathogen in question. The most common routes of infection are inhalation of infectious aerosols or dusts, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, or percutaneous inoculation (injection, incision or animal bite). Appropriate precautions can be implemented to avoid such exposures.

C. Exposure Sources

1. Clinical and Pathological Specimens

Any specimen from human patients or animals may contain infectious agents. Specimens most likely to harbor such microorganisms include blood, sputum, urine, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, feces, and tissues. Personnel in laboratories and clinical areas handling human blood, body fluids, non-human primate material, or even human cell lines that have been screened for pathogens should practice *general universal precautions,* an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), Hepatitis C (HCV) and other blood-borne pathogens. Such personnel are required by law (OSHA 29 CFR 1910.1030) to undergo blood-borne pathogen training. At the University of Chicago, this training requirement can be satisfied either online or by attending an in-class training session. For information on obtaining this training, go to https://training.uchicago.edu/course_detail.cfm?course_id=32.

Animals may harbor indigenous pathogens that are virulent for humans. For personnel handling these animals or their tissues/body fluids, we recommend an analogous approach to infection control, *general precaution,* which assumes these animals and their blood and body fluids to be potentially infectious.

2. Cultures

Accidental spilling of liquid infectious cultures is an obvious hazard due to the generation of aerosols and/or small droplets. However, even routine manipulations of cultures may release microorganisms via aerosol formation:

- Popping stoppers from culture vessels.
- Opening closed vessels after vigorous shaking.
- Spattering from flame-sterilized utensils.
- Expelling the final drop from a pipette.
- Spinning microfuge tubes in a standard microfuge.
- Immediately following vortexing.

Manipulate cultures of infectious material carefully to avoid the uncontrolled release of aerosols or the generation of large droplets or spills. Centrifugation should involve the use of gasket-sealable tubes, carriers, and rotors, when available. Seal microplate lids with tape or replace them with adhesive-backed Mylar film. Load, remove, and open tubes, plates, and rotors within a biological safety cabinet or fume hood, although keep in

mind that the fume hood will protect you from your sample but will not protect your sample from potential room contamination.

When preparing aliquots of infectious material for long-term storage, consider that lyophilization of viable cultures may release high concentrations of dispersed particles if ampules are not properly sealed. Breakage of ampules in liquid nitrogen freezers may also present hazards because of survival of pathogens in the liquid phase.

Equipment used for manipulations of infectious materials, such as cell sorters and automated harvesting equipment, must be evaluated to determine the need for secondary containment and to consider decontamination issues. Costly equipment of this type are often operated as multi-user or core facilities; the inherent variability in risk from one project to another makes it imperative that operators and users of these facilities understand risks and methods for risk mitigation.

Use of human or animal cell cultures in laboratories requires special consideration. Cell or tissue cultures in general present few biohazards, as evidenced by their extensive use and lack of infection transmitted to laboratory personnel. Clearly, when a cell culture is inoculated with or known to contain an etiologic agent, it should be classified and handled at the same biosafety level as the agent.

Biosafety Level 2 containment conditions should be used for cell lines of human origin, even those that are well established like HeLa and HEK293, and for all human clinical material (e.g., tissues and fluids obtained from surgery or autopsy). Non-human primate cell cultures derived from lymphoid or tumor tissue, cell lines exposed to or transformed by a non-human primate oncogenic virus, and all non-human primate tissue should also be handled at BSL2. **When manipulations of these types of cell cultures present a potential to create aerosols, use a biological safety cabinet, not a clean bench or fume hood.**

3. Animals

Exercise care and thoughtfulness when using animals to isolate and propagate microorganisms, study pathology, or produce antibodies. Laboratory animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted material. In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols. During surgical procedures, necropsies, and processing of tissues, aerosols can be produced unintentionally, or the operator can inflict self-injury with contaminated instruments. Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols and dust when changing bedding and cleaning cages. The Animal Resources Center (ARC) offers required training for any personnel working with animals. For information on obtaining this training, contact the ARC at http://arc.bsd.uchicago.edu/.

D. Laboratory Exposure Potential

1. Teaching Laboratories

Whenever possible, we recommend the use of avirulent strains of infectious microorganisms in teaching laboratories. However, even attenuated microbes should be handled with care. Students should be cautioned against and trained to prevent unnecessary exposure, as exposure to “avirulent” strains may become problematic in the immunocompromised individual. Establishment of safety consciousness is integral to the conduct of good science.

2. Research Laboratories

Experiments in research laboratories using high concentrations or large quantities of pathogens increase the risk of exposure. The use of animals in research on infectious diseases also presents greater opportunities for exposure.

3. Clinical Laboratories

Personnel in laboratories performing diagnostic work-up of clinical specimens from human or animal patients are often at risk of exposure to infectious agents. The absence of an infectious disease diagnosis does not preclude the presence of pathogens. This is especially true of materials from patients who have received immunosuppressive therapy since such treatment may activate latent infections or make hosts more likely to harbor infectious agents.

E. Health Status

Some unusual circumstances warrant special considerations or measures to prevent infection of laboratory personnel by certain microorganisms:

It is good practice to inform your personal physician about your occupational risks, especially work with biohazardous or potentially biohazardous agents, so he or she may have a record of this information. Certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include, but are not limited to: diabetes, pregnancy, certain autoimmune diseases, immunodeficiency or immunosuppression, animal-related allergies, chronic skin conditions or respiratory disorders, and steroid therapy, even if only temporary.

IV. BIOHAZARD CONTAINMENT

Although the most important aspect of biohazard control is the awareness and care used by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can prevent dissemination of pathogens should their accidental release occur.

A. Biosafety Levels

Biosafety Levels consist of combinations of laboratory practices and procedures, safety equipment and laboratory facility design features appropriate for the operations to be performed within the lab, and are based on the potential hazards imposed by the agents used and for the specific lab activity. It is the combination of practice, equipment, and facility that form the basis for physical containment strategies for infectious agents. There are four biosafety levels, with Biosafety Level 1 (BSL1 or BL1) being the least stringent and Biosafety Level 4 (BSL4 or BL4) being the most stringent. Generally speaking, BSL1 is recommended for work with non-pathogenic microorganisms, BSL2 is recommended for disease agents transmitted by direct contact (percutaneous inoculation, ingestion, or mucous membrane exposure), BSL3 is recommended for disease agents with a potential for aerosol transmission, and BSL4 is recommended when total separation between the infectious agent and investigator is critical. Roughly speaking, Risk Group designations often correlate directly with the physical containment level appropriate for a given research activity. This booklet is designed to focus on Biosafety Level 2, but a brief description of the correlation between Risk Group and Biosafety Level and the facility design features appropriate for labs operating at the various biosafety levels is presented in the Tables 1 and 2.

Table 1
RELATIONSHIP OF RISK GROUPS TO BIOSAFETY LEVELS, PRACTICES, AND EQUIPMENT

Risk Group	Biosafety Level	Examples of Laboratories	Laboratory Practices	Safety Equipment
1	Basic - BSL1	Basic teaching	GMT[a]	None; open bench work
2	Basic - BSL2	Primary health services; primary level hospital; diagnostic, teaching, and public health	GMT plus protective clothing; biohazard sign	Open bench plus BSC[b] for potential aerosols
3	Containment - BSL3	Special diagnostic	As BSL2 plus special clothing, controlled access, directional air flow	BSC and/or other primary containment for all activities
4	Maximum Containment - BSL4	Dangerous pathogen units	As BSL3 plus airlock entry, shower exit, special waste disposal	Class III BSC or positive pressure suits, double-ended autoclave, filtered air

[a] GMT, Good Microbiological Technique.
[b] BSC, Biological Safety Cabinet

Table 2
SUMMARY OF BIOSAFETY LEVEL REQUIREMENTS

	Biosafety Level			
	1	2	3	4
Isolation of laboratory	No	No	Desirable	Yes
Room sealable for decontamination	No	No	Yes	Yes
Inward air flow ventilation	No	Desirable	Yes	Yes
Mechanical ventilation via building system	No	Desirable	Desirable	No
Mechanical, independent ventilation	No	No	Desirable	Yes
Filtered air exhaust	No	No	Yes	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Effluent treatment	No	No	No	Yes
Autoclave on site	Yes	Yes	Yes	Yes
Autoclave in laboratory room	No	No	Yes	Yes
Double-ended autoclave	No	No	Desirable	Yes
Class I or II BSC[1]	No	Yes	Yes	Desirable
Class II BSC	No	No	Desirable	Yes

[1] BSC, Biological Safety Cabinet

For a more comprehensive description of each of these biosafety levels, please consult the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories, 5th edition,* (2009) http://www.cdc.gov/biosafety/publications/bmbl5/.

Experiments involving recombinant DNA are also governed by another method of providing containment, namely *biological containment.* For biological containment, highly specific biological barriers are considered in the risk assessment process. Specifically, biological containment considers natural barriers that limit either (1) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (2) its dissemination and survival in the environment. For additional information on biological containment, please consult the NIH *Guidelines for Research Involving Recombinant DNA Molecules* (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html).

B. Practices and Procedures

The following practices, **corresponding to BSL2,** are important for the prevention of laboratory infection and disease, as well as for the reduction of the potential for contamination of experimental material. These practices and procedures provide the foundation for the more restrictive containment of RG3 organisms. If you are considering research with a RG3 organism, contact the Office of Biological Safety at 773-834-2707 for additional BSL3 containment information.

1. Personal Hygiene

(a) Do not eat, drink, chew gum, use tobacco, apply cosmetics, or handle contact lenses in the laboratory.
(b) Do not store food for human consumption in laboratory refrigerators.
(c) Wash hands frequently after handling infectious materials, after removing latex/nitrile gloves and protective clothing, and always before leaving the laboratory.
(d) Keep hands away from mouth, nose, eyes, face, and hair.
(e) Do not store personal items such as coats, boots, bags, and books in the laboratory.

2. Laboratory Procedures for Handling Infectious Microorganisms

(a) A laboratory safety manual should be assembled outlining activities and defining standard operating procedures. In most cases, your lab's Institutional Biosafety Committee (IBC) protocol, together with this BSL2 Biosafety Manual, will provide you with the necessary information to work safely.

(b) If you are working with recombinant DNA and/or working with agents at BSL2 or higher, you must obtain approval by the UC IBC.

The IBC can be reached at 773-834-4765 or online at: http://ibc.uchicago.edu/.

(c) Principal Investigators and/or laboratory supervisors are responsible for training employees and ensuring that all personnel are informed of hazards.

(d) Plan and organize materials/equipment before starting work.

(e) Keep laboratory doors closed; limit access to lab personnel.

(f) When RG2 (or higher) pathogens are used in long-term studies, post a biohazard sign at the laboratory entrance, identifying the agents in use and the appropriate emergency contact personnel. Electronic versions of these biohazard signs will be generated by the Office of Biological Safety based upon the information provided in your lab's IBC Protocol.

(g) Wear a fully fastened laboratory coat when working with infectious agents. Wear protective gloves whenever handling potentially hazardous materials, including human blood and body fluids.

(h) Remove and leave all protective clothing, including gloves, within the laboratory before exiting.

(i) Never mouth-pipette; use mechanical pipetting devices.

(j) When practical, perform all aerosol-producing procedures such as shaking, grinding, sonicating, mixing, and blending in a properly operating biological safety cabinet. Note that some equipment may compromise cabinet function by disturbing the air curtain.

(k) Centrifuge materials containing infectious agents in unbreakable, closable tubes. Use a centrifuge with sealed heads or screw-capped safety cups. After centrifugation, open the tubes in a biological safety cabinet.

(l) Avoid using needles and syringes whenever possible. When necessary, discard used syringe-needle units in a sharps container without removing or recapping the needles.

(m) Cover countertops where hazardous materials are used with plastic-backed disposable paper to absorb spills.

(n) Wipe work surfaces with an appropriate disinfectant after experiments and immediately after spills.

(o) Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal (See Section V of this Manual).

(p) Report all accidents and spills to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol and the location of clean-up equipment.

(q) Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Establish the habit of cleaning weekly at a minimum. Do not forget to routinely decontaminate all shared equipment and equipment in common areas.

(r) Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate biohazard signs.

C. ENGINEERING CONTROLS

1. LABORATORY DESIGN

The more virulent an organism, the greater the degree of physical containment required. Proper safety equipment provides primary containment; laboratory design provides secondary containment. The Office of Biological Safety (773-834-2707) is available for consultation on these matters.

2. LABORATORY VENTILATION

To control containment it is important that laboratory air pressure be lower than that in the adjacent spaces. This negative air pressure differential insures that air will enter the laboratory and not egress to the hallway. **To maintain negative room pressure, laboratory doors must be kept closed.**

Exhaust air from biohazardous laboratories should not be recirculated in the building. It should be ducted to the outside and released from a stack remote from the building air intake. In certain special situations including many BSL3 labs, air exhausting from a containment facility should be filtered through HEPA (high efficiency particulate air) filters, which can capture microorganisms.

3. BIOLOGICAL SAFETY CABINETS

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. BSCs, when functioning correctly and used in conjunction with good microbiological techniques, are very effective at controlling infectious aerosols. BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed.

The following are brief descriptions of BSC types and guidelines for their use. For more in-depth descriptions, including diagrams of airflow and more detailed usage parameters, please visit this site: http://safety.uchicago.edu/pp/labsafety/biosafety/cabinets.shtml.

(a) BSC TYPES

Three kinds of biological safety cabinets, designated as Class I, II, and III, have been developed to meet varying research and clinical needs. Two varieties of Class II biological safety cabinets are used on campus. Both are adequate for manipulations of RG2 or RG3 pathogens:

CLASS II TYPE A—recirculates 70% of the internal air and exhausts 30% of filtered air into the laboratory.

CLASS II TYPE B—either recirculates 30% of internal air and exhausts 70% of filtered air through a duct to the outside atmosphere or has 100% total exhaust cabinets. Because of the greater safety margin, small amounts of nonvolatile chemical carcinogens or radioactive materials can be used in this cabinet.

CLASS III cabinets are totally enclosed glove boxes and are used only for the most hazardous biological operations. Class III BSCs have dedicated, independent air handlers and exhaust fans. These enclosures should not be confused with anaerobic chambers.

Horizontal laminar flow clean benches are not biological safety cabinets and should never be used for work with potentially hazardous materials, whether biological or chemical. These devices protect the material in the cabinet but not the worker or the environment. Similarly, chemical fume hoods are not biological safety cabinets. They draw air in, potentially protecting the worker, but do not protect the material in the cabinet (your sample), and exhaust aerosolized material and vapors/gases into the environment.

The ultraviolet lamps within some biosafety cabinets provide only limited ability to inactivate microbes. Efficacy is limited to exposed surfaces and penetration of organic material is poor. Note that effectiveness decreases as the lamp ages. Furthermore, exposure to the ultraviolet light may cause eye damage. Therefore, ultraviolet lamps are not recommended to be the sole source of decontamination of biosafety cabinet surfaces.

(b) BSC OPERATION

- **LOADING MATERIALS AND EQUIPMENT**
  - Disinfect interior surfaces of the BSC.
  - Load only items needed for the procedure.
  - Do not block the rear or front exhaust grills.
  - Disinfect the exterior of all containers prior to commencing.
  - Arrange materials to minimize movement within the cabinet.
  - Arrange materials within the cabinet from CLEAN to DIRTY.
  - Materials should be placed at least six inches back from the front BSC grill.
  - Never place non-sterile items upstream of sterile items.
  - Lower the viewscreen

- **START UP**
  - Turn on blower and fluorescent light; close drain valve.
  - Wait at least two minutes before loading equipment. This is to purge the BSC of contaminated air.
  - Check grills for obstructions and disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.

- Adjust the viewscreen to proper position; NEVER use above the 8-inch mark.
- RESTRICT traffic in the BSC vicinity.

- **RECOMMENDED WORK TECHNIQUES**
  - Wash hands thoroughly with soap and water before and after procedure.
  - Wear sterile gloves and lab coat/gown; use aseptic technique.
  - Avoid blocking front grill. Work only on or over solid surface; adjust chair so armpits are at elevation of lower window edge.
  - Avoid RAPID movement during procedures, particularly within the BSC, but in the vicinity of the BSC, as well.
  - Move hands and arms straight into and out of work area; never rotate hand/arm out of work area during procedure.

- **FINAL PURGING AND WIPE-DOWN**
  - After completing work, run the BSC blower for two minutes before unloading materials from the cabinet.
  - Disinfect the exterior of all containers BEFORE removal from the work zone.
  - Decontaminate interior work surfaces of the BSC with an appropriate disinfectant effective against the agent in question.

- **DECONTAMINATION AND SPILLS**
  - All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. The final surface decontamination of the cabinet should include a wipe-down of the work zone. Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.
  - Small spills within the BSC can be handled immediately by covering the spill with absorbent paper towels, carefully pouring an appropriate disinfectant onto the towel-covered spill, and removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated. Hands should be washed whenever gloves are changed or removed.
  - Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. The drain pan should be emptied into a collection vessel containing disinfectant. Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

(c) MAINTENANCE

To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be verified as leak-proof. The University of Chicago requires that all biological safety cabinets be certified annually. **This is imperative for BSCs intended for work at BSL2 or above**.

(d) DRIP PAN MAINTENANCE

Beneath the BSC work surface is a drip pan to collect large spills. This area ought to be routinely checked for cleanliness and, if a major spill has occurred, appropriately cleaned and disinfected (see **DECONTAMINATION AND SPILLS** above).

(e) PURCHASING A BSC

Before ordering a biological safety cabinet, consult the Office of Biological Safety (773-834-2707) for an evaluation of its suitability for the intended research and the available space.

(f) BSC TRAINING

BSC training is offered quarterly by Environmental Health and Safety and can be arranged online at: https://training.uchicago.edu/course_detail.cfm?course_id=639.

V. DISPOSAL OF WASTES CONTAMINATED WITH INFECTIOUS AGENTS

These biohazardous waste disposal guidelines are designed to protect not only the public and the environment, but also laboratory and custodial personnel, waste haulers, and landfill/incinerator operators at each stage of the waste-handling process. Generators of biohazardous waste in the laboratory must assure that the labeling, packaging, and intermediate disposal of waste conforms to these guidelines.

"**Decontamination**" means a process of removing disease-producing microorganisms and rendering an object safe for handling.
"**Disinfection**" means a process that kills or destroys most disease-producing microorganisms, except spores.
"**Sterilization**" means a process by which all forms of microbial life, including spores, viruses, and fungi, are destroyed.

A. WHAT IS INFECTIOUS WASTE

The following items usually are considered to be infectious waste.

1. Microbiological laboratory wastes such as cultures derived from clinical specimens and pathogenic microorganism, and laboratory equipment, which have come into contact with the cultures.
2. Tissues, bulk blood, and body fluids from humans.
3. Tissues, bulk blood, or body fluids from an animal that is carrying an infectious agent that can be transmitted to humans.
4. Contaminated sharps (needles, broken glass, etc.).

Regulated recombinant DNA organisms and exotic or virulent plant and animal pathogens also require decontamination before disposal.

The following are usually not included in the definition of infectious waste, but should be placed in containers such as plastic bags prior to disposal to contain the waste. If these items were mixed with infectious wastes, they would have to be managed as though they were infectious. For this reason, you should **segregate infectious waste from other waste**.

1. Items soiled or spotted, but not saturated, with human blood or body fluids. Examples: blood-spotted gloves, gowns, dressings, etc.
2. Containers, packages, waste glass, laboratory equipment, and other materials that have had no contact with blood, body fluids, clinical cultures, or infectious agents.
3. Noninfectious animal waste, such as manure and bedding, and tissue, blood, and body fluids or cultures from an animal that is not known to be carrying an infectious agent that can be transmitted to humans.

B. PACKAGING OF WASTE

Laboratory materials used in experiments with potentially infectious microorganisms, such as discarded cultures, tissues, media, plastics, sharps glassware, instruments, and laboratory coats, must be either handed off to a contractor licensed as a infectious waste treatment facility or be decontaminated before disposal or washing for reuse. Collect contaminated materials in leak-proof containers labeled with the *Universal Biohazard Symbol*; autoclavable biohazard bags are recommended.

After autoclaving, biohazard symbols on containers are defaced to assure custodial/waste disposal personnel that containers are safe to handle.

Uncontaminated sharps and other noninfectious items that may cause injury require special disposal even if they need not be decontaminated. Sharps need to be collected in rigid puncture-proof containers to prevent wounding of coworkers, custodial personnel, and waste handlers. If a package is apt to be punctured because of sharp-edged contents, double bagging or boxing may be necessary.

C. METHODS OF DECONTAMINATION

Choosing the right method to eliminate or inactivate a biohazard is not always simple. The choice depends largely on the treatment equipment available, the target organism, and the presence of interfering substances (*e.g.*, high organic content) that may protect the organism from decontamination. A variety of treatment techniques are available, but practicality and effectiveness govern which is most appropriate.

Ideally, biohazardous waste should be decontaminated before the end of each working day unless it is to be collected for treatment off-site. In the latter case, the waste should be packaged and stored (frozen if pathological waste) until the scheduled pick-up by the off-site contractor. Biohazardous waste should never be compacted. Ordinary lab wastes should be disposed of as routinely as possible to reduce the amount requiring special handling.

1. STEAM STERILIZATION

Decontamination is best accomplished by steam sterilization in a properly functioning autoclave that is routinely monitored with a biological indicator such as spores of *Bacillus stearothermophilus*. The tops of autoclavable biohazard bags should be opened to allow steam entry. For dry materials, it may be necessary to add water to the package.

Although we recommend autoclaving all biohazardous wastes for at least one hour, the nature of the waste in a batch should determine cycle duration. For example, if the waste contains a dense organic substrate such as animal bedding or manure, one hour may be insufficient to inactivate certain pathogens buried within. A considerably longer exposure time may be required to effectively decontaminate such waste. Since there is a practical limit to the time that can be spent autoclaving waste, in such a case alternative treatment options may be more effective and economical. However, as with most generalizations, it is difficult to prescribe methods that meet every contingency. Such decisions are best left to the personnel directly involved, provided they are well informed and prepared to verify the effectiveness of the treatment.

Use extreme caution when treating waste that is co-contaminated with volatile, toxic, or carcinogenic chemicals, radioisotopes, or explosive substances. Autoclaving this type of waste may release dangerous gases (*e.g.*, chlorine) into the air. Such waste should be chemically decontaminated, incinerated, or sent to a hazardous waste landfill. Consult Environmental Health and Safety (773-702-9999) for more information.

2. SEWAGE TREATMENT

Most fluid waste, including human blood or infectious cultures that have been decontaminated with the appropriate germicide, can be discarded by pouring into the sanitary sewer, followed by flushing with water. Care should be taken to avoid generation of aerosols. The routine processing of municipal sewage provides chemical decontamination. However, if the fluid is contaminated with infectious agents or biological toxins, it must be rendered safe by chemical or autoclave treatment before sewer disposal.

3. CHEMICAL DISINFECTION

Where autoclaving is not appropriate, an accepted alternative is to treat material with a chemical disinfectant, freshly prepared at a concentration known to be effective against the microorganisms in use. The disinfectant of choice should be one that quickly and effectively kills the target pathogen at the lowest concentration and with minimal risk to the user. Other considerations, such as economy and shelf life, are also important. Allow sufficient exposure time to ensure complete inactivation.

Halogens such as hypochlorite (household bleach) are the least expensive and are also highly effective in decontaminating large spills. Their drawbacks include short shelf life, easy binding to nontarget organic substances, and corrosiveness, even in dilute forms. Hypochlorite typically is diluted 1:10 to 1:100 such that the available halogen is 0.01-5.0%. Also be aware that using chlorine compounds to disinfect substances co-contaminated with radioiodine may cause gaseous release of the isotope.

Alcohol (ethanol or isopropanol), usually used at 70%, is effective against vegetative forms of bacteria and fungi, and enveloped viruses, but will not efficiently destroy spores or non-enveloped viruses. Characteristics limiting its usefulness are its flammability, poor penetration, presence of protein-rich materials, and rapid evaporation, making extended contact time difficult to achieve.

It is important to be aware that common laboratory disinfectants can pose hazards to users. For example, ethanol and quaternary ammonium compounds may cause contact dermatitis. Further information about chemical disinfectants can be obtained from the Office of Biological Safety.

Properties of common classes of disinfectants are summarized in Table 3a and 3b below:

Table 3a.

	Active Against						Dilution
	Fungi	Bacteria (Gram positive and negative)	Myco-bacteria	Spores	Lipid Viruses	Non-lipid Viruses	Optimal working concentration
Phenolic Compounds	+++	+++	+++	-	+	v	1-5%
Hypochlorites	+	+++	++	++	+	+	0.05-0.5% free chlorine (~1-10% household bleach)
Alcohols	-	+++	+++	-	+	v	70-85%
Formaldehyde	+++	+++	+++	+++[a]	+++	+	2-8%
Glutaraldehyde	+++	+++	+++	+++[b]	+	+	2%
Iodophores	+++	+++	+++	+	+	+	0.5%

+++, good; ++, fair; +, slight; -, nil; v, depends on virus.
[a] above 40 °C.
[b] above 20 °C.

Table 3b.

	Inactivated by			Toxicity			Stable?	Corrosive?	Flammable?
	Protein	Hard water	Detergent	Skin	Eyes	Lungs	>1 week (away from light/air)		
Phenolic Compounds	+	+	C	Y	Y	N	Y	Y	N
Hypochlorites	+++	+	C	Y	Y	Y	N	Y	N
Alcohols	+	+	-	N	Y	N	Y	N	Y
Formaldehyde	-	+	-	Y	Y	Y	Y	N	Y/N
Glutaraldehyde	-	+	-	Y	Y	Y	Y	N	N
Iodophores	+++	+	A	Y	Y	N	Y	Y	N

+++, good; ++, fair; +, slight; -, nil; C, inactivated by cationic detergent; A, inactivated by anionic detergent; Y, yes; N, no; Y/N, depends on physical form and other conditions.

Adapted from *Laboratory Safety Monograph. A Supplement to the NIH Guidelines for Recombinant DNA Research.* pp 104-105. National Institutes of Health, Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, Bethesda, MD (January 1979).

Large volume areas such as fume hoods, biological safety cabinets, or rooms may be decontaminated using vapors or gases such as hydrogen peroxide, ethylene oxide, or peracetic acid. These gases, however, must be applied with extreme care. **Only experienced personnel who have the specialized equipment and protective devices to do it effectively and safely should perform gas decontamination.**

VI. EMERGENCY PLANS AND REPORTING

No matter how carefully one works, laboratory accidents occur and necessitate emergency response. Emergency plans should be tailored for a given biohazardous situation. The laboratory supervisor should prepare instructions specifying immediate steps to be taken. These instructions should be displayed prominently in the laboratory and periodically reviewed with personnel. No single plan will apply to all situations but the following general principles should be considered:

A. SPILL PROTOCOLS

1. In the event of an extensive or explosive spill of virulent pathogens, everyone should leave the affected area **immediately**. Clothing, if contaminated, should be removed. Exposed skin should be washed thoroughly.

2. Close the laboratory door and post a "No Entry" sign indicating the hazard. Notify the laboratory supervisor and the Office of Biological Safety (773-834-2707).

3. Determine the necessity and extent of medical treatment for persons exposed to infectious microorganisms. Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation of an infectious agent should be given appropriate first aid and, if necessary, transported to the University Hospital emergency room. UC Occupational Medicine (UCOM) should be notified. If after hours, the Infectious Diseases/BBP Hotline should be contacted (773-753-1880, enter pager number 9990, followed by #). Your supervisor, principal investigator and Office of Biological Safety should be notified.

4. Do not reenter the room until large droplets have settled and aerosols have been cleared by the building ventilation system (30 minutes, minimum), and the extent of the hazard and its dissemination has been determined.

5. Each person who enters the laboratory for clean-up should wear proper protective clothing.

6. Use an appropriately concentrated disinfectant to clean up and decontaminate the area. A supply of stock disinfectants should always be available.

7. Decontaminate all materials used in clean-up procedures.

In any emergency situation, attention to immediate personal danger overrides containment considerations. Currently, there is no known biohazard on the University of Chicago campus that would prohibit properly garbed and masked fire or security personnel from entering any biological laboratory in an emergency.

B. EXPOSURE PROTOCOLS

Determine the necessity and extent of medical treatment for persons exposed to infectious microorganisms. Personnel accidentally exposed via ingestion, skin puncture, or obvious inhalation of an infectious agent should be given appropriate first aid and, if necessary, transported to the University Hospital emergency room. For exposures to the eyes or mucous membranes, the exposed area should be flushed with running water for a minimum of 15 minutes. UC Occupational Medicine (UCOM) should be notified.

If after hours, the Infectious Diseases/BBP Hotline should be contacted (**773-753-1880, enter pager number 9990, followed by #). From a campus phone, 1) dial 188#, 2) at tone, dial 9990#, 3) at the tone, enter your callback number followed by the pound sign and hang up**. Your supervisor, principal investigator and Office of Biological Safety should be notified.

C. REPORTING

The importance of reporting accidental spills or exposure events is obvious. Not only is this important in terms of personal health, but it is also important for the health of our coworkers, the research community, and the general public.

The secure and responsible conduct of life sciences research depends, in part, on observation and reporting by peers, supervisors, and subordinates. Individuals working with potentially infectious material and/or molecular recombinant DNA constructs with either direct or indirect, acute or latent disease potential (*e.g.*, insertional mutagenesis due to exposure to a viral vector) must understand and acknowledge their responsibility to report activities that are inconsistent with a culture of responsibility or are otherwise troubling. Likewise, institutional and laboratory leadership must acknowledge their responsibility to respond to reports of concerning behavior and undertake actions to prevent retaliation stemming from such reports.

The University of Chicago Office of Risk Management has established a program to enable the anonymous reporting of troubling behavior. Information about this program can be found at: http://rmas.uchicago.edu/whistleblower.shtml. In addition, reports can be provided to UC at: Whistleblower hotline: 1-800-971-4317.

Reports of concerning behavior within the lab can also be reported to the Office of Biological Safety, the Department of Environmental Health and Safety, the Institutional Biosafety Committee, and the University Safety Committee. Please see Chapter II and Appendix 2 of this Manual for additional information on reporting of concerning behavior in the laboratory.

Finally, persons with health conditions, whether chronic or acute, that have the potential to place them at risk in the laboratory should self-report to their personal physician and/or UC Occupational Medicine. A decision about continued involvement with research involving infectious agents must be an informed decision that includes appropriate medical expertise.

VII. SHIPPING HAZARDOUS BIOLOGICAL MATERIALS

Hazardous materials capable of posing an unreasonable risk to health, safety, and property, are commonplace in University facilities. Amongst them are chemicals and solvents, cleaning agents, radionuclides, infectious agents, and toxins. When hazardous materials are transported in commerce, complex federal regulations for shipping hazardous materials must be followed. Seemingly minor technical violations can result in major fines while more serious violations can endanger the public.

The U.S. Department of Transportation requires all persons involved in shipping hazardous materials to be trained and certified in proper handling of these materials. Activities for which training is required include:

- Preparing shipping papers
- Loading and unloading trucks
- Marking and labeling packages
- Filling packages
- Supervising these activities

Required training for shipping of hazardous biological materials is offered by Environmental Health & Safety. Information for obtaining this training can be found here: http://safety.uchicago.edu/tools/faqs/training.shtml.

VIII. VIRAL VECTORS

Viral vectors have become standard tools for molecular biologists. For this reason, it is necessary that researchers using these biological agents are aware of their origins and the consequences of their use.

The following contains pertinent information for commonly used viral vectors at UC:

A. Adenovirus

Virology: Medium-sized (90–100 nm), non-enveloped icosahedral viruses containing double-stranded DNA. There are more than 49 immunologically distinct types (6 subgenera: A–F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body.

Cultivation: Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors is capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

Clinical features: Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash-associated illnesses. Symptoms of respiratory illness caused by adenovirus infection range from common cold symptoms to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection that can cause more systemic diseases.

Epidemiology: Although epidemiologic characteristics of the adenoviruses vary by type, all are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission. Some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years. Some adenoviruses (*e.g.*, serotypes 1, 2, 5, and 6) have been shown to be endemic in parts of the world where they have been studied, and infection is usually acquired during childhood. Other types cause sporadic infection and occasional outbreaks; for example, epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Epidemics of febrile disease with conjunctivitis are associated with waterborne transmission of some adenovirus types. ARDS is most often associated with adenovirus types 4 and 7 in the United States. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower respiratory tract disease, whereas oral transmission of the virus typically causes no or mild disease.

Treatment: Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

Laboratory hazards: Ingestion; droplet exposure of the mucous membrane.

Susceptibility to disinfectants: Susceptible to Clidox, 5% household bleach (freshly prepared), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate.

Source: http://www.drs.illinois.edu/bss/factsheets/viralvectors.aspx#adenovirus

B. Adeno-Associated Virus (AAV)

Virology: Adeno-associated virus gets its name because it is often found in cells that are simultaneously infected with adenovirus. Parvoviridae; icosahedral, 20–25 nm in diameter; single stranded DNA genome with protein capsid. AAV is dependent for replication on presence of wild type adenovirus or herpesvirus; in the absence of helper virus, AAV will stably integrate into the host cell genome. Coinfection with helper virus triggers lytic cycle, as do some agents that appropriately perturb host cells. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theoretical risk of insertional mutagenesis.

Clinical features: No known pathology for wild type AAV serotype 2.

Epidemiology: Not documented definitively. Infection apparently via mouth, esophageal, or intestinal mucosa.

Treatment: No specific treatment.

Laboratory hazards: Ingestion, droplet exposure of the mucous membrane, direct injection.

Susceptibility to disinfectants: Susceptible to Clidox, 5% household bleach (freshly prepared), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate.

Source: http://www.med.upenn.edu/gtp/vectorcore/BiosafetyInformation.shtml.

C. Epstein-Barr Virus (EBV)

Virology: Double-stranded linear DNA, 120–150 nm diameter, enveloped, icosahedral; types A and B; Herpesviridae (Gammaherpesvirinae). A ubiquitous B- lymphotrophic herpesvirus, EBV has been found in the tumor cells of a heterogeneous group of malignancies (Burkitt's lymphoma, lymphomas associated with immunosuppression, other non-Hodgkin's lymphomas, Hodgkin's disease, nasopharyngeal carcinoma, gastric adenocarcinoma, lymphoepithelioma-like carcinomas, and immunodeficiency-related leiomyosarcoma). EBV is a transforming virus and can immortalize B-cells and cause lymphoma in various animal models.

Clinical Features: Infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly, and lymphadenopathy; one to several weeks, rarely fatal/ Burkitt's lymphoma - monoclonal tumor of B cells, usually involving children's jaw involvement is common; AIDS patients (25%–30% are EBV related) / Nasopharyngeal carcinoma - malignant tumor of epithelial cells of the nasopharynx involving adults between 20 and 40 years.

Epidemiology: EBV infects 80–90% of all adults worldwide; mononucleosis is common in early childhood worldwide, typical disease occurs in developed countries, mainly in young adults; Burkitt's tumor is worldwide but hyperendemic in highly malarial areas such as tropical Africa; carcinoma is worldwide but highest in Southeast Asia and China.

Transmission: Mononucleosis - person-to-person by oropharyngeal route via saliva, possible spread via blood transfusion (not important route); Burkitt's lymphoma - primary infection occurs early in life or involves immunosuppression and reactivation of EBV later, malaria an important co-factor; NPC infection occurs in early life and reactivation later with epithelial invasion.

Treatment: No specific treatment

Laboratory hazards: Ingestion, accidental parenteral injection, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials. Note that cell lines are often immortalized by transformation with EBV.

Susceptibility to disinfectants: Susceptible to many disinfectants – Clidox, 5% household bleach (freshly prepared), 70% ethanol, 2% glutaraldehyde/formaldehyde.

Source:
http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/EBV.pdf.

D. Lentivirus

Virology: The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. Five serogroups are recognized, reflecting the mammalian hosts with which they are associated. HIV-1 is the type species.

Available constructs: Most of the lentiviral vectors presently in use are HIV-derived vectors. The *cis*- and *trans*-acting factors of lentiviruses are often on separate plasmid vectors, with packaging being provided *in trans*. The vector constructs contain the viral *cis* elements, packaging sequences, the Rev response element (RRE), and a transgene.

Lentiviral Pseudotyping: Replacement of the HIV envelope glycoprotein with VSV-G provides a broad host-range for the vector and allows the viral particles to be concentrated by centrifugation.

Clinical Features: In terms of the pathogenesis of lentivirus, some key properties are:

- **Lifelong persistence -** This is a function both of their ability to integrate into the host chromosome and evade host immunity. This ability to evade host immunity may be related both to the high mutation rates of these viruses, and to their ability to infect immune cells (macrophages, and in the case of HIV, T-cells).
- **Lentiviruses have high mutation rates**. Lentiviruses replicate, mutate, and undergo selection by host immune responses.
- **Infection proceeds through at least three stages**.

(A) Initial (acute) lentivirus infection is associated with rapid viral replication and dissemination, which is often accompanied by a transient period of disease.

(B) This is followed by a latent period, during which the virus is brought under immune control and no disease occurs.

(C) High levels of viral replication then resume at some later time, resulting in disease.

Epidemiology: Transmitted from person to person through direct exposure to infected body fluids (blood, semen), sexual contact, sharing unclean needles, etc.; transplacental transfer can occur.

Treatment: Specific measures for the opportunistic diseases that result from AIDS; multidrug treatment for HIV.

Laboratory Hazards: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral injection; ingestion; hazard of aerosols exposure unknown.

Susceptibility to disinfectants: Susceptible to many disinfectants – Clidox, 5% household bleach (freshly prepared), 70% ethanol, 2% glutaraldehyde/formaldehyde.

Source: http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Lentivirus.pdf.

E. Retrovirus (Other than Lentivirus)

Infectious viruses that integrate into transduced cells with high frequency and may have oncogenic potential in their natural hosts. They are usually based on murine viruses. They include ecotropic viruses (infect murine cells only), amphotropic viruses (infect murine and human cells), or pseudotyped viruses, when vector particles express glycoproteins derived from other enveloped viruses (usually can infect human cells). The most common glycoprotein currently used is VSV-g; however, there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola, and Marburg.

Virology [Moloney Murine Leukemia Virus (MoMuLV), Murine Stem Cell Virus (MSCV), etc.]: Retroviridae; subfamily oncovirinae type C, enveloped, icosahedral core, virions 100 nm in diameter, diploid, single-stranded, linear RNA genome. MoMuLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

Virus is not lytic. Data suggest a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk.

The host range is dependent upon the specificity of the viral envelope. The ecotropic *env* gene produces particles that infect only rodent cells. The amphotropic *env* gene allows infection of rodent and non-rodent cells, including human cells.

VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells.

Clinical features: None to date.

Epidemiology: MMLV infects only actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germline infection*. In vivo* transduction in humans appears to require direct injection with amphotropic or pseudotyped virus.

Treatment: No recommended treatment.

Laboratory Hazards: Contact with feces or urine from infected animals for 72 hours post-infection. Contact with tissues and body fluids of infected animals. Direct injection.

Susceptibility to disinfectants: Susceptible to many disinfectants – Clidox, 5% household bleach (freshly prepared), 70% ethanol, 2% glutaraldehyde/formaldehyde.
Source: http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Moloney_Murine_Leukemia_Virus.pdf.

F. Poxvirus/Vaccinia

Poxvirus vectors include avian viruses (avipox vectors) such as NYVAC and
ALVAC, which cannot establish productive infections in humans, as well as mammalian poxviruses, which can productively infect humans—such as vaccinia virus and modified vaccinia viruses (MVA). Poxviruses are highly stable, and vaccinia virus can cause severe infections in immuno-compromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus.

Virology: The poxviruses are the largest known DNA viruses and are distinguished from other viruses by their ability to replicate entirely in the cytoplasm of infected cells. Poxviruses do not require nuclear factors for replication and, thus, can replicate with little hindrance in enucleated cells. The core contains a 200-kilobase (kb), double-stranded DNA genome, and is surrounded by a lipoprotein core membrane.

Recombinant Vaccinia vectors: Vaccinia virus can accept as much as 25 kb of foreign DNA, making it useful for expressing large eukaryotic and prokaryotic genes. Foreign genes are integrated stably into the viral genome, resulting in efficient replication and expression of biologically active molecules. Furthermore, post-translational modifications (*e.g.*, methylation, glycosylation) occur normally in the infected cells.

Vaccinia is used to generate live recombinant vaccines for the treatment of other illnesses. Modified versions of vaccinia virus have been developed for use as recombinant vaccines. The modified Ankara strain (MVA) of vaccinia virus was developed by repeated passage in a line of chick embryo fibroblasts. NYVAC is another attenuated form of the vaccinia virus that has been used in the construction of live vaccines. NYVAC has a deletion of 18 vaccinia virus genes that render it less pathogenic.

Clinical Features: Virus disease of skin induced by inoculation for the prevention of smallpox; vesicular or pustular lesion; area of induration or erythema surrounding a scab or ulcer at inoculation site; major complications - encephalitis, progressive vaccinia (immunocompromised susceptible), eczema vaccinatum, fetal vaccinia; minor complications - generalized vaccinia with multiple lesions; auto-inoculation of mucous membranes or abraded skin, benign rash, secondary infections; complications are serious for those with eczema or who are immunocompromised.

Epidemiology: Communicable to unvaccinated contacts via contact with mucosal membranes or cuts in skin.

Treatment: Vaccinia immune globulin and an antiviral medication may be of value in treating complications.

Susceptibility to disinfectants: Susceptible to Clidox, 5% household bleach (freshly prepared), 2% glutaraldehyde/formaldehyde

Source: http://emedicine.medscape.com/article/231773-overview.

G. Baculovirus

Non-mammalian virus that usually infects insects. They can be very stable, lasting in the environment for years. Able to transduce mammalian cells, but cannot usually replicate within them. Work is usually done at BSL1. Note: Even though this vector is non-pathogenic it must still be inactivated by heat or chemical methods following use because it is a recombinant agent.

UC Biosafety Management of Viral Vectors
To determine what biosafety level to use and what method of viral vector testing is mandated by the UC IBC, please go to this link: http://ibc.**uchicago**.edu/docs/ibc_Testing_Requirements_**Viral_Vectors**.pdf.

IX. BIOLOGICAL TOXINS

BASIC CHARACTERISTICS

Biological toxins are natural, poisonous substances produced as by-products of microorganisms (exotoxins, endotoxins, and mycotoxins, such as T-2 and aflatoxins), plants (plant toxins such as ricin and abrin), and animals (zootoxins such as marine toxins and snake venom). Unlike pathogenic microorganisms, including those that produce toxins, the toxins themselves are not contagious and do not replicate. In this regard, toxins behave more like chemicals than infectious agents. However, unlike many chemical agents, biological toxins are not volatile and are odorless and tasteless. The stability of toxins varies greatly, depending on the toxin structure (low molecular weight toxins are quite stable).

Most biological toxins, with the exception of T-2 Mycotoxin, are NOT dermally active; i.e., intact skin is an excellent barrier against most toxins. That said, mucous membranes of the eyes, nose, and mouth serve as portals of entry, as do breaks in the skin. Aerosol transmission, ingestion, and percutaneous transmission are also a concern for most biological toxins.

Bacterial toxins can be exotoxins (including enterotoxins) or endotoxins. Exotoxins are cellular products excreted from certain viable Gram positive and negative bacteria, highly toxic (i.e., microgram quantities) and are relatively unstable (destroyed rapidly when heated to $\geq 60^{o}C$). Bacterial endotoxins are lipopolysaccharide complexes derived from the cell membrane of Gram negative bacteria which are released upon bacterial death. Endotoxins are relatively stable (can withstand heating at $60^{o}C$ for hours without losing activity) and moderately toxic (tens to hundreds of micrograms required for animal fatality).

The modes of action of biological toxins vary, but include damage to cell membranes or cell matrices (e.g. *Staphylococcus aureus* alpha toxin), inhibition of protein synthesis (e.g. Shiga toxin), or via activation of secondary messenger pathways (e.g. *Clostridium botulinum* and *C. difficile* toxins).

LABORATORY REQUIREMENTS AND SAFETY OPERATIONS

Most work with biological toxins can be safely managed in a BSL2 setting. In some cases (*e.g.,* large scale production, manipulation of large quantities of powder form of toxin) management at BSL3 may be required, depending on the toxin in question and the quantities used. The most hazardous form of any toxin is the dry, powder form. Manipulations of dry forms of toxins should be performed in a biological safety cabinet or in a fume hood. In some cases a glove box may be recommended for such operations.

Once reconstituted into an aqueous form, BSL2 management is usually sufficient for work with most biological toxins. Your lab should exhibit negative, directional air-flow and like all BSL2 labs, have a hand-washing sink available within the lab. Access to the lab should be controlled when toxin is in use. Biohazard warning signs displaying the biosafety level, toxin in use, emergency contact information, and entrance requirements (available upon request from the Office of Biological Safety) should be posted at the lab entrance. If vacuum lines are used, it is advisable to protect the vacuum system with an in-line disposable HEPA filter. Personal protective equipment should include a lab coat, gloves and mucous membrane protection. You should routinely confirm the operational status of your lab eye wash station and safety shower. All personnel in the lab should be trained about the specific hazards associated with the toxin in use. At UC, an IBC protocol is required for research utilizing any of the toxins listed in Table 4.

Table 4
TOXINS THAT REQUIRE AN IBC PROTOCOL

Toxin	LD_{50} (µg/kg)*
Abrin	**0.7**
Aerolysin	**7**
Botulinum toxin A	**0.0012**
Botulinum toxin B	**0.0012**
Botulinum toxin C1	**0.0011**
Botulinum toxin C2	**0.0012**
Botulinum toxin D	**0.0004**
Botulinum toxin E	**0.0011**
Botulinum toxin F	**0.0025**
b-bungarotoxin	**14**
***Clostridium difficile* enterotoxin A**	**0.5**
***Clostridium perfringens* lecithinase**	**3**
***Clostridium perfringens* perfringolysin O**	**13-16**
***Clostridium perfringens* delta toxin**	**5**
Clostridium perfringens epsilon toxin	**0.1**
Conotoxin (Only short, paralytic alpha conotoxins with specific sequences are considered Select Agents)	**12-30**
Diacetoxyscirpenol	**1000-10,000**
Diphtheria toxin	**0.1**
Listeriolysin	**3-12**
Modeccin	**1-10**
Pertussis toxin	**15**
Pneumolysin	**1.5**
***Pseudomonas aeruginosa* toxin A**	**3**
Ricin	**2.7**
Saxitoxin	**8**
Shiga toxin	**0.25**
***Shigella dysenteriae* neurotoxin**	**1.3**
***Staphylococcus* enterotoxin B**	**25**
***Staphylococcus* enterotoxin F**	**2-10**
***Staphylococcus* enterotoxins A, C, D, and E**	**20(A); <50(C)**
Streptolysin O	**8**
Streptolysin S	**25**
T-2 toxin	**5,000-10,000**
Taipoxin	**2**
Tetanus toxin	**0.001**
Tetrodotoxin	**8**
Volkensin	**1.4**
***Yersinia pestis* murine toxin**	**10**

***Note that the LD_{50} values are from a number of sources (see below). For specifics on route of application, animal used, and variations on the listed toxins, please go to the references listed below. (Table courtesy, in part, of University of Florida EHSO).**

Toxins are noted in RED are considered Select Agents if being stored in large enough quantities. For more information please consult (http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html).

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3. Registry of toxic effects of chemical substances (RTECS): comprehensive guide to the RTECS. 1997. Doris V. Sweet, ed., U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, OH.

SECURITY

It is important that stocks of biological toxins be maintained in locked freezers and refrigerators. Since biological toxins are not self-replicating as are microorganisms, it is prudent to maintain an inventory of toxins present in a lab at any given time. This inventory should display the current quantity of a particular toxin in site, the date and amount removed from storage, the person removing the aliquot from storage, the purpose of use, and the quantity remaining. Inventory forms are available from the Office of Biological Safety upon request.

DECONTAMINATION METHODS

The majority of biological toxins can be inactivated or decontaminated with household bleach (i.e., a 1:2 dilution of household bleach or a 2.5% sodium hypochlorite solution) or autoclaving. Tables 5 and 6 below describe the inactivation regimens for biological toxins in common use:

Table 5

COMPLETE INACTIVATION OF DIFFERENT TOXINS WITH A 30-MINUTE EXPOSURE TIME TO VARYING CONCENTRATIONS OF SODIUM HYPOCHLORITErite (NaOCl) +/- SODIUM HYDROXIDE (NaOH)

Toxin	2.5% NaOCl + 0.25 N NaOH	2.5% NaOCl	1.0% NaOCl	0.1% NaOCl
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

(Wannemacher, 1989)

Table 6

COMPLETE INACTIVATION OF TOXINS BY AUTOCLAVING OR 10-MINUTE EXPOSURE TO VARYING TEMPERATURES OF DRY HEATt

Toxin	Autoclaving	Dry Heat°F			
		200	500	1000	1500
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

(Wannemacher, 1989)

For exposure events involving skin exposure to minute quantities of toxin, soap and water are effective in removing the toxin burden (toxins are not dermally active, except for T-2 mycotoxin). For significant exposures to biological toxins, contact Occupational Medicine immediately.

X. SELECT AGENTS AND TOXINS

The federal government has published a list of infectious agents and biological toxins that it strictly regulates due to their potential for use as bioterror agents. Shipping, manipulation, and even possession of these “Select Agents” are heavily regulated at the Federal and Institutional level. Currently, the only work with Select Agents at the University of

Chicago occurs at the Howard Taylor Ricketts Laboratory on the campus of Argonne National Laboratory. There are no Select Agents that are currently approved for use at the Hyde Park campus.

For more information about the National Select Agent Program, including a list of the agents that are currently regulated, please visit this site:
http://www.selectagents.gov/index.html.

If you think you may be in possession of agents on this list or intend to study them, please contact the Office of Biological Safety.

XI. DUAL USE RESEARCH

Broadly defined, "dual use" refers to the malevolent misapplication of technology or information initially developed for benevolent purposes. In the realm of life sciences, "dual use" refers to the potential misuse of microorganisms, toxins, recombinant DNA technology or research results to threaten public health or national security. "Dual Use Research of Concern," referred to as DURC, is research that has a potential to be DIRECTLY misapplied.

The National Science Advisory Board on Biosecurity (NSABB) is an advisory board to the U.S. Government on issues of biosecurity. The NSABB is administered through the NIH Office of Biotechnology Activities (NIH-OBA), which publishes the most recent NSABB discussions and NSABB reports on issues involving Dual Use Research. A video prepared by the NSABB is available on the NIH-OBA web-site, which can be accessed here:
http://oba.od.nih.gov/biosecurity/biosecurity.html.

If you think someone may be misusing biological agents or data in a manner that may be harmful to public health or national security or wish to learn more about DURC, please contact the Biosafety Officer by phone (773-834-7496) or visit the Office of Biological Safety (Abbott Hall, Rm. 120). Your identity will be kept confidential.

The Institutional Biosafety Committee invites persons who have questions or concerns regarding biosafety aspects of their work to contact the Office of Biological Safety (773-834-2707) or go to 947 E. 58th St, Abbott Hall 120.

Additional copies of this booklet can be obtained from the Office of Biological Safety, 947 E. 58th St; Abbott 120. 773-834-2707.

Appendix 1
SAFETY INFORMATION AND ASSISTANCE

Office of Biological Safety	773-834-2707
Biosafety Officer	773-834-7496
Assistant Biosafety Officer (Hyde Park)	773-834-6756
Assistant Biosafety Officer (H.T. Ricketts Laboratory)	630-252-1742
Environmental Health & Safety	773-702-9999
Institutional Biosafety Committee (IBC) Office	773-834-4765
Biohazard information, Laboratory spills or accidents involving biological agents, Biological safety cabinet, Recombinant DNA guidelines, Shipping Requirements, Permits	Office of Biological Safety (See numbers above) or http://biologicalsafety.uchicago.edu/
Hazardous waste disposal (chemical, toxic, animal), laboratory spills, or accidents involving chemicals, Chemical hygiene plans	Environmental Health & Safety (See number above)
Ventilation (HVAC) problems, Fume hood operation, Laboratory safety design	Environmental Health & Safety (See number above)
Radioactive materials	Radiation Safety Associate Director 773-834-8876
Research animal use	Animal Resources Center (ARC) General Questions 773-702-6756 **Emergencies** 773-702-1342
Bloodborne pathogens training and information	Environmental Health & Safety (See number above)
UC Occupational Medicine Needlestick Hotline	1-773-753-1880 enter pager: 9990#

Appendix 2

IBC PROCEDURES FOR DEALING WITH BIOSAFETY/BIOSECURITY CONCERNS